Curtin Medical School

Physicochemical Compatibility of Parenteral Medications Used in Neonatal Intensive Care

Dadallage Thisuri Nayanthi De Silva

0000-0003-2508-9550

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

March 2024

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: …….

Date: 25/06/2024

Abstract

Background: Patients in neonatal intensive care unit (NICU) settings, often receive multiple, high concentration intravenous (IV) medications through a single IV access point, via three-way (Y-site) connectors. Due to fluid restriction, IV medications are infused at a very slow flow rate to avoid adverse effects of fluid overload. As such, the drugs remain in contact with each other for long periods of time, hence, their physicochemical compatibility is an important consideration. Physical incompatibility can present as precipitation, turbidity, particle formation, colour change, and evolution of gas, leading to adverse consequences such as infusion line occlusion and thromboembolism. Chemical incompatibility can lead to reduction in drug concentrations resulting in suboptimal clinical outcomes or adverse effects if toxic compounds are formed. Hence, for optimal neonatal drug therapy, reliable physicochemical compatibility data, pertaining to NICU drugs and parenteral nutrition (PN) solutions, should be available.

Amongst the many drugs used in NICU's, sildenafil and caffeine are two life-saving medications. Sildenafil is effectively used to treat pulmonary hypertension and caffeine is a respiratory stimulant used to treat apnoea in premature neonates.

Aims: The research project consisted of three components. The first was conducting a systematic review of physicochemical compatibility of IV drugs used in NICU settings. Secondly, the physicochemical compatibility of sildenafil with 45 other NICU drugs and 2-in-1 PN solutions was evaluated. The third component involved investigating the physicochemical compatibility of caffeine citrate and caffeine base injection with 43 secondary IV drugs and 2-in-1 PN solutions used in NICU settings.

Methods: In conducting the systematic review, the 'SPIDER' systematic review model was used to formulate the research question. The search strategy included a predetermined list of NICU drugs prepared by a clinical expert panel. Selection of abstracts from database search results, was facilitated by a semi-automated, machine learning tool, 'Research Screener'. The selected articles were then subjected to full-text reading to include in the review, based on pre-determined inclusion criteria. Using a pre-tested data extraction sheet and a quality assessment instrument, data pertaining to the type of compatibility studied, study setting, drug(s), concentrations tested, diluents used, conditions used to simulate contemporary neonatal setting, method of mixing drugs, test conditions, methods to test physical and chemical compatibility, key results, and conclusions, were collected. Statistical and narrative synthesis of data was carried out to have implications for future research.

To investigate the physicochemical compatibility of sildenafil with other NICU drugs and 2-in-1 PN solutions, sildenafil 600 µg/mL or 60 µg/mL was mixed 1:1 with the secondary drug solution to simulate Y-site co-administration procedures. Physical compatibility was evaluated by visual observation against a black and white background and under polarized light for 2 hours, for changes in colour, precipitation, haze and evolution of gas. Chemical compatibility was determined from sildenafil concentrations, using a validated, stability-indicating high performance liquid chromatography (HPLC) assay.

To investigate the physicochemical compatibility of caffeine citrate and caffeine base injections with other NICU drugs and 2-in-1 PN solutions, caffeine citrate (20 mg/mL or 10 mg/mL) or caffeine base injection (10 mg/mL) were mixed with the secondary

drug solutions and physicochemical compatibility testing was carried out in a similar experimental procedure as outlined above.

Results: In the systematic review process, data base searching, and deduplication produced 27,597 articles for initial screening, of which, 118 were selected for the review. The majority (72%) had only evaluated physical compatibility, 2% evaluated chemical compatibility only, and 26% evaluated both physical and chemical compatibility of selected IV drug combinations. Physical compatibility has been evaluated by both visual and subvisual methods. HPLC was the most widely used technique to assess chemical compatibility. Although physical compatibility data are available for crucial NICU drugs such as inotropes and prostaglandins, there are limited chemical compatibility data for several drugs, including epinephrine and alprostadil. Sildenafil and caffeine have been limitedly studied for combined physicochemical compatibility.

Sildenafil 600 µg/mL was physicochemically compatible with 29 of the 45 drugs tested at 'high-end' clinical concentrations and physically incompatible with 16 drugs and six 2-in-1 PN solutions. Sildenafil 600 µg/mL was compatible with lower, clinically relevant concentrations of calcium gluconate, heparin, and hydrocortisone. Aciclovir, amoxicillin, ampicillin, ibuprofen lysine, indomethacin, phenobarbitone and rifampicin were incompatible with sildenafil 600 µg/mL, but compatible with sildenafil 60 µg/mL. Amphotericin, flucloxacillin, furosemide, ibuprofen, meropenem and sodium bicarbonate were incompatible with sildenafil 600 µg/mL and 60 µg/mL.

In the caffeine compatibility evaluation, six of the 43 secondary drugs tested (aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine) were physically incompatible with caffeine citrate undiluted injection (20

mg/mL), at their high end, clinically relevant concentrations for NICU settings. However, when tested at lower concentrations, hydrocortisone (1 mg/mL) was physicochemically compatible, whereas furosemide (0.2 mg/mL) was physically incompatible with caffeine citrate. The six drugs which showed physical incompatibility with caffeine citrate 20 mg/mL injection were also physically incompatible with caffeine citrate 10 mg/mL solution. All 43 secondary drugs tested were physicochemically compatible with caffeine base injection. The 2-in-1 PN solutions tested were physicochemically compatible with both caffeine citrate (20 mg/mL) and caffeine base (10 mg/mL) injections.

Conclusions: By its machine learning-aided article ranking system, Research Screener tool significantly reduced the burden of article screening, resulting in only 10% of abstracts within a large search database (~ 25000 articles) having to be evaluated in order to identify eligible studies. Although physicochemical compatibility information is imperative for clinical decisions, these combined data are reported in <30% of published literature. Therefore, an increased focus on physicochemical compatibility studies with direct relevance to contemporary healthcare settings will enhance the existing databases and provide greater support for clinical decisions.

Sildenafil 600 µg/mL was physicochemically compatible with approximately 70% of the 45 clinically relevant IV drugs used in NICU settings that were tested in the present study. Most secondary test drugs were physicochemically compatible with caffeine citrate injection. Caffeine base injection was physicochemically compatible with all 43 test drugs tested.

Publications and Presentations

Publications

- **Paper 1** – **De Silva DTN**, Moore BR, Strunk T, Petrovski M, Varis V, Chai K, Ng L, Batty KT (2024) Development of a pharmaceutical science systematic review process using a semi-automated machine learning tool: Intravenous drug compatibility in the neonatal intensive care setting. Pharmacology Research & Perspectives. 12:e1170. https://doi.org/10.1002/prp2.1170
- **Paper 2** – **De Silva DTN**, Strunk T, Petrovski M, Page-Sharp M, Moore BR, Batty KT (2024) The Physicochemical Compatibility of Sildenafil Injection with Parenteral Medications Used in Neonatal Intensive Care Settings. Pharmaceutics. 16:419. http://dx.doi.org/10.3390/pharmaceutics16030419
- **Paper 3 – De Silva DTN,** Petrovski M, Strunk T, Mukadam N, Page-Sharp M, Moore BR, Batty KT (2024) Physicochemical compatibility of caffeine citrate and caffeine base injections with parenteral medications used in neonatal intensive care settings. European Journal of Clinical Pharmacology. https://doi.org/10.1007/s00228-024-03678-6

Note: Publication waivers and attribution statements for all published papers can be found in Appendix 10.

Conference abstracts – Oral presentation

- **Australasian Pharmaceutical Science Association - Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists Joint Conference, Perth, Australia, December 2022**
  Systematic review of intravenous drug compatibility in neonatal intensive care setting, won the award for the best oral presentation in the 'Pharmaceutical Sciences' stream

Conference abstracts – Poster presentations

- **81st FIP (International Pharmaceutical Federation) world congress held in Brisbane, September 2023**
  Systematic review of intravenous drug compatibility in neonatal intensive care setting

- **81st FIP (International Pharmaceutical Federation) world congress held in Brisbane, September 2023**

  Physicochemical compatibility of sildenafil injection with intravenous drugs used in the neonatal intensive care setting

Acknowledgements

I express my heartfelt gratitude to my incredibly supportive supervisory team Prof. Kevin Batty, A/Prof. Brioni Moore, Prof. Tobias Strunk and Mr. Michael Petrovski. I consider myself absolutely privileged and blessed, to have their endless support throughout this remarkable journey. Without your support, this project would not have been possible. Thank you for bringing out the best in me.

I am very much grateful to Prof. Kevin Batty, the principal supervisor of my PhD, for giving me this wonderful postgraduate study opportunity and introducing me to drug compatibility research. Your guidance was instrumental.

Special thanks to A/Prof. Brioni Moore, for her continuous guidance and motivation throughout the project and the thesis writing process.

I wish to acknowledge my co-supervisors from King Edward Memorial Hospital, Prof. Tobias Strunk and Mr. Michael Petrovski, for their invaluable guidance with regard to clinical aspects of the project.

I would like to express my sincere appreciation to the Sri Lankan 'Accelerating Higher Education Expansion and Development Operation' (AHEAD) Scholarship and Curtin Medical School, for providing financial support for my PhD program. Further, I am very much thankful to The Open University of Sri Lanka, for granting me study leave to complete the postgraduate studies.

My sincere thanks to Dr. Kylie Munyard (Director Graduate Research), A/Prof. Cyril Mamotte, Dr. Hendra Gunosewoyo (Thesis chairs) and to distinguished thesis examiners for their guidance during my thesis preparation and examination.

I would like to express my thanks to Dr. Madhu Page-Sharp for her support with her expertise in Analytical Chemistry. Further the contribution of Dr. Giuseppe Luna and Mr. Jorge Martinez was remarkable. Thank you so much!

I appreciate the invaluable assistance by the Pharmacy staff of King Edward Memorial Hospital in completion of the laboratory experiments, by providing the required drugs and intravenous solutions.

Sincere thanks to my colleagues in the laboratory, and the Sri Lankan friends in Perth, for their excellent company and support.

I am very grateful for my beautiful family in Sri Lanka – my parents and the two younger siblings for their continuous support throughout, particularly my mother for inspiring me in science.

Last but certainly foremost, my deepest thanks to Dineth, for his unconditional love and support. Thank you for believing in me and accepting me for being me. You were my strongest motivation.

Dedication

to Dineth……
for seeing my dream as his own…….

Table of Contents

Declaration ii
Abstract iii
Publications and Presentations vii
Acknowledgements ix
Dedication xi
Table of Contents xii
Abbreviations xvi
List of Figures xviii
List of Tables xxi
Chapter 1 Introduction & Literature Review 1
1.1 Background 1
1.1.1 Neonates and neonatal intensive care 1
1.1.2 Parenteral drug/fluid delivery in neonates 3
1.1.2.1 Fluid restriction and subsequent high standard drug concentrations used in neonates 3
1.1.2.2 Multidrug therapy 3
1.1.3 Importance of drug compatibility during co-administration of multiple drugs 5
1.1.4 Availability of physical and chemical drug incompatibility information 6
1.1.5 Information sources for drug compatibility evaluation in hospitals 6
1.2 Drug incompatibility 7
1.2.1 Types of IV drug incompatibilities 7
1.2.2 Complications of drug incompatibilities 12
1.2.3 IV drug compatibility in neonates – What is already known? 14
1.2.3.1 Types of drugs/ admixtures investigated for stability and compatibility 14
1.2.3.2 Physical and chemical compatibility testing 15
1.2.3.3 Methods used to test physical compatibility 16
1.2.3.4 Methods used to test chemical compatibility 21
1.2.3.5 Compatibility/stability of ingredient components of PN solutions 21
1.2.3.6 Simulation of contemporary NICU setting 25
1.2.4 Role of chromatography and stability indicating methods in chemical analysis 26
1.2.4.1 Sample generation using forced degradation 27
1.2.4.1.1 Hydrolysis 30
1.2.4.1.2 Oxidation conditions 31
1.2.4.1.3 Photolysis 31
1.2.4.1.4 Thermal conditions 32
1.2.4.2 Method development and optimization 33
1.2.4.3 Method validation 34
1.2.4.3.1 Selectivity or specificity 35
1.2.4.3.2 Linearity, limit of detection and lower limit of quantification 35

1.2.4.3.3 Accuracy 36
1.2.4.3.4 Precision 37
1.2.4.3.5 Robustness 38
1.3 Aims of the thesis 39
Chapter 2 Systematic review of physicochemical compatibility of intravenous drugs: application to neonatal intensive care setting 41
2.1 Development and validation of a pharmaceutical science systematic review process using a semi-automated machine learning tool 44
2.1.1 Background 44
2.1.1.1 Research Screener 46
2.1.2 Methods 49
2.1.2.1 Development of the research question and search strategy 49
2.1.2.2 Pilot testing success of search strategy 50
2.1.2.2.1 Testing the search strategy 50
2.1.2.2.2 Feasibility and reliability of manual screening of abstract titles 51
2.1.2.2.3 Pilot evaluation of Research Screener 51
2.1.2.3 Database searching and the Research Screener process of the main systematic review 52
2.1.3 Results 53
2.1.3.1 Manual screening versus semi-automated screening (Research Screener – pilot study) 53
2.1.3.2 Main review 55
2.2 Investigating the physicochemical drug compatibility data pertaining to NICU setting 61
2.2.1 Methods 61
2.2.1.1 Data Extraction and Presentation 61
2.2.1.2 Quality assessment of selected studies 62
2.2.2 Results 63
2.2.2.1 Type of compatibility tested and clinical focus of the selected studies 63
2.2.2.2 Preparation of samples for compatibility testing 64
2.2.2.3 Testing temperatures 64
2.2.2.4 Contact (dwelling) time of test drug combinations 64
2.2.2.5 Use of control samples and baseline testing 65
2.2.2.6 pH testing 65
2.2.2.7 Use of more than one assessor 65
2.2.2.8 Physical compatibility testing methods 66
2.2.2.9 Chemical compatibility testing methods 67
2.2.2.10 Physical and chemical compatibility 67
2.3 Discussion 81
Chapter 3 Physicochemical compatibility of sildenafil injection with parenteral medications used in neonatal intensive care settings 90
3.1.1 Pharmacology of sildenafil 90
3.1.2 Effects of sildenafil in pulmonary hypertension 92
3.1.3 Pulmonary hypertension in the newborn and the use of sildenafil as treatment 93

3.1.4 Administration of IV sildenafil in NICU settings 94
3.1.5 Development of assays to evaluate stability and compatibility of sildenafil formulations 94
3.1.6 Investigations of the physicochemical compatibility of sildenafil with commonly used NICU drugs 98
3.2 Materials and methods 100
3.2.1 Stability-indicating HPLC assay development and validation 102
3.2.2 Preparation of samples for physical and chemical compatibility testing 105
3.2.3 Physical compatibility testing 107
3.2.4 Chemical compatibility testing 108
3.2.5 Physical compatibility testing of sildenafil with a lipid emulsion 108
3.2.6 Evaluation of absorption/adsorption loss of sildenafil by syringe filters 110
3.3 Results 111
3.3.1 HPLC method validation 111
3.3.2 Sildenafil compatibility 117
3.3.2.1 Sildenafil 600 µg/mL 117
3.3.2.2 Sildenafil 60 µg/mL 119
3.3.3 Physical compatibility of sildenafil with lipid emulsion 122
3.3.4 Absorption/adsorption loss of sildenafil by filter material 123
3.4 Discussion 127
Chapter 4 Physicochemical compatibility of caffeine citrate and caffeine base injections with parenteral medications used in neonatal intensive care settings 133
4.1 Introduction and background 133
4.1.1 Methylxanthines and caffeine 133
4.1.2 Mechanism of action of caffeine and other methylxanthines in neonatal apnoea 134
4.1.3 Pharmacokinetics of caffeine 135
4.1.4 Oral and IV administration of caffeine 136
4.1.5 Use of caffeine in neonatal intensive care settings, and its co-administration with other drugs 136
4.1.6 Physical and chemical compatibility of caffeine with other NICU drugs 137
4.2 Materials and methods 141
4.2.1 Stability indicating HPLC assay for chemical compatibility testing of caffeine 143
4.2.2 Preparation of samples for physicochemical compatibility testing 145
4.2.3 Physical compatibility testing 146
4.2.4 Chemical compatibility testing 147
4.2.5 Physical compatibility testing of caffeine citrate and caffeine base injection with a lipid emulsion 148
4.2.6 Evaluation of absorption/adsorption loss of caffeine by syringe filters 149
4.3 Results 150
4.3.1 HPLC method validation 150
4.3.2 Physicochemical compatibility testing 152
4.3.3 Physical compatibility of caffeine with lipid emulsion 155
4.3.4 Evaluation of absorption/adsorption loss of caffeine by syringe filters 158

4.4 Discussion 159
4.5 Conclusion 165
Chapter 5 General conclusions and recommendations for future work 166
References 173
Appendix 1 200
Appendix 2 203
Appendix 3 207
Appendix 4 209
Appendix 5 210
Appendix 6 226
Appendix 7 229
Appendix 8 236
Appendix 9 238
Appendix 10 243

Abbreviations

%RSD	percentage relative standard deviation
AIBN	azobisisobutyronitrile
API	active pharmaceutical ingredient
cGMP	cyclic guanosine monophosphate
CI	confidence interval
CV	coefficient of variation
D10W	glucose 10% w/v
D5W	glucose 5% w/v
ECLIPSE	Expectation, Client group, Location, Impact, Professionals, ServicE
EMA	European Medicines Agency
FNU	Formazin Nephelometric Unit
HPLC	high performance liquid chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	intensive care unit
ILE	injectable lipid emulsion
IV	intravenous
KEMH	King Edward Memorial Hospital
LC-MS	liquid chromatography–mass spectrometry
LC-NMR	liquid chromatography and nuclear magnetic resonance
LLD	largest lipid droplet
LLOQ	lower limit of quantification
LOD	limit of detection
MDD	mean droplet diameter
MMD	mass median diameter
MW	molecular weight
NICU	neonatal intensive care unit
NO	nitric oxide
NS	sodium chloride 0.9% w/v/ normal saline
NTU	Nephelometric Turbidity Units
PAH	pulmonary arterial hypertension

PDE	phosphodiesterase
PDE5	phosphodiesterase type 5
PDI	poly dispersity index
PFAT5	percentage of lipid droplets >5 μm
PH	pulmonary hypertension
PICO	Population, Intervention, Comparison, Outcomes
PICU	paediatric intensive care unit
PKG	protein kinase G
PN	parenteral nutrition
PPHN	persistent pulmonary hypertension of the newborn
QA	quality assurance
QC	quality control
RH	relative humidity
SD	standard deviation
SIM	stability indicating method
SMOFlipid 20%	soybean oil 6%, medium chain triglycerides 6%, olive oil 5% and fish oil 3%
SPICE	Setting, Population, Intervention, Comparison, and Evaluation
SPIDER	Sample, Phenomenon of Interest, Design, Evaluation, Research type
SRA	Systematic Review Accelerator
TLC	thin layer chromatography
TPN	total parenteral nutrition
USFDA	United States Food and Drug Administration
USP	United States Pharmacopoeia
UV	ultraviolet
WFI	water for injection

List of Figures

Figure 1.1 An illustration of a Y-site connector used to infuse two medications simultaneously to the patient (adapted from IV Sets and Access Devices Product Catalog—B. Braun Medical Inc., effective June 2017) 4

Figure 1.2 An illustration of a multi-lumen connector used to infuse multiple medications simultaneously to the patient (adapted from IV Sets and Access Devices Product Catalog—B. Braun Medical Inc., effective June 2017)..... 4

Figure 1.3 An illustration of a general protocol for forced degradation of drug substances and drug products; adopted from Ngwa (2010) 29

Figure 2.1 Research Screener assisted screening process − adapted from Chai *et al*. (2021) 47

Figure 2.2 Conflict resolution interface in Research Screener with the comments pane for each reviewer 54

Figure 2.3 PRISMA* flow diagram for the systematic review search, screening and selection process (* PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses)..... 56

Figure 2.4 Number of abstracts flagged by each reviewer for full-text review in the Research Screener process. Reviewers 1 and 2 completed 52 and 35 cycles, respectively 59

Figure 2.5 Number of papers selected for the review (after full-text read) from each screening cycle..... 60

Figure 2.6 Trend in conducting compatibility studies with time..... 63

Figure 2.7 Physical compatibility data for selected drug combinations (Piperacillin-tazo.–Piperacillin-tazobactam; Sodium bicarb. – Sodium bicarbonate; Sodium nitro.– Sodium nitroprusside; Trimethoprim-SMX–Trimethoprim-sulfamethoxazole; C– Compatible; I– Incompatible; #– compatible in special situations) 70

Figure 2.8 Chemical compatibility data for selected drug combinations (Piperacillin-tazo.–Piperacillin-tazobactam; Sodium bicarb.–Sodium bicarbonate; Sodium nitro.–Sodium nitroprusside; Trimethoprim-SMX–Trimethoprim-sulfamethoxazole; C–Compatible; I–Incompatible; #–compatible in special situation. 71

Figure 3.1 Nitric oxide/ cyclic Guanosine monophosphate signalling pathway, illustrating the role of cGMP in decreasing intracellular Ca^{2+} levels and subsequent smooth muscle relaxation..... 90

Figure 3.2 Sildenafil citrate chemical formula; molecular weight of citrate salt=666.7; molecular weight of base=474.6 91

Figure 3.3 Sildenafil 600 µg/mL exposure to 20% v/v hydrogen peroxide (1:1 v/v), stored at 45°C; Sample diluted 1-in-50 with water at the point of assay; injection volume 20 µL. (− Time 0; − Day 7); Degradation products were detected at 1.5, 1.7 and 2.9 min, at Day 7 112

Figure 3.4 Sildenafil 600 µg/mL exposure to 4 M NaOH (1:1 v/v), stored at 45°C; Sample neutralized with 4 M HCl and diluted 1-in-50 with water at the point of assay; Injection volume 20 µL. (− Time 0; − Day 7); A degradation product was detected 0.9 min, at Day 7..... 113

Figure 3.5 Sildenafil 600 µg/mL exposure to 4 M HCl (1:1 v/v), stored at 45°C; Sample neutralized with 4 M NaOH and diluted 1-in-50 with water at the point of assay; Injection volume 20 µL. (− Time 0; − Day 7); No degradation products observed 113

Figure 3.6 Sildenafil 600 µg/mL in water (1:1 v/v) exposure to heat at a temperature of 60°C in a water bath; Injection volume 5 µL (− Time 0; − Day 3); No degradation products observed..... 114

Figure 3.7 Sildenafil 600 µg/mL in water (1:1 v/v) exposure to laboratory fluorescent lighting 24/7 and normal daylight (indirect sunlight) for approximately 12 hours per day at 22°C (room temperature); Injection volume 5 µL; (− Time 0; − Day 7); No degradation products observed..... 114

Figure 3.8 Linearity curve for sildenafil solution in aqueous solution within the concentration range 3-800 µg/mL (n=3); Correlation coefficient (r^2) >0.999; Regression equation Y = 14.86x – 10.036 115

Figure 3.9 Recovery (%) of sildenafil by different filters using the 600 µg/mL solution; The coloured bars represent the recovery in five separate, consecutive millilitre portions – pilot study..... 123

Figure 3.10 Recovery (%) of sildenafil by different filters using the 60 µg/mL solution; The coloured bars represent the recovery in five separate, consecutive millilitre portions – pilot study..... 124

Figure 3.11 Recovery (%) of sildenafil 600 µg/mL injection solution from sterilising filters. Sildenafil concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○

polyethersulfone; ▼ mixed cellulose esters; △ inline polyethersulfone; see Table 3.3 for further details). Data are mean ± SD (n=3). 125

Figure 3.12 Recovery (%) of sildenafil 60 µg/mL injection solution from sterilising filters. Sildenafil concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ polyethersulfone; ▼ mixed cellulose esters; △ inline polyethersulfone; see Table 3.3 for further details). Data are mean ± SD (n=3) 126

Figure 4.1 Chemical structure of caffeine and other xanthines: theobromine; theophylline; paraxanthine; adenine and guanine. 133

Figure 4.2 Chromatograms of caffeine standard 5 mg/mL (—), caffeine citrate 10 mg/mL (—), caffeine base injection 5 mg/mL (—), a mixture of caffeine standard, caffeine citrate and caffeine base injection in aqueous solution (—); caffeine retention time approximately 5.8 minutes..... 150

Figure 4.3 Linearity curve for caffeine solution in aqueous solution within the concentration range 1-5 mg/mL (n=3); correlation coefficient (r^2) > 0.999; regression equation Y=2907x+217.13..... 151

Figure 4.4 Recovery (%) of caffeine 10 mg/mL solution from syringe filters. Caffeine concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ regenerated cellulose; ▼ polyether sulfone; △ inline polyether sulfone;). Data are mean ± SD (n=3). 158

Figure 4.5 Recovery (%) of caffeine 5 mg/mL solution from syringe filters. Caffeine concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ regenerated cellulose; ▼ polyether sulfone; △ inline polyether sulfone;). Data are mean ± SD (n=3) 159

List of Tables

Table 1.1 Physical and chemical compatibility testing by selected studies 15

Table 1.2 Methods used to evaluate physical compatibility of IV drugs with other drug solutions and admixtures 18

Table 1.3 Evaluation of physical stability of lipid emulsions in lipid containing PN solutions 19

Table 1.4 Methods employed (other than visual evaluation) to evaluate physical compatibility of ingredient components in PN solutions 24

Table 1.5 Methods used to determine the concentrations of different constituents of PN solutions 25

Table 1.6 Conditions generally employed in forced degradation studies (Adopted from Ngwa 2010) 33

Table 2.1 Search strategy concepts and key words 50

Table 2.2 Abstract flagging by reviewers in selection rounds 54

Table 2.3 Number of combinations for which each drug has physical (indicated in green font) and chemical (indicated in blue font) compatibility data available, i.e., Adenosine has no physical compatibility data with any of the other drugs of concern while caffeine base has physical compatibility data with one other drug) 68

Table 2.4 Drug combinations reported as both compatible and incompatible under different testing conditions by selected studies. All data are related to physical compatibility unless specified. (D5W – glucose 5% w/v; NS – normal saline (0.9% w/v sodium chloride); U – undiluted; v/v – volume/volume ratio; RT – room temperature) 72

Table 2.5 Quality assessment for physical and chemical compatibility study components 80

Table 3.1 Manufacturers/ suppliers of injectable products used for compatibility studies 101

Table 3.2 Composition of the 2-in-1 PN solutions, manufactured at King Edward Memorial Hospital 102

Table 3.3 Syringe filter types tested, the membrane and mesh size description.. 110

Table 3.4 Accuracy, intra-assay and inter-assay precision data for selected sildenafil concentrations..... 116

Table 3.5 Robustness test results for deliberate changes in method parameters.. 116

Table 3.6 Physicochemical compatibility of sildenafil 600 µg/mL with secondary drugs and 2-in-1 PN solutions (see Table 3.2)..... 118

Table 3.7 Physicochemical compatibility of secondary drugs and 2-in-1 PN solutions tested with sildenafil 60 µg/mL, their concentrations, and diluents. 120

Table 3.8 Re-testing of drug combinations with sildenafil 60 µg/mL in which the SIL ratio (Table 3.7) was > 102%. Combinations were considered compatible if the sildenafil filtered ratio was in the range of 90-110% (nylon filters; see Methods for further details). 121

Table 3.9 Mean and median droplet diameter data (MDD and Dv50, respectively) at 0 and 2 hours after mixing combinations of lipid emulsion and IV drugs/fluids 122

Table 4.1 Manufacturers/ suppliers of injectable products used for compatibility studies. 142

Table 4.2 Composition of the 2-in-1 PN solutions, manufactured at King Edward Memorial Hospital 143

Table 4.3 Syringe filter types tested, the membrane, mesh size description and manufacturer details..... 149

Table 4.4 Accuracy, intra-assay, and inter-assay precision data for selected caffeine concentrations (caffeine citrate and caffeine base injection) 151

Table 4.5 Robustness test results for deliberate changes in method parameters.. 152

Table 4.6 Physicochemical compatibility of caffeine citrate 20 mg/mL (10 mg/mL caffeine base) with secondary drugs 2-in-1 PN solutions (see Table 4.2 for details).... 153

Table 4.7 Physicochemical compatibility of caffeine citrate 10 mg/mL (5 mg/mL caffeine base) with secondary drugs 154

Table 4.8 Physicochemical compatibility of caffeine base injection 10 mg/mL with secondary drugs 2-in-1 PN solutions 156

Table 4.9 Mean and median droplet diameter data (MDD and Dv50, respectively) at 0 and 2 hours after mixing combinations of lipid emulsion and IV drugs..... 157

Chapter 1

Introduction & Literature Review

1.1 Background

Worldwide, 1 in 10 babies is born preterm (babies born alive before 37 weeks of pregnancy are completed). This is an estimated one baby every two seconds [1]. An estimated 13.4 million newborn babies were born preterm in 2020 compared with 13.8 million in 2010 (9.8% of all births) worldwide [2]. According to the Australian Preterm Birth Prevention Alliance, established in 2018, more than 26,000 Australian babies are born preterm each year [3]. According to the 2021 statistics, in Australia, a proportion of 8.2% of births were preterm, and 90% of babies who required resuscitation were more likely to be born preterm [4]. Preterm birth related complications are the leading cause of death among children under 5 years of age, responsible for approximately 900 000 deaths in 2019 [5]. Ensuring the safe care and survival of this extremely vulnerable population is therefore of upmost importance.

1.1.1 Neonates and neonatal intensive care

'Neonate' is an umbrella term used to represent term, post-term and preterm babies. Term and post-term includes births after 37 weeks of gestation whilst any birth occurring before 37 weeks of gestation, or fewer than 259 days since the first day of the woman's last menstrual period, is considered 'pre-term' [6]. The neonatal period of a term or post-term baby is defined as the first 28 completed days of life. Whereas for a preterm infant, the neonatal period spans the 28 completed days post the expected delivery date [7].

High-risk premature babies, and full-term neonates with serious medical conditions, are often admitted to neonatal intensive care units (NICU) for specialised treatment. Common conditions requiring NICU admission include, but are not limited to, prematurity, low birth weight (<2500 g), requirements of medication or resuscitation, congenital heart defects, respiratory distress, infections, seizures, jaundice and additional support requirements such as intravenous (IV) therapy and blood transfusions [8-14]. Some of these conditions may coexist in this group of patients, demanding co-administration of multiple medications.

The vast majority of medications available for administration to neonates are designed as oral liquid or parenteral formulations. Overall, oral drug delivery is preferred in paediatrics due to its non-invasiveness, the low risk of pain, thus likely improved compliance [15]. However, it has several drawbacks for use in unwell neonates; for example, difficulties in drug taste masking, inability to swallow, drug absorption and metabolism changes due to reduced gastric emptying and altered expression of drug transporters and enzymes in neonates. As most neonates in the NICU are too small, or sick, to receive medicines or fluids by mouth, IV drug administration is required.

In addition to IV drugs, parenteral nutrition (PN) solutions and admixtures form an integral part of neonatal care. PN, the IV infusion of a specialised form of liquid nutrition, is critical in many preterm and low-birthweight babies who are unable to receive adequate nutrition by mouth. Each PN solution provides full nutritional requirements, including all essential macro and micronutrients; carbohydrates, vitamins, amino acids, lipids, electrolytes, and trace elements. Carbohydrates and lipids are utilized as a non-protein energy source, whilst lipids also provide essential fatty acids and long chain poly unsaturated fatty acids which are crucial for neonatal brain and retina development [16].

1.1.2 Parenteral drug/fluid delivery in neonates

IV drug administration in neonates is a complex process, both technically and pharmacokinetically. Fluid restriction, slow flow rates, high drug concentrations and the use of multiple drugs in treatment regimens are key challenges in neonatal IV drug administration.

1.1.2.1 Fluid restriction and subsequent high standard drug concentrations used in neonates

Fluid overload in neonates may give rise to haemodynamic instability, thus increasing the risk of morbidity and mortality [17]. Extreme care should be taken to avoid fluid overload in neonates as the blood volumes range from approximately 250 mL for a term neonate and to less than 60 mL for a pre-term neonate [18, 19]. The fluid allowance of a full-term neonate is 100-140 mL/kg/day, with a typical fluid infusion rate of 10-20 mL/h [20], and for neonates weighing less than 1 kg, it is 3-5 mL/h (compared to 100 mL/h in adults) [21]. Consequently, in fluid restricted neonates, IV solutions must be concentrated so that slow infusion rates and low volumes of IV fluids are administered [22].

1.1.2.2 Multidrug therapy

In critical care settings, neonates may receive in the order of 3 to 11 IV medications, many of which may be unlicensed for paediatric use or used off-label [23]. Due to the lack of reliable data around physicochemical compatibility of these multitude of medications, dedicated IV cannulas must be used for drug administration for neonates [24, 25]. However, obtaining multiple IV access in neonates is difficult due to the potential complications (e.g., pain, embolism, phlebitis, extravasation and infections) [26]. Therefore, to avoid mixing different drug solutions in one IV container, and to

administer multiple IV medicines via a single access point with minimum contact time between drugs, Y-site (three-way) and multi-lumen connectors are used (Figure 1.1 and 1.2).

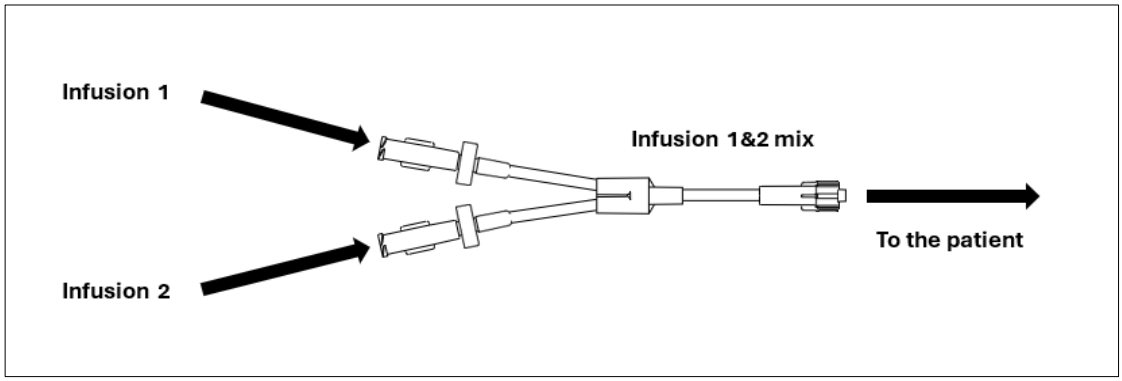


Figure 1.1 An illustration of a Y-site connector used to infuse two medications simultaneously to the patient (adapted from IV Sets and Access Devices Product Catalog—B. Braun Medical Inc., effective June 2017)

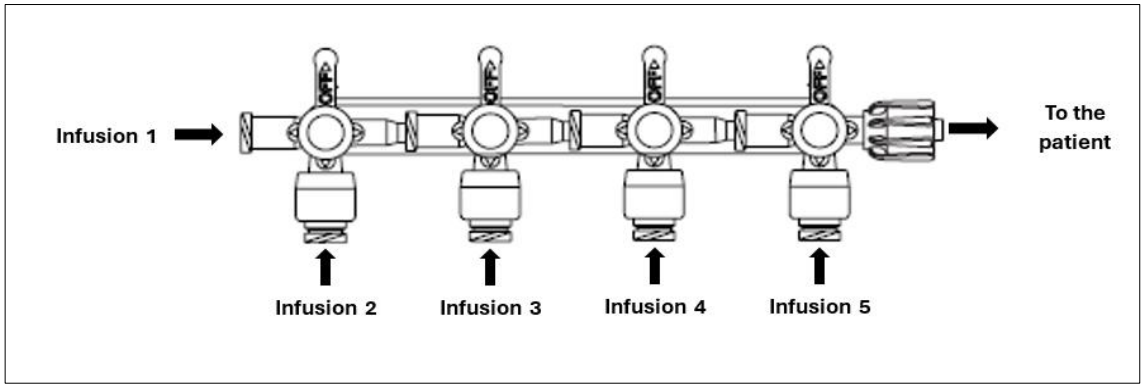


Figure 1.2 An illustration of a multi-lumen connector used to infuse multiple medications simultaneously to the patient (adapted from IV Sets and Access Devices Product Catalog—B. Braun Medical Inc., effective June 2017)

In clinical practice, Y-site connectors and multi-lumen connectors are used to simultaneously administer multiple drugs through a single port, if compatibility information is available.

1.1.3 Importance of drug compatibility during co-administration of multiple drugs

Drug incompatibilities are undesirable interactions that take place during drug preparation or administration process, when two or more drugs are mixed in a single syringe, tube or container [27].

Due to the very slow flow rates, and the dead space of drug infusion connectors and tubing, drugs may remain in contact with each other for considerably long periods of time before entering the neonatal circulation. As the infused drugs are of high concentration (due to fluid restriction in neonates) and the nutrition solutions are complex and unstable [28], there is potential for drug incompatibilities to occur within these connectors [29]. Incompatibilities can present as physical and/or chemical incompatibility reactions and may contribute to adverse events in patients. The nature of drug incompatibilities and subsequent complications are discussed in section 1.2.

A study has reported that drug incompatibilities account for 14.3% of all medication errors in intensive care unit (ICU) settings [30]. Further, a NICU study has shown that potential drug incompatibilities are extremely common, with half of the population susceptible to simultaneous administration of incompatible medications [31]. It further highlights the need of further research to determine all potential drug incompatibilities and their clinical outcomes.

In the adult setting, drugs should be at least physically compatible for Y-site administration, due to typically low contact time as a result of method of administration and medium to high flow rates. Chemical compatibility is of concern if the drugs are combined in the same container (syringe or bag), with a longer expected contact time [32] . However, in the neonatal setting, consideration of both physical and

chemical compatibility is critical for Y-site administration, due to both longer contact duration and higher concentration of drugs. If reliable compatibility data is unavailable, medications or PN solutions may have to be temporarily stopped to prevent the risk of incompatibility. This may lead to suboptimal clinical outcomes, including malnutrition [33].

Against this background, there is a critical need for robust data on the combined physical and chemical compatibility for a diverse range of IV drugs, PN solutions and other IV fluids, commonly used in NICU settings.

1.1.4 Availability of physical and chemical drug incompatibility information

The review conducted by Kanji *et al.,* concluded that studies conducted to provide Y-site compatibility data are limited for common medicines used in ICU patients, and this may contribute to unsafe medication practices [32]. In a study conducted in a paediatric intensive care setting, 10.3% of commonly used drug combinations did not have any compatibility data documented [34]. A study in a NICU reported that 15% of the drug combinations studied had no compatibility data available [35]. This critical lack of IV drug incompatibility data in neonates, particularly for co-administered drugs, was highlighted by Kalikstad and colleagues, who concluded more research on IV drug stability at the concentrations and combinations used for neonates is warranted [25].

1.1.5 Information sources for drug compatibility evaluation in hospitals

Apart from compatibility study findings reported in literature, health professionals access different compatibility decision support tools such as contemporary handbooks [27], institutional or national electronic drug compatibility databases [36-38] and in-

house IV compatibility cross-tables [39, 40] which compile currently available drug compatibility information. However, it is of paramount importance that these sources are regularly reviewed and updated by relevant authorities.

1.2 Drug incompatibility

1.2.1 Types of IV drug incompatibilities

Drug incompatibilities are mainly categorized into physical and chemical incompatibilities. Physical incompatibilities are visually observable changes e.g. colour change, turbidity, precipitation, haze, gas formation; and sub-visible changes such as particle formation. Chemical incompatibilities are sub-visible and include chemical degradation of the drug and formation of toxic products [41]. These incompatibilities are dependent not only on the drug molecule chemistry itself, but also on other factors such as drug concentration, temperature, infusion solution and order of mixing.

Abdelkader and colleagues [42], have conducted a detailed review of different mechanisms involved in physical and chemical drug incompatibilities.

Mechanisms involved in physical drug incompatibilities include, acid-base reactions, ionic interactions, dilution-related incompatibilities, gas generating reactions, and emulsion cracking [43].

Since a vast majority of drug molecules are organic weak electrolytes, acid-base reaction is the most common mechanism of drug incompatibility. This reaction occurs frequently in reconstituted drugs which are in their ionized or salt form. In these reactions, the pH change that occur in reconstitution or mixing with another solution leads to precipitation or insoluble drug. This process is influenced by the drug concentration. The pH dependent precipitation is usually rapid and is visually

observable as crystals, haze or turbidity [27, 43]. Walker *et al.* [44], investigated the physical compatibility of pantoprazole, an alkaline solution, with several acidic drug solutions including dobutamine, esmolol, midazolam, norepinephrine and octreotide. A precipitate was observed in the combination of pantoprazole with dobutamine and norepinephrine separately and a colour change in the combinations with esmolol, midazolam and octreotide. The authors have explained the pH difference in the solutions (pH>8 in pantoprazole and pH<4.5 in other drug solutions) as the probable cause for physical incompatibility. Furthermore, the incidence of physical incompatibility was found to be concentration-dependent, hence, recommendations were made to avoid higher concentrations in Y-site administration.

'Salting out' or ionic interaction is another mechanism involved in incompatibility. This occurs when ionic bonds are generated between two opposing ions in the solution. Salts containing anions and cations (e.g. calcium, magnesium or sulfate) can form strong bonds and they are less soluble than salts with monovalent ions (e.g. sodium, potassium, chloride). Therefore it is recommended to avoid mixing drug salts of calcium or magnesium with phosphates, carbonates, bicarbonates or sulfates [43]. Ambados (2002) has demonstrated the incidence of physical incompatibility between magnesium sulfate injection and solutions containing calcium at various concentrations [45]. The author stated that the incompatibility between calcium and sulfate ions would have been influenced by the concentration, pH and the contact duration.

Precipitation of drugs in concentrated solution upon dilution with water or other IV fluid (e.g. saline) have been reported in a limited number of injection solutions (i.e. diazepam). These drug solutions are originally formulated in water miscible organic solvents such as alcohol, to improve solubility. Dilution of these non-aqueous

injections with water or water based diluents, may precipitate the drug until enough solution is added to dissolve the drug [46]. Morris investigated the compatibility of diazepam injection following dilution to different concentrations with diluents. The study has demonstrated that dilutions of diazepam ≤1:20 resulted in immediate precipitation [47]. Similar findings were obtained by the work of Onuki and colleagues who explained that precipitation might occur at diazepam concentrations that exceeds the solubility limit [48].

Gas-generating reactions is another mechanism of physical drug incompatibility. Mixing acidic drug solutions with a parenteral solution containing carbonate or bicarbonate ions may generate insoluble carbonate precipitates and evolution of carbon dioxide. Several authors reported the incompatibility of acidic drugs with sodium bicarbonate solution [49-52].

Emulsion cracking is another mechanism of physical drug incompatibility. Lipid emulsions, total parenteral nutrition (TPN) and certain drug formulations (i.e. diazepam) can be cracked (destabilized) if mixed with solutions containing high concentrations of positively charged ions. TPN contains oil droplets (pH 5-7), which have a coating of negatively charged phospholipids. Acidic pH reduces the negative charge on the lipid droplet surface, creating repulsion forces between particles, leading to coalescence. Similar flocculation can occur upon addition of electrolytes or cations, due to a change in the surface charge [53].

Various chemical reactions including oxidation, reduction and hydrolysis, involve in drug incompatibility. Exposure to water (i.e. in reconstitution), oxygen, ultraviolet (UV) light, changes in pH and temperature can potentially accelerate these reactions [46].

Oxidation may occur as a loss of electrons, as an addition of oxygen to a compound or as a loss of a hydrogen ion such as from a phenolic hydroxy group. Phenols and catechols in solution are oxidized to quinones and other products. The oxidation process is catalysed by light, oxygen, alkaline pH, heavy metal ions (Co^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}), amines such as theophylline, and high temperatures. Such oxidized products are coloured pink or brown and therapeutically inactive [54]. Examples for drug oxidation in admixtures include aminophylline mixed with epinephrine or isoproterenol and addition of a trace mineral injection to any phenolic drug [55]. Common injectable phenolic drugs include morphine and phenylephrine, and catecholamines include dopamine, epinephrine, norepinephrine and isoproterenol [54].

Hydrolysis (attack by water) occurs to amides, esters, imines, and lactams. The products of hydrolysis may or may not have therapeutic activity, and may be toxic or sensitising [54].

Studies conducted by Uccello-Barretta *et al.* [56] and Bouchoud *et al.* [57] have shown that chemical instability issues are associated with the addition of some electrolytes, trace elements and vitamins to TPN admixtures.

Of the ingredient components of TPN admixtures, vitamins are the most susceptible for chemical decomposition. Photodegradation, oxidation and interaction with the container material are the mechanisms by which these vitamins are degraded. Vitamin C is degraded by oxidation, vitamin B1 by reduction and vitamin A and E by exposure to daylight. Oxidation/reduction capacity, pH, temperature, presence of calcium and magnesium salts, all contribute to the stability of vitamins in TPN admixtures [58-60].

Various physicochemical determinants lead to incompatibility in injectable drug solutions and admixtures. Factors leading to physicochemical incompatibilities in

admixtures include pH, acid-base character, solvent system, colour change, complexation, adsorption and salting-out [54].

Acidic or alkaline solutions with a pH of two or more units below or above the pKa of the weakly acidic or basic drug should be avoided due to the risk of precipitation of calcium, potassium or sodium salts of acidic drugs and precipitation of acid salts of amine drugs [61-63].

Ionic interaction of organic anions and cations in solution may result in precipitates, turbidity or transparent complexes with each other. Furthermore, the buffer effect of one ion may create an unfavourable pH for the opposite ion [61-63]. Generally, salts of weakly ionized acidic drugs (e.g. sodium and potassium salts) are incompatible with salts of weakly basic drugs (e.g. phosphates and sulfates) [64, 65].

The presence of approximately 10% or more of a water miscible non-aqueous solvent (e.g. ethanol, propylene glycol) can affect the ionic equilibrium of a weakly ionizing drug. The resulting dielectric constant of the mixed solvent system favours dissolution of the nonionized drug species [63]. Injectable drug formulations containing such solvent systems include phenytoin, digoxin, phenobarbitone and diazepam [27]. Dilution of such hydroalcoholic solutions in aqueous solutions is undesirable as the solubility of the drug in water may be inadequate depending on the final concentration. These drugs should be administered by IV bolus injection or through Y-site (if the compatibility is previously determined) [66].

Colour changes (e.g. darkening) can occur in antibiotics (aminoglycosides, cephalosporins, tetracyclines), catecholamines and phenolic drugs. However, these colour changes are not definitive indications of chemical degradation or loss of drug activity. For example, darkened cephalothin is acceptable until 24 hours of

preparation, slightly yellowed chlorpromazine solutions and darkened kenamicin solutions are usable [27]. However, dextrose can react with amino acid groups of amino acids and darken TPN solutions and subsequently lead to precipitation [67].

Complexation is another phenomenon that leads to drug incompatibility. Tetracyclines form insoluble chelates with polyvalent metal cations such as Al^{3+}, Ca^{2+}, Fe^{2+} and Mg^{2+} [54]. Furthermore, metal chelating agents (e.g. EDTA which is used as a preservative in some injectables) should not be mixed with drugs containing polyvalent metal cations such as calcium chloride, calcium gluconate, iron dextran and magnesium sulfate [54].

Some antibiotics and protein products adhere to plastic leading to an incompatibility. For example insulin admixed in amino acid or protein solutions may separate from the solution and adhere to the plastic administration sets, syringes or needles [27].

Certain electrolytes (e.g. sodium chloride), dextrose and antibacterial preservatives (e.g. benzyl alcohol) decrease the solubility of some drugs. For example, amphotericin B and erythromycin should be reconstituted only with unpreserved sterile water for injection prior to admixing [68].

1.2.2 Complications of drug incompatibilities

Previous studies have highlighted the adverse effects of drug incompatibilities on the safety and effectiveness of drug therapy in patients [69, 70]. The impact is more severe in intensive care settings, and particularly in high-risk patient populations such as neonates and children [31, 71].

Harmful effects of concomitant administration of physically incompatible drugs can have adverse consequences including, catheter lumen occlusion, thromboembolism,

impaired microcirculation and immune response modulation [69, 72, 73]. Chemical incompatibility and subsequent changes in drug concentrations can give rise to suboptimal therapeutic outcomes due to reduced drug levels, and there is a potential risk of toxicity [42, 74].

Benlabed *et al.* [69], have reported life-threatening complications of drug incompatibilities such as ischaemia, hypoxia, irritation, thrombophlebitis and pulmonary embolism. Pulmonary complications mainly result from micro-emboli of precipitates blocking pulmonary blood vessels and causing pneumonitis and pulmonary arteritis. Diffusion of multiple emboli would give rise to severe arterial pulmonary hypertension associated with cardiac arrest. In 2009, Bradley *et al.* [75] reported the death of neonates upon simultaneous administration of ceftriaxone and calcium. Autopsy findings revealed crystalline material in vascular beds in lungs.

Pulmonary complications caused by TPN have been reported in previous studies. The high concentration of cations present in TPN results in the degradation of lipid emulsion causing aggregation of lipid droplets. High concentrations of calcium, phosphate and magnesium has led to the formation of calcium phosphate crystals. McNearney *et al*. [76] and Reedy *et al.* [77] have reported such TPN associated crystalline precipitates resulting in pulmonary vascular occlusion.

Studies in paediatric intensive care setting have demonstrated that particles generated by incompatibilities can activate the immune system, causing systemic inflammatory response syndrome, which is a major cause of organ failure and death [78-80].

1.2.3 IV drug compatibility in neonates – What is already known?

A narrative literature review was undertaken to evaluate the current knowledge and findings regarding IV compatibility of drugs/solutions used in neonatal intensive care settings. This was carried out using 73 selected original research studies which intended to evaluate compatibility of IV drugs used in neonatal settings. The selected articles were analysed based on types/classes of drugs/solutions evaluated, their aims (physical/chemical compatibility studies), methods used evaluate physicochemical compatibility and key findings pertaining to compatibility.

1.2.3.1 Types of drugs/ admixtures investigated for stability and compatibility

Among the 73 studies selected, 37 studies involved mixing of commonly used NICU drugs with other drugs or admixture solutions, of which 4 studies have compared the drug stability in different diluents used in the neonatal setting; for example, sodium chloride 0.9%/ normal saline (NS), glucose 5% (D5W), glucose 10% (D10W), and Plasma-Lyte 148. The rest of the 36 studies involved PN solutions (lipid free or 3-in-1) and investigated their compatibility or stability of ingredient contents. As PN solutions are complex in composition, and critically ill neonates are often treated with IV drugs and PN solutions in combinations, their compatibility should be an important consideration. Co-infusing certain drugs with PN admixtures poses many advantages in the neonatal clinical setting such as maintaining the consistency of planned daily nutrition intake, decreasing the non-PN fluid intake and decreasing nursing time for IV line manipulation [81]. Furthermore, PN mixtures can serve as vehicles for drug administration [82]. Safe co-administration of IV drugs also avoids the necessity of a second IV administration site in neonates. In addition, plasma drug levels can be kept

constant, and there is no need for repeated injections of the drug, which may cause fluctuations in serum drug concentrations with potential adverse effects [83].

1.2.3.2 Physical and chemical compatibility testing

Only 15 out of the 37 studies which involve compatibility testing of drugs/ PN solutions and diluents investigated both physical and chemical aspects of compatibility. Fifteen investigated the physical compatibility aspect only. One study investigated the chemical compatibility aspect only. Five studies determined the changes in antibiotic concentrations using microbiological assays, amongst which, four included physical compatibility testing aspect as well. One study included physical compatibility testing and an immune assay to determine the drug concentration.

Of the 36 studies which investigated compatibility between PN components, only 13 studies investigated both chemical and physical compatibility aspects. Twenty-two studies investigated only the physical compatibility between PN components. One study investigated the chemical compatibility aspect only (Table 1.1).

Table 1.1 Physical and chemical compatibility testing by selected studies

	Studies investigating compatibility of drugs with other drugs and solutions (n=37)	**Studies investigating compatibility of ingredient components of PN solutions (n=36)**
Combined physical and chemical compatibility	15	13
Physical compatibility only	15	22
Chemical compatibility only	1	1
Microbiological assay methods for antibiotic concentrations	1	-
Physical compatibility and microbiological assay	4	-
Physical compatibility and immunoassay	1	-

Against the above background information, it is evident that combined physical and chemical compatibility testing of IV medicines in the context of NICU settings would address gaps in the literature.

1.2.3.3 Methods used to test physical compatibility

Visual inspection by unaided human eye, for precipitate formation, visual particulate matter, turbidity, colour change, haze, evolution of gas has been conducted in studies that evaluated physical compatibility. In some studies, visual observation has been carried out against a black and white background under normal fluorescent light and using a high-intensity monodirectional light (Tyndall beam) for sub visual particle analysis [84, 85]. Turbidimetry using a laboratory grade turbidimeter has been utilized to quantify turbidity, as a part of physical compatibility testing. Measurement of pH has been used both as a physical and chemical stability predictor in different studies. According to Newton and Driscoll (2008), a change in pH by more than one unit can result in drug precipitation and pH greater than 7.2 could induce the risk of calcium phosphate precipitation [86]. Furthermore, it is reported that emulsion destabilization is highly likely to occur at pH values less than 5.5 [87]. Sub visual particle counting techniques have also been carried out using light obscuration techniques [53, 88].

Studies which investigated the compatibility of drugs with lipid containing-PN solutions, have evaluated the emulsions' physical stability by visual inspection for discolouration, creaming, phase separation, precipitates and analysis of lipid droplet size. Techniques such as light microscopy [89], dynamic light scattering [88, 90], coulter counter technique [91], light obscuration [53, 92] and laser diffraction [93] have been used for lipid droplet size analysis. Zeta potential measurement [90] and

calculation of Poly dispersity Index (PDI) [88] have also been used for lipid stability evaluation.

The different methods used by authors to evaluate physical compatibility of IV drugs with other drugs, solutions and admixtures (including PN solutions) are listed in Table 1.2. Methods employed to evaluate physical stability of lipid emulsions in lipid containing PN solutions are listed in Table 1.3.

Table 1.2 Methods used to evaluate physical compatibility of IV drugs with other drug solutions and admixtures

Method	Studies involved	Acceptance criteria for compatibility/ Points considered
Visual inspection by unaided human eye for precipitate formation, visual particulate matter, turbidity, colour change, haze, evolution of gas	[53, 81, 84, 85, 89, 90, 93-114]	• Incompatibility – Precipitates, particles, turbidity, colour changes, haze, evolution of gas
Gross temperature changes	[99]	• Incompatibility – Gross temperature changes
Subvisual particle analysis using a Tyndall beam (a high-intensity monodirectional light)	[53, 84, 85, 88, 90, 93, 113]	• Incompatibility – Visible signs of precipitation or Tyndall effect
Turbidimetry using a laboratory turbidimeter	[53, 84, 88, 93, 94, 101, 102, 104]	• Incompatibility – a turbidity change by 0.5 nephelometric turbidity units (NTU) compared to controls [84, 94, 101, 102] • Compatibility – no any increase in turbidity [104] • Upper limits are 0.2-0.3 Formazine Nephelometry Units (FNU) [53, 88, 93]
Measurement of pH using pH meter/ reagent strips	[53, 84, 88-91, 93, 94, 98, 102, 103, 108, 114, 115]	• Incompatibility – a pH change by > 0.5 units from the initial pH is incompatible [94] • A change in pH by one pH unit or more indicate the presence of chemical reactions hence physically unstable [84, 88, 102] • A change in pH by more than 1.0 pH unit could induce the risk of precipitation and pH more than 7.2 could induce the risk of calcium phosphate precipitation [88] • Safe pH for peripheral infusion is any between 5.0 and 9.0 [103] • pH <5.5 – Increased risk of emulsion destabilization [53, 88, 93] • Absolute variation of pH more than 15% was considered significant [91]
Sub visual particle counting using light obscuration	[53, 88, 93]	• particles ≥0.5µm/ml should be below 2000; larger particles –within pharmacopoeial limits for large volume parenterals: no more than 25 particles/ml for particles ≥10µm and no more than 3 particles/ml for particles ≥25µm

Method	Studies involved	Acceptance criteria for compatibility/ Points considered
Osmolality measurements	[91]	• Absolute variation of osmolality more than 15% – significant

Table 1.3 Evaluation of physical stability of lipid emulsions in lipid containing PN solutions

Method	Studies involved	Acceptance criteria for compatibility/ Points considered
		Visual emulsion stability
Visual inspection for discolouration, creaming, phase separation, precipitates	[89]	No visual signs for discolouration, creaming, phase separation and precipitates
		Presence of calcium crystals
Microscopy for calcium crystals	[90]	Comparison against reference calcium mono hydrogen phosphate dehydrate crystals
Testing of precipitate for presence of calcium and phosphorus	[115]	Qualitative analysis for calcium by atomic absorption spectroscopy, and phosphorus by phosphomolybdate reduction
		Lipid droplet size analysis
Light microscopy	[89]	Largest lipid droplet (LLD) in 15 fields: ≤8 µm; Mean LLD: 4.5 µm; standard deviation: ≤2.0 µm; number of lipid droplets >5 µm: ≤9
Dynamic light scattering	[88, 90]	Stable emulsions have a mean droplet diameter (MDD) of less than 500 nm
Coulter counter technique	[91]	Lipid particle size variation by 15% was considered significant
Light obscuration	[53, 88, 92, 93]	Weighted volume percentage of lipid droplets > 5 µm (PFAT5) < 0.40%
Laser diffraction	[53, 93]	MDD should be <500 nm; % Size fraction >5 µm should be zero
		Lipid droplet stability

Method	Studies involved	Acceptance criteria for compatibility/ Points considered
Zeta potential	[88, 90]	Minimal changes in zeta potential suggest electrical stability
PDI	[88, 90]	PDI on average 0.125 indicates monodispersity [90]; PDI below 0.2 – regarded as monodisperse samples [88]

1.2.3.4 Methods used to test chemical compatibility

Chemical compatibility testing of drug and other admixture combinations include determination of drug concentration changes with time, under the experimental conditions. The most common method used for chemical compatibility testing is high performance liquid chromatography (HPLC) with UV detection [81, 90, 91, 94-96, 98, 103, 105, 106, 109, 112, 114]. However, liquid chromatography coupled with mass spectroscopy (LC-MS) and other analytical techniques have been used to determine drug concentrations for IV compatibility studies [89, 108, 116]. Of the 16 studies which evaluated chemical compatibility, a majority (n=13, 81%) utilized HPLC to determine drug concentrations.

Two studies which included chemical compatibility testing, have determined the concentrations of different ingredients in PN solutions involved, in addition to the drug concentrations [91, 112]. Gellis *et al.* [91] evaluated the concentrations of the main nutrients by colorimetry. Calcium, chloride, glucose, iron, magnesium, potassium, and sodium were assayed with a biochemical analyser. Total nitrogen was quantified using an automatic analyser. A nutrient concentration change of more than 15% was considered significant. In the work of Tounian *et al*. [112], concentrations of calcium, chlorine, copper, glucose, iron, magnesium, potassium, phosphorus, sodium, and zinc were measured using an automated analyser. A high-performance amino acids analyser was used to measure the concentrations of different amino acids studied.

1.2.3.5 Compatibility/stability of ingredient components of PN solutions

PN admixtures designed for neonatal and paediatric patients are very complex in nature. Due to IV fluid restrictions in neonates (as discussed in section 1.1.2.1.), PN components are contained in a very small fluid volume making them highly

concentrated solutions. Low IV fluid rate in neonates allows longer contact times for the PN components, increasing the possibility of incompatibility. Precipitation of calcium phosphate is known to be a major physical incompatibility in PN particularly for premature infants who require high electrolyte concentrations in a small volume of fluid. The insoluble precipitates can give rise to serious clinical consequences such as cannula occlusion and pulmonary embolism [117].

Precipitation of calcium phosphate is an endothermic reaction and the use of incubators for neonates increase the possibility of precipitation. The temperature increase will have two effects. First, organic calcium will dissociate to release free calcium ions to react with phosphate. Second, the high temperature may also shift the phosphate equilibrium from a monobasic salt to a dibasic salt [118]. Using organic phosphorus in neonatal PN, has partially addressed the problem of calcium precipitation [119, 120].

In lipid containing PN admixtures, the Injectable Lipid Emulsion (ILE) is the most sensitive component. The repulsion of negatively charged particles which reduces the aggregation and coalescence of oil droplets, maintains the stability of the emulsion. However, other additives of the admixture (e.g., electrolytes, glucose and amino acids) can alter the surface charge and decrease the physical stability of ILEs. As PNs containing ILEs are concentrated and may be unstable, 2-in-1 PN solutions and ILEs are normally administered separately via a Y-site in NICU settings [121].

Fat droplet diameter is the main indicator of emulsion stability. The droplet size limits for commercial IV nutritional lipids established by the United States Pharmacopoeia (USP): the mean droplet diameter (MDD) cannot exceed 500 nm, and the percentage of large fat globules (>5 μm) (PFAT5) cannot exceed 0.05% [122]. Protection from

droplets >5 μm is important as these can accumulate in capillary beds in lungs causing life threatening pulmonary embolism [123, 124].

Amongst the 36 studies which evaluated the compatibility of component ingredients of neonatal PN solutions, only 13 have assessed both physical and chemical aspects of compatibility. Twenty-two studies have only assessed physical compatibility and one study had assessed chemical compatibility only. Visual observation for macroscopic evidence of precipitation, colour change, particulate matter, emulsion instability (i.e., creaming, phase separation, coalescence) has been the mainstay of physical compatibility evaluation in all studies. Other methods used for physical compatibility testing are detailed in Table 1.4. Methods used to determine the concentrations of different constituents of PN solutions such as vitamins, amino acids, glucose are listed in Table 1.5.

Table 1.4 Methods employed (other than visual evaluation) to evaluate physical compatibility of ingredient components in PN solutions

Method	Studies involved
Turbidity measurement	[125-128]
Microscopic observation of the solution for particles or the filter disks for microcrystals	[120, 124, 125, 129-140]
Physical characterization of the precipitate in the filter by gross appearance	[134]
Infrared spectroscopy of the precipitate in the filter	[134]
Differential interference contrast microscopy	[141]
Laser diffraction	[16, 136, 137, 142-145]
Optical particle counter	[146]
Osmolality measurement	[57, 120, 126, 128, 136, 138, 146]
Coulter counter for lipid droplet size analysis	[126, 147, 148]
Lipid droplet size analysis using dynamic light scattering (DLS)/ Photon correlation spectroscopy method (PCS)	[16, 57, 128, 130, 137-140, 142, 147, 149, 150]
Integrated light scattering	[151]
Micro flow imaging methods	[130]
Light obscuration/ light extinction methods	[57, 124, 130, 131, 134, 138, 149, 152, 153]
Zeta potential measurement	[137-140, 143]
Light scattering by a UV-Vis spectrophotometer	[132]
Viscosity measurement	[154]
Surface tension measurement using a tensionometer	[140]

Table 1.5 Methods used to determine the concentrations of different constituents of PN solutions

Method	Ingredient component/s analysed and the corresponding study
HPLC	• Amino acids [57, 146, 149] • Vitamins [16, 57, 148, 151, 155]
Spectrophotometry	• Glucose [57, 149] • Phosphate [57] • Peroxide [16, 57, 120, 151] • Non-Esterified Fatty Acids [149] • L-Cysteine [149]
Titrimetry	• Peroxide [138, 149] • Vitamin C [155]
Ion specific electrodes	• Potassium, sodium, phosphate [57] • Calcium [57, 143]
Inductively coupled plasma–mass spectrometry (ICP-MS)	• Iron [133]
Atomic absorption spectrometry	• Sodium and potassium [146] • Calcium [127, 135, 146]
Colourimetry	• Calcium [128]
UV photometric method	• Glucose and phosphorus [146]

1.2.3.6 Simulation of contemporary NICU setting

Use of humidicribs/ incubators (with typical temperatures of 35-37°C), slow flow rates of infusion, clinically relevant neonatal concentrations, contact time of two or more drug/solution/admixture during Y-siting are unique features of IV drug therapy in a NICU setting. Among the studies which evaluated compatibility of different drug/solution combinations, five tested the drug combinations in an incubator temperature. Interestingly, Watson *et al.*, have used a 38°C water bath, to simulate the temperature of a febrile patient [115].

All studies except Hammond *et al.* [103], and Dawson *et al.* [98], (which tested drug compatibility in Plasma-Lyte 148), have used clinically relevant neonatal or paediatric drug concentrations and PN solutions.

Four studies simulated the neonatal setting of IV Y-site drug administration using syringe pumps and IV infusion sets [95, 115, 116, 156]. All others followed static mixing of drugs and solutions in vials. In comparison to the simulation of Y-site using IV tubing, static mixing is less costly, more feasible, efficient and suitable to test a large range of drug combinations.

Different studies have used a range of time periods as the duration of a drug/solution combination being in contact with each other in a Y-site connector. Some authors have concluded it to be 1 hour [96, 99, 109], and some as long as four hours [90, 92, 93]. Testing for longer durations (i.e. 24 hours to several days), has been utilized by authors to conclude storage stability, but the data generated could be useful to predict Y-site compatibility of drugs [89, 106].

1.2.4 Role of chromatography and stability indicating methods in chemical analysis

By definition, a stability indicating method (SIM) is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product". A SIM accurately measures the active ingredients, without interference from degradation products, potential impurities, and excipients [157].

Chromatographic methods (i.e. HPLC) are widely employed in developing SIMs for a variety of analytes including highly polar compounds, heat labile compounds and non-volatile compounds. Additionally, chromatography normally has advantages over other analytical methods as it separates all compounds in a single run with good sensitivity, selectivity, accuracy, precision, and robustness [158]. Structure elucidation of degradation products and impurities are often supported by combined techniques

such as LC-MS and liquid chromatography and nuclear magnetic resonance (LC-NMR) [159].

Reverse phase HPLC coupled with a UV detector is a widely used analytical method for separation and quantifying impurities [160]. According to the review of Blessy *et al.*, [157] there is a series of steps involved in development of SIM on HPLC which meets the regulatory requirements. These steps include sample generation, method development and optimization and validation of the developed SIM.

1.2.4.1 Sample generation using forced degradation

To generate the samples for SIMs, the drug is degraded using forced degradation conditions (conditions more severe than accelerated degradation conditions). These conditions include hydrolytic, oxidative, photolytic, thermal, and light exposure. As the term implies, the aim of 'forced degradation' is to force the generation of degradation products which are likely to be formed in actual storage or processing conditions. This degraded sample could then be used to develop the SIM [157].

The importance of the use of forced degradation (stress testing) approach in designing SIMs to determine drug concentrations and validation of such methods for individual drugs has been well reported and reviewed. As per the International Council for Harmonisation (ICH) Technical Requirements for Pharmaceuticals for Human Use guidelines, stress testing is useful to identify the likely degradation products, determine their intrinsic stability, establish degradation pathways and to describe and validate the analytical methods used to develop SIM [161].

The review of Blessy *et al.*, [157] provides guidance on the practical performance of forced degradation and its application for the development of SIM. The authors have identified several objectives of forced degradation studies as follows.

- Establishing degradation pathways for drug substances
- Differentiation of drug degradation products from and products generated from non-drug products
- Structure elucidation of degradation products
- Determination of intrinsic drug stability in a formulation
- Understanding the degradation mechanism of drug substances
- Understanding the stability indicating nature of a developed method
- Understanding the chemical properties of the drug molecule
- Generation of more stable formulations
- Constructing degradation profiles
- Solving stability related problems

These stress studies should assess the stability of the drug substance at different pH solutions, in the presence of oxygen and light and at elevated temperatures and humidity levels. Furthermore, scientists are encouraged to ensure the stress conditions are consistent with product decomposition under manufacturing, storage and intended use conditions of individual drug substances and products [162].

The limit of drug substance degradation to be achieved by forced degradation has been controversial, although a degradation of 10-15% is considered acceptable for chromatographic methods [163]. A limit of 10% is regarded as optimal for use in analytical method validation for small drug molecules with a label claim of 90% as acceptable stability limit [164]. No such limits are established for degradation during shelf life for biological products [165]. However, it is not essential to produce a

degradation product during forced degradation. Testing can be terminated if no degradation is observed during the exposure to standard conditions in the accelerated stability protocol [166]. This indicates that the drug molecule is stable. Over-stressing a sample may lead to secondary degradation products and under-stressing would not generate sufficient degradation products [167]. Protocols for drug-product degradation may differ from that of individual substances due to differences in matrices and concentrations [168].

A general protocol for degradation of drugs and drug products suggested by Ngwa (2010) [162] is given in Figure 1.3.

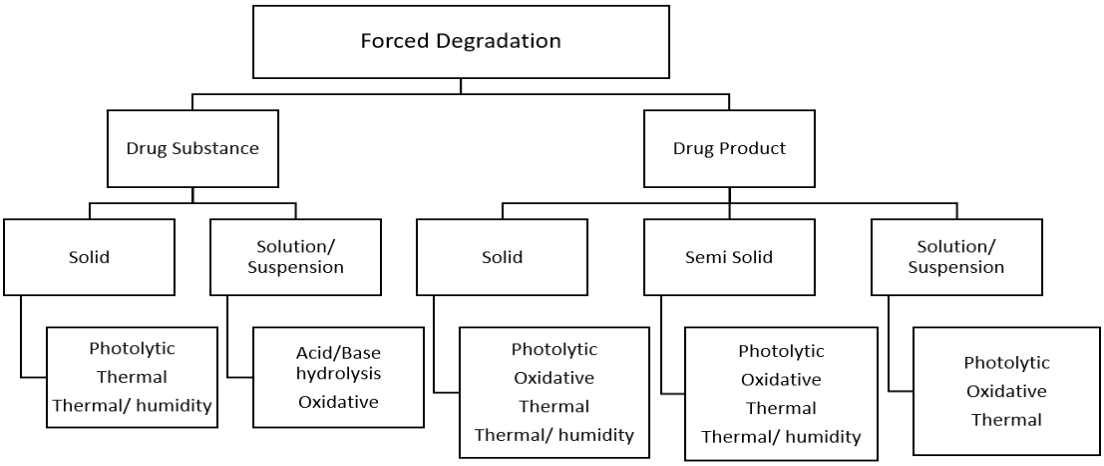


Figure 1.3 An illustration of a general protocol for forced degradation of drug substances and drug products; adopted from Ngwa (2010)

Despite the importance of forced degradation in drug development, regulatory guidelines do not clearly specify the conditions of pH, temperature, and oxidizing agents to be used in forced degradation studies [158, 162], however, a minimal list of factors suggested include, acid and base hydrolysis, thermal degradation, photolysis, oxidation [161]. Comprehensive documents providing guidance to conduct stress

testing under a variety of ICH prescribed conditions were lately published by several authors [157, 169, 170].

1.2.4.1.1 Hydrolysis

In hydrolysis, a chemical compound is decomposed by a reaction with water, and it occurs over a wide range of pH. Acid or base hydrolysis catalyses the ionizable functional groups in the drug molecule, to permit generation of primary degradants in a desirable range [157]. The type and concentration of the acid or base should be based on the stability of the drug molecule. For acid hydrolysis, 0.1-1 M hydrochloric acid or sulfuric acid, and for base hydrolysis, 0.1-1 M sodium hydroxide or potassium hydroxide is suggested [170, 171]. If compounds are poorly soluble in water, co-solvents could be included to aid dissolution in the acid or base. Stress testing is generally started at room temperature, but if there is no degradation taking place the temperature can be elevated to 50-70°C. Furthermore, testing should not exceed 7 days. The acid/base degraded sample is then neutralized using a suitable acid/base to avoid further degradation [157].

Other than higher temperatures, the use of higher concentration acid/alkali and exposure to longer durations are also suggested if degradation is not evident in lower concentrations and short exposure times [169]. Alternatively, if a complete degradation is observed upon the drug exposure to initial condition, acid/alkali strength, reaction time and reflux temperature can be reduced [169].

Degradation under neutral conditions is generally started by refluxing the drug in water for 12 hours and reflux time can be increased if no degradation occurs. If the drug degrades completely, exposure time and temperature can be reduced [169].

1.2.4.1.2 Oxidation conditions

In oxidative degradation, an electron transfer mechanism operates to form reactive anions and cations which results in generation of degradation products [172].

For degradation by oxidation, hydrogen peroxide is widely used. Other oxidizing agents used include metal ions, oxygen, and radical initiators (e.g., azobisisobutyronitrile; AIBN). The choice of oxidizing agent depends on the drug substance. According to previous reports, exposure to 0.1-3% hydrogen peroxide at neutral pH and room temperature for seven days or until 20% degradation could generate potential degradation products [171], however, a hydrogen peroxide concentration range of 3-30% is also suggested [169].

1.2.4.1.3 Photolysis

Photo stability testing of drugs should be evaluated to demonstrate that exposure to light does not result in adverse change to the substance of concern. Stress testing by exposure to light (photo stability testing) is carried out to generate primary degradants of drugs upon exposure to UV or fluorescent light, ICH guidelines have recommended some conditions for photostability testing [166].

Ideally, drug samples should be exposed to a minimum of 1.2 million lx h (1.2 million lux for 1 hour) and 200 W h/m^2 light. A wavelength of light in the range of 300-800 nm is recommended to cause photolytic degradation [173, 174]. If no decomposition observed, the intensity can be increased by five times, where the maximum illumination recommended is 6 million lx h. In case of no decomposition takes place, even at the highest light intensity, the drug can be declared photostable [169, 175].

1.2.4.1.4 Thermal conditions

Degradation by exposure to elevated temperature (thermal degradation) should be carried out at more strenuous conditions than recommended ICH Q1A [161] accelerated testing conditions. Dry heat and wet heat can be used for this purpose. Solid samples should be exposed to dry and wet heat, while liquid samples should be exposed to dry heat. Studies can be conducted at higher temperatures for a shorter period [171]. Thermal degradation study is carried out at 40-80°C [157].

The review of Bakshi and Singh (2002) provides a detailed account of the control samples, sampling times and processing of samples in degradation studies [169]. A minimum of four samples is recommended for a forced degradation experiment, namely, the blank solution stored under normal condition, the blank subjected to stress, time zero sample of the drug stored under normal condition and the drug sample subjected to stress condition. These samples will provide a comparative assessment of the changes that occur upon exposure of the drug to stress conditions. Furthermore, withdrawing samples at different time points during the experiment is also recommended, as it would provide a clear idea of the number of degradation products formed with time [169]. This information is crucial in developing a SIM, particularly if the end-goal is to establish the degradation profile of the drug substance of concern. Unless otherwise relevant, a drug concentration of 1 mg/mL has been recommended to initiate forced degradation experiments as it would enable even minor decomposition products in the range of detection. The samples can be stored at low temperatures (i.e. freezers) to stop further degradation. Aliquots may require dilution or neutralization prior to injecting into HPLC, thus protecting the integrity of the columns [169].

Some conditions commonly used for forced degradation studies are presented in table 1.6 (adopted from Ngwa, 2010), however, the decision on adequate stress is based on the stability of the drug compound [162].

Table 1.6 Conditions generally employed in forced degradation studies (Adopted from Ngwa 2010)

Degradation type	Experimental conditions	Storage conditions	Sampling time (Days)
Hydrolysis	Control (no acid or base)	40°C, 60°C	1,3,5
	0.1 M Hydrochloric acid	40°C, 60°C	1,3,5
	0.1 M Sodium Hydroxide	40°C, 60°C	1,3,5
	Acid control (no API)	40°C, 60°C	1,3,5
	Base control (no API)	40°C, 60°C	1,3,5
	pH: 2,4,6,8	40°C, 60°C	1,3,5
Oxidation	3% H_2O_2	25°C, 60°C	1,3,5
	Peroxide control	25°C, 60°C	1,3,5
	Azobisisobutyronitrile (AIBN)	40°C, 60°C	1,3,5
	AIBN control	40°C, 60°C	1,3,5
Photolytic	Light 1 × ICH	Not Applicable	1,3,5
	Light 3 × ICH	Not Applicable	1,3,5
	Light control	Not Applicable	1,3,5
Thermal	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75% RH	1,3,5
	Heat chamber	80°C	1,3,5
	Heat chamber	80°C/75% RH	1,3,5
	Heat control	Room Temperature	1,3,5

(API – active pharmaceutical ingredient; AIBN – azobisisobutyronitrile; RH – relative humidity)

1.2.4.2 Method development and optimization

Prior to HPLC method development, the physicochemical parameters of the drug molecules of interest should be considered (i.e. pKa value and solubility), as these properties have an impact on selection of mobile phase, solvents, and mobile phase pH in HPLC methods [169]. The pH-related retention of a compound occurs at pH values within ±1.5 units of the pKa value. The ionization value is also crucial in selecting the pH of the buffers to be used in the mobile phase [176].

A reverse phase column is commonly used for HPLC assays of drug molecules. The choice of organic phase (e.g., methanol, acetonitrile, water) may depend on the solubility of the drug molecule, and the aqueous phase usually comprises a buffer to improve peak separation and peak symmetry [177]. Column temperature in the range of 30-40°C is commonly recommended to obtain good method reproducibility [176]. It is preferred to have the drug peak further in chromatogram as it permits all degradation products to be separated [169].

If degradant peaks and the drug peak are co-eluted, a peak purity analysis is required to determine the specificity of the method, which can be performed by photo diode array detection, if available [178]. The method is then optimized by changing parameters such as flow rate, injection volume, mobile phase ratio and column type, in order to separate closely eluting peaks [157].

1.2.4.3 Method validation

The developed SIM should then be validated using accepted guidelines. Method validation is extensively covered by several international guidelines such as the ICH [179], the United States Food and Drug Administration (USFDA) [180, 181], The United States Pharmacopoeia (USP) [182], the American Association of Official Analytical Chemists (AOAC) [183], and the European Medicines Agency (EMA) [184]. Furthermore, informative reviews provide detailed insights to validation of analytical methods of interest [185-187].

Important validation characteristics in analytical methods employed in pharmaceutical analysis include selectivity (specificity), linearity, accuracy, precision and robustness [179], which are described below.

1.2.4.3.1 Selectivity or specificity

Selectivity or specificity is the ability to detect the analyte in the presence of other components (e.g. degradants). Results of forced degradation experiments with acidic, alkali and oxidative stress conditions (described above) can be used to demonstrate the selectivity of the method for the specific drug molecule in the presence of degradants [179].

1.2.4.3.2 Linearity, limit of detection and lower limit of quantification

Linearity is the ability to obtain test results which are directly proportional to the concentration of analyte in the sample. To assess linearity, a series of dilutions is prepared to construct a calibration curve (with a recommended minimum of five calibration standard concentration levels). Calibration curve parameters should be reported, and regression analysis is performed to find out the correlation coefficient (r^2) [179].

The calibration curve parameters will also inform the determination of the limit of detection (LOD) and lower limit of quantification (LLOQ) of the analytical procedure. The LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LLOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the LOD are described in ICH guidelines [179], depending on whether the method is a non-instrumental or instrumental.

Visual evaluation may be used for determining LOD for both non-instrumental and instrumental methods. The LOD is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum concentration of analyte that can be reliably detected.

Determination of LOD based on Signal-to-Noise can only be applied to analytical procedures which exhibit baseline noise. Signals from samples with known low concentrations of analyte are compared with those of blank samples and the minimum concentration at which the analyte can be reliably detected is established. A signal-to-noise ratio between 3 or 2:1 is considered acceptable for estimating the LOD.

The next approach is based on the standard deviation of the response and the slope in the calibration curve. The equation 1.1 (below) is used to estimate LOD, where σ is the standard deviation of the response and S is the slope of the calibration curve.

$$\text{Equation 1.1; } \text{LOD} = (3.3 \times \sigma)/S$$

The estimate for σ can be obtained by the residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines of the calibration curve.

Similarly, the LLOQ can be estimated based on visual evaluation, the signal-to-noise approach or using the calibration curve. For calculating LLOQ, a typical signal-to-noise ratio is 10:1. The equation 1.2 (below) is used to estimate LLOQ.

$$\text{Equation 1.2; } \text{LLOQ} = (10 \times \sigma)/S$$

1.2.4.3.3 Accuracy

Accuracy is the closeness of agreement between the determined value obtained by the method to the nominal concentration (expected concentration) of the analyte. Accuracy should be assessed on quality control samples (QC samples; samples spiked with known amounts of the analyte). The QC samples should be spiked independently from the calibration standards, using separate stock solutions. The accuracy should be reported as a percentage of the nominal value. Accuracy should be evaluated for the values of the QC samples obtained within a single run (the within run accuracy) and in different runs (the between-run accuracy). Within-run accuracy should be

determined by analysing a minimum of five samples per concentration level at a minimum of four concentration levels. The mean concentration should be within 15% of the nominal value for the QC samples. For LLOQ, accuracy should be within 20% of the nominal value. For between-run accuracy, LLOQ, low, medium and high QC samples from at least three runs analysed on at least two different days should be evaluated. The mean concentration should be within 15% of the nominal values for the QC samples, except for the LLOQ which should be within 20% of the nominal value [184].

1.2.4.3.4 Precision

Precision is the "closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample". Three levels of precision are considered: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the coefficient of variation (CV) or percentage relative standard deviation (%RSD) of a series of measurements [179]. Repeatability (intra-assay/ within run precision) is the precision under the same operating conditions over a short interval of time. Intermediate (inter-assay) precision is within-laboratory variation over different days.

According to the EMA guidelines, precision should be demonstrated for the LLOQ, low, medium and high QC samples, within a single run and between different runs (same runs and data accuracy testing can be used). For within-run precision, there should be a minimum of five samples per concentration level at LLOQ, low, medium and high QC samples in a single run. The within-run and between-run precision, CV value should not exceed 15% for the QC samples, and for LLOQ the CV should not exceed 20%. For the validation of the between-run precision, LLOQ, low, medium and high QC samples from at least three runs analysed on at least two different days should

be evaluated [184]. Reproducibility is assessed by means of an inter-laboratory trial and its considered in case of standardisation of an analytical procedure [179].

1.2.4.3.5 Robustness

Robustness is a measure of the method's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In the case of liquid chromatography, examples of typical variations are composition and pH of the mobile phase, different columns (different lots and/or suppliers), temperature and flow rate. In the case of gas-chromatography, examples of typical variations are different columns (different lots and/or suppliers), temperature, flow rate [179].

1.3 Aims of the thesis

Given the scarcity of information of physicochemical drug compatibility, the overall objective of the thesis was to further investigate the physicochemical compatibility of commonly used NICU drugs, using standard methods. The specific objectives included:

1. **To explore the current knowledge and findings regarding physicochemical compatibility of common IV drugs used in NICUs, using a systematic review process.**

   A pharmaceutical science systematic review process using a semi-automated machine learning tool was developed and validated (Chapter 2; Paper 1). Studies for the systematic review were selected using the validated method, the types of compatibility tested in the studies (physical /chemical), the drugs/ drug classes/ admixtures/ solutions which are tested for compatibility, the conditions used to simulate contemporary neonatal setting, the different techniques/methods used to test the chemical and physical compatibility were investigated. Further, physical and chemical compatibility data reported for NICU medicines were quantified, and quality of the studies which provide these data were evaluated.

2. **To investigate the physicochemical compatibility of sildenafil injection with parenteral medications used in neonatal intensive care settings**

   Physicochemical compatibility of sildenafil with a range of NICU drugs was investigated, clinically relevant concentrations, and with a selection of 2-in-1 PN solutions. Sildenafil compatibility with commonly used syringe filters also was investigated. A stability-indicating HPLC method was developed and validated for determination of sildenafil concentrations (Chapter 3; Paper 2).

3. **To investigate the physicochemical compatibility of caffeine citrate and caffeine base injection with parenteral medications used in neonatal intensive care settings**

   Physicochemical compatibility of caffeine citrate and caffeine base injection with a range of NICU drugs was investigated, at clinically relevant concentrations, and with a selection of 2-in-1 PN solutions. Caffeine compatibility with commonly used syringe filters also was investigated. (Chapter 4; Paper 3)

Chapter 2

Systematic review of physicochemical compatibility of intravenous drugs: application to neonatal intensive care setting

As physicochemical drug incompatibilities can give rise to adverse clinical outcomes in neonates, it is essential that healthcare professionals have access to compatibility information prior to co-administration of drugs. Several research designs have attempted collating compatibility information and data availability, to assist clinical decisions. These designs include prospective [31, 34] and retrospective [24, 35] observational studies in paediatric/neonatal intensive care unit settings (PICU and NICU), and critical reviews of current literature [25]. Furthermore, although not specifically pertaining to the paediatric or neonatal setting, two systematic reviews have also evaluated the published compatibility information of ICU drugs [32, 188]. A common theme across these studies was established, a paucity of available compatibility data for commonly used drugs in ICU settings.

Gikic and colleagues [34] performed an open, prospective study in a PICU setting, and reported that 10.3% of drug combinations co-administered during the study period had no available compatibility data. Likewise, in the prospective NICU cohort study conducted by Leopoldino and colleagues [31], a total of 1114 potential drug incompatibilities were identified, of which 31.2% had no compatibility information.

In a retrospective observational study conducted by Hani and colleagues [35], concurrent PICU drug administration data for 100 patients was analysed over a period of two months. Among the 1447 co-administered continuous infusions, 207 combinations (15%) had no drug compatibility data available. Furthermore, Gaetani and colleagues [24] retrospectively evaluated concurrent IV drug administration in

children admitted to a single centre, and it was concluded that 21% of concurrent infusions had 'unknown' compatibility, adding complexity to routine bedside management.

Critical and systematic reviews of this subject area have rendered similar findings. Kalikstad and colleagues [25] conducted a critical review which sought to investigate the compatibility of co-infusions for a selected group of NICU drugs and nutrition solutions. To evaluate requirements for compatibility, thirteen critical care drugs commonly used in NICU were reviewed against a list of 66 frequently used IV co-infusion drugs, two PN solutions and albumin. A total of 1042 co-infusions were studied: 820 drug-drug, 131 drug-nutrition, and 65 drug-albumin combinations. Interestingly, there was no previous documentation on compatibility for almost 60% of the evaluated drug-drug co-infusions. The review further highlighted that whilst information on the chemical compatibility of drug infusions is vital, it was rarely analysed in the reports included within the review. A limitation of this review was that they only considered drug co-infusions that were relevant to their specific NICU setting. Hence, the list of drug combinations was not exhaustive and they did not review studies reporting data for a number of commonly used drug combinations in alternative NICU settings, specifically those including calcium and other divalent anions.

A systematic review conducted by Kanji *et al*. [32] sought to quantify the physical and chemical stability data for common ICU medications, and to evaluate the quality of the selected studies. Research reports of chemical or physical compatibility, involving 820 possible two-drug combinations, were sought from three scientific databases (1966 to 2009), yielding 1945 citations of which 93 studies were included within the systematic review. Of the selected studies, 92% (n=86) evaluated physical whilst 38%

(n=35) evaluated chemical compatibility. Furthermore, it was identified that only 54% (n=441) of the possible 820 drug-drug combinations had physical and/or chemical compatibility data available, whilst of concern, chemical compatibility data only existed for 9% (n=75) of the evaluated drug combinations. Similarly, the systematic review conducted by Lao *et al*. [188], collated compatibility data of 42 commonly used ICU drugs and 2 PN solutions reported in 29 studies. Of the 27 original studies included in the review, 21 (78%) reported the physical compatibility and 6 (22%) reported combined physicochemical compatibility. Furthermore, drug stability data was only available for 50.3% of the studied combinations.

Against this background information, the aim of the present systematic review was to collate the current evidence on IV drug compatibility as applicable to Y-site administration in NICU settings. To the best of our knowledge, no systematic reviews have been conducted to evaluate peer-reviewed physicochemical compatibility studies in this context.

The following chapter will be presented in two distinct sections. Chapter 2; section 2.1. reports the process of establishing and testing a robust literature search strategy in accordance with the SPIDER (Sample, Phenomenon of Interest, Design, Evaluation, Research type) model and the use of a semi-automated, machine learning, abstract screening tool Research Screener in the reference selection process for a systematic review in pharmaceutical sciences. Chapter 2; Section 2.2. describes the process of data extraction, presentation and synthesis of physicochemical compatibility data from the selected studies of the systematic review, and the quality assessment procedure of the selected studies.

2.1 Development and validation of a pharmaceutical science systematic review process using a semi-automated machine learning tool.

2.1.1 Background

The number of studies published in medical and health journals has increased strikingly over the past few decades, making clinical decision making extremely complex. Well-conducted systematic reviews and meta-analyses are considered the highest level evidence for informed decisions in clinical practice, however, the methodological rigour required is associated with significant time and economic demands [189].

The process for constructing a research question and search strategy for systematic reviews is typically defined by established models, including PICO (Population, Intervention, Comparison, Outcomes) [190], SPIDER (Sample, Phenomenon of Interest, Design, Evaluation, Research type) [191], SPICE (Setting, Population, Intervention, Comparison, and Evaluation) search strategy [192] and ECLIPSE (Expectation, Client group, Location, Impact, Professionals, ServicE) [193].

a) PICO is the most common fundamental tool in both evidence-based practice and systematic reviews, enabling researchers to define their quantitative research question and search terms. Furthermore, it is the best method of question formulation to use when conducting a quantitative systematic literature review. However, the PICO tool is not an optimal working strategy for qualitative evidence synthesis [191].
b) SPICE was developed in the context of evidence-based librarianship and subsequently promoted for qualitative systematic reviews [192].

c) SPIDER is reported to be more suited for qualitative and mixed-methods research [191].

d) ECLIPSE was introduced to handle health management topics [193].

In conducting systematic reviews, screening of titles and abstracts is considered to be the most time and labour-intensive component of the review process [194]. Hence, there is a growing interest for automated solutions to facilitate systematic reviews [195]. The introduction of new technologies, such as machine learning tools, for streamlining the screening process has provided promising results by substantially reducing the time for initial screening. Machine learning-based screening tools, including Rayyan [196], Abstrackr [197], RobotAnalyst [198], and ASReview [199], offers approaches to overcome the manual and time-consuming process of screening large numbers of studies by prioritising relevant studies through a process of active learning. Increasing evidence supports the use of these semi-automated tools in increasing the feasibility of conducting robust systematic reviews [194]. Nevertheless, due to the fast-paced evolution of machine learning, new methods and techniques may highlight previously unidentified limitations in early versions of the technology.

For example, the use of traditional machine learning (using manual coding instructions) and natural language programming methods (use of syntactic parsing) is one such limitation. However, more recent techniques, that teaches algorithms to learn from data and make its own predications, have proven to outperform these traditional methods and improve overall performance [194]. Another limitation has been the need to screen numerous articles to initially 'train' the program model. As this is a laborious process, completing this 'training' phase may significantly reduce the intended time savings, thus the use of machine learning becomes less desirable when conducting small targeted systematic reviews [200]. Furthermore, as the estimated reliability of

existing tools varies largely, there is a need to develop reliable thresholds for when reviewers can stop screening [201].

A further limitation of some machine-learning tools is the requirement of a computer or a dedicated server to install the screening tool [199]. This can present a barrier for adoption by non-expert users, such as students. However, though not machine-learning assisted, the use of programs such as Covidence reduces this barrier providing easy entry for inexperienced users [194]. Covidence is a cloud-hosted and web-based software whose functionality mirrors the multiphase review process; including data extraction, project management, and conflict resolution during the abstract revision phase. This provides significant benefit over existing semi-automated tools, which primarily focus on abstract screening thus limiting their widespread adoption [202]. Furthermore, newer technologies have enabled the abstract screening tools to be designed with more visually appealing and user-friendly interfaces, that can adapt to multiple platforms including both desktop computers and mobile devices. This has advantages over older generation screening tools, that were developed only for single specific platforms such as Microsoft Windows [194].

2.1.1.1 Research Screener

Research Screener is a semiautomated abstract screening tool designed to assist researchers through the process of selecting relevant articles for inclusion in systematic reviews (Figure 2.1). The tool has been designed to address several limitations of previously existing tools, as highlighted above. Research Screener (https://researchscreener.com) is a cloud-hosted web application and algorithm that uses deep learning and natural language processing (ability of computers to analyse

human language) methods. Key features of Research Screener that increase its utility as a systematic review tool include:

- Automated removal of duplicate articles
- Ability for multiple reviewers to collaborate in the systematic review process
- Conflict resolution for disagreements between multiple reviewers
- Ability to export de-duplication and screening results
- Desktop, mobile and tablet friendly user interface

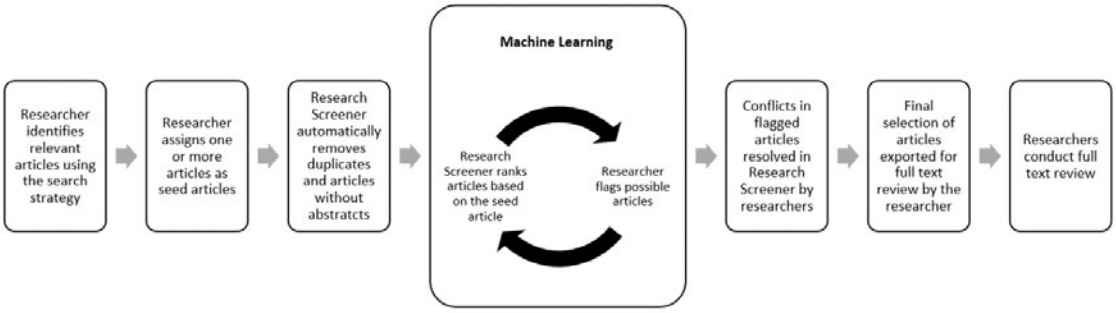


Figure 2.1 Research Screener assisted screening process − adapted from Chai *et al.* (2021)

The semiautomated screening process of Research Screener is outlined in Figure 2.1. Briefly, to initiate the screening process the researcher must upload two separate files to Research Screener, from their reference management software, to initiate the process:

1. All potentially eligible articles retrieved from the systematic review search strategy.
2. At least one seed article abstract that the researcher has identified as highly relevant for inclusion in the review.

Using the abstract/s of the seed article(s), the Research Screener algorithm ranks all identified eligible articles (uploaded in file 1) by relevance and presents the articles in groups of 50 (a cycle) to the review team for consideration of eligibility. Independently, each member of the review team screens each of the 50 abstracts presented in that cycle, and flags those which they deem relevant: according to predetermined inclusion criteria for the systematic review. The titles of the selected articles are retained for full article screening and, in conjunction with the irrelevant (discarded) articles, are used to refine the Research Screener algorithm. Using machine learning, Research Screener will then re-rank the remaining articles into the next cycle of abstracts (n=50) that are most relevant to those papers selected for review in cycle 1. This process will continue in cycles of 50 articles until either all selected articles (uploaded in file 1) are reviewed, or the team decides that they have screened an appropriate number of cycles that ensures (to a level of confidence) that all relevant articles have been identified (e.g., several cycles with no article selected as relevant). To note, each reviewer will be presented with abstracts in a varying order, determined by their own individual selection preferences (based on articles they have included, and excluded, in previous cycles). Upon completion of the screening process, the principal reviewer can access the combined results (all reviewer decisions) and identify any abstracts that would be considered conflicts (i.e., selection disagreements between the individual reviewers). The conflicts are resolved in Research Screener, by an open process of consideration by the reviewers and/or an independent third reviewer [194]. The final selected articles (flagged by both reviewers and the resolved conflicts) are then exported for full-text review.

Chapter 2; section 2.1.2 reports the process of establishing and testing a robust literature search strategy in accordance with the SPIDER model and the use of

Research Screener in the reference selection process for a systematic review in pharmaceutical sciences.

2.1.2 Methods

2.1.2.1 Development of the research question and search strategy

The research question, "In-vitro studies conducted to evaluate the physical and chemical compatibility of IV drugs used in NICUs," was defined in consultation with members of the research team. The SPIDER model (Sample, Phenomenon of Interest, Design, Evaluation, Research type) for systematic reviews was adapted for the research question formulation as below.

Sample – *In-vitro* experimental studies conducted to evaluate physicochemical compatibility of IV drugs in neonatal settings and IV drug compatibility studies conducted focusing non-neonatal settings but includes commonly used NICU drugs.

Phenomenon of Interest – Physical and chemical compatibility of IV drugs.

Design – Physical compatibility methods (e.g., visual observation; light viewer; turbidimeter) and chemical compatibility (HPLC; other).

Evaluation – Information on physicochemical drug compatibility of IV drugs.

Research type – Experimental (In-vitro)

The search strategy (Table 2.1) was structured as three concepts (categories), the first of which focused on compatibility, incompatibility, and stability terms. The second concept focused on IV, injection, and Y-site terms, and the third comprised a list of drugs based on expert panel review of a compilation of neonatal drug protocols from seven health-care institutions (four different countries); see Appendix 1.

Table 2.1 Search strategy concepts and key words

Concept 1	Concept 2	Concept 3
compatib*	intravenous*	NICU drugs
incompatib*	intra-venous*	(6 drugs in pilot study, 95 drugs in full
stability	iv	review; see Appendix 1)
instability	y-site	
	y- site	
	ysite	
	injection*	
	infusion*	
	parenteral	
	injectable*	
	mixture*	

The systematic review protocol was registered in Open Science Framework (https://doi. org/ 10. 17605/ OSF. IO/ XGK6V).

2.1.2.2 Pilot testing success of search strategy

As the proposed strategy was ambitious (including 95 drugs), and Research Screener had yet to be used for a systematic review within the field of pharmaceutical sciences, it was decided that a pilot study should be undertaken to test the success of both the proposed search strategy (against a smaller panel of drugs) and sensitivity of Research Screener in this context. The pilot study was conducted in a series of phases, as outlined below:

1. Testing of search strategy

2. Feasibility and reliability of manual screening of abstract titles

3. Pilot evaluation of Research Screener

2.1.2.2.1 Testing the search strategy

To ensure the proposed search strategy would successfully identify relevant content, the search concepts were pilot tested in iterative stages using the Embase database and

various terms within concepts 1 and 2, and a panel of six drugs (aminophylline, indometacin, ketamine, pentoxifylline, caffeine, and sotalol). The six drugs were selected on the basis of their potential relevance to the planned systematic review and a total known list of 59 articles, which was determined from a standard reference source [27] and an independent manual literature search. The Embase database was selected for pilot testing due its high search functionality and content of biomedical and pharmaceutical references, including all Medline content [203]. 'English Language' was the only limiter used. The search strategy generated 1622 results (excluding duplicates). The optimum search strategy captured 1622 articles and included all known articles of interest.

2.1.2.2.2 Feasibility and reliability of manual screening of abstract titles

The first stage of evaluating the screening process was to test the feasibility and reliability of title reading only. Two independent reviewers manually screened a random selection of 400 titles from the set of 1622 references (25% of the articles) and the kappa coefficient [204] was calculated to determine the inter-reviewer reliability associated with title reading as a screening process for the systematic review.

2.1.2.2.3 Pilot evaluation of Research Screener.

As Research Screener had not previously been used in a pharmaceutical sciences systematic review, the full set of 1622 articles was then used to pilot test the tool. Three seed articles were selected by the research team for uploading into Research Screener, and two reviewers conducted the screening process, with the kappa coefficient calculated to assess the inter-reviewer reliability.

2.1.2.3 Database searching and the Research Screener process of the main systematic review

Based on the favourable pilot study results, which validated the proposed search strategy, the full literature search to identify articles for inclusion in the systematic review was executed. This included the use of all keywords captured in concept 1, 2 and all 95 drugs in concept 3 (Table 2.1 and Appendix 1), with the systematic search undertaken across five databases; comprising two inter-disciplinary (Proquest and Web of Science) and three intra-disciplinary databases (Embase, Medline, and Cinahl). Subject headings were generated according to the different databases used (e.g. medical subject headings; MeSH headings in Medline database). Full details (key search terms, subject headings, limiters, number of hits retrieved) of the search strategy are outlined in Appendix 2. The retrieved references were initially deduplicated using a validated deduplication tool "Systematic Review Accelerator" (SRA). SRA project is based at the Bond University Institute for Evidence-Based Healthcare and was originally conceived with the aim of reducing the amount of time it takes to construct a systematic review using Information Technology (https://sr-accelerator.com). SRA is a suite of purpose-built automation tools, which speed up multiple steps in the systematic review process. SRA tools assist with many steps of a systematic review, including searching for citations, citation screening and write-up of review findings. Amongst the SRA tools, 'Deduplicator' identifies and removes duplicate studies from search results, decreasing the reference screening workload. The accuracy of SRA for utility of systematic reviews has been previously documented [205].

The deduplicated references were separately exported to Endnote and the final library was uploaded into Research Screener. Eight articles were identified as seed abstracts for the screening process, which were also uploaded to Research Screener. Following

automatic exclusion of articles by Research Screener (conference proceedings, duplicates, and articles with no abstracts), the reviewers proceeded with independent cyclical screening of the captured articles. The reviewers also manually screened (by title) the articles excluded by Research Screener due to lack of abstracts. The kappa coefficient was determined to quantify reviewer agreement for each relevant process.

2.1.3 Results

2.1.3.1 Manual screening versus semi-automated screening (Research Screener – pilot study)

The pilot search strategy study identified a total of 1622 articles, that were used to test each facet of the screening process. For the manual screening pilot study, the kappa coefficient determined in the reviewers title screening process of 400 titles (25% of identified articles) was 0.75, suggesting "moderate agreement" [204].

In the Research Screener pilot study, 98 references (out of 1622) were immediately removed due to a lack of abstracts and were exported to the reference manager for later manual evaluation. because they did not contain abstracts (e.g., letters, editorials, and short communications), because abstracts are essential for the Research Screener machine learning cycles). However, the excluded titles were separately exported back to the reference manager software for manual screening by the reviewers at a later time. The 1524 remaining abstracts were reviewed independently by Reviewer 1 and Reviewer 2, with their selection outcomes presented in Table 2.2.

Table 2.2 Abstract flagging by reviewers in selection rounds

Abstract selection round	Reviewer 1	Reviewer 2
1	34	38
2	23	25
3	09	12
4	01	01
5	0	0

Fifteen conflicts were identified and resolved by the reviewers. Figure 2.2 presents an example of a conflict resolution, as undertaken within Research Screener. Each reviewer can provide comments regarding the final decision of flagging/ unflagging an abstract.

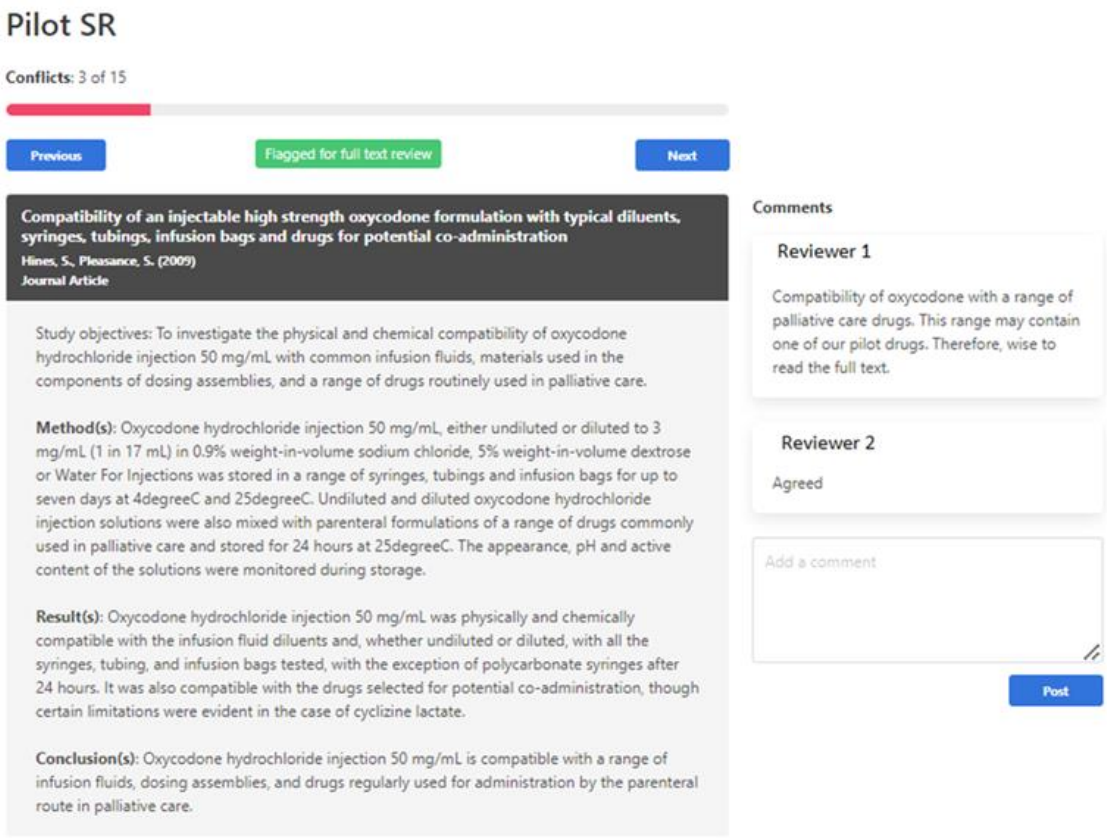


Figure 2.2 Conflict resolution interface in Research Screener with the comments pane for each reviewer

The kappa coefficient determined at the end of the Research Screener evaluation was 0.86, which was indicative of "strong" agreement between the two reviewers [204].

2.1.3.2 Main review

A total of 42,814 results were retrieved from the selected databases (Embase – 21,880, Medline - 8526, Cinahl - 1262, Proquest - 1843, Web of Science - 9303) and the Systematic Review Accelerator deduplication process retained 27,597 references for further screening. The flow diagram for the systematic review search, screening, and selection process, generated according to the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [189] is presented in Figure 2.3.

Research Screener initially removed 15 long abstract articles (i.e., conference proceedings in which the reference manager record contains all conference abstracts combined), 451 duplicated titles/abstracts, and 1269 articles with missing abstracts/titles, from the full set of 27,597 records (Figure 2.3). The 1269 articles with no abstract/title included short reports, editorials, letters, and notes, and were directed for manual screening by the reviewers. The remainder (25,862) were subject to screening by the two independent reviewers in cycles of 50, as outlined above. A third reviewer was assigned to be involved in conflict resolution if required (if the two independent reviewers were unable to resolve the conflict at the Research Screener conflict resolution stage). To be flagged for full text reading, the title and the abstract had to indicate that the article described the physical and/or chemical compatibility of at least one two-drug combination involving drugs listed in the predetermined NICU drug list (Appendix 1).

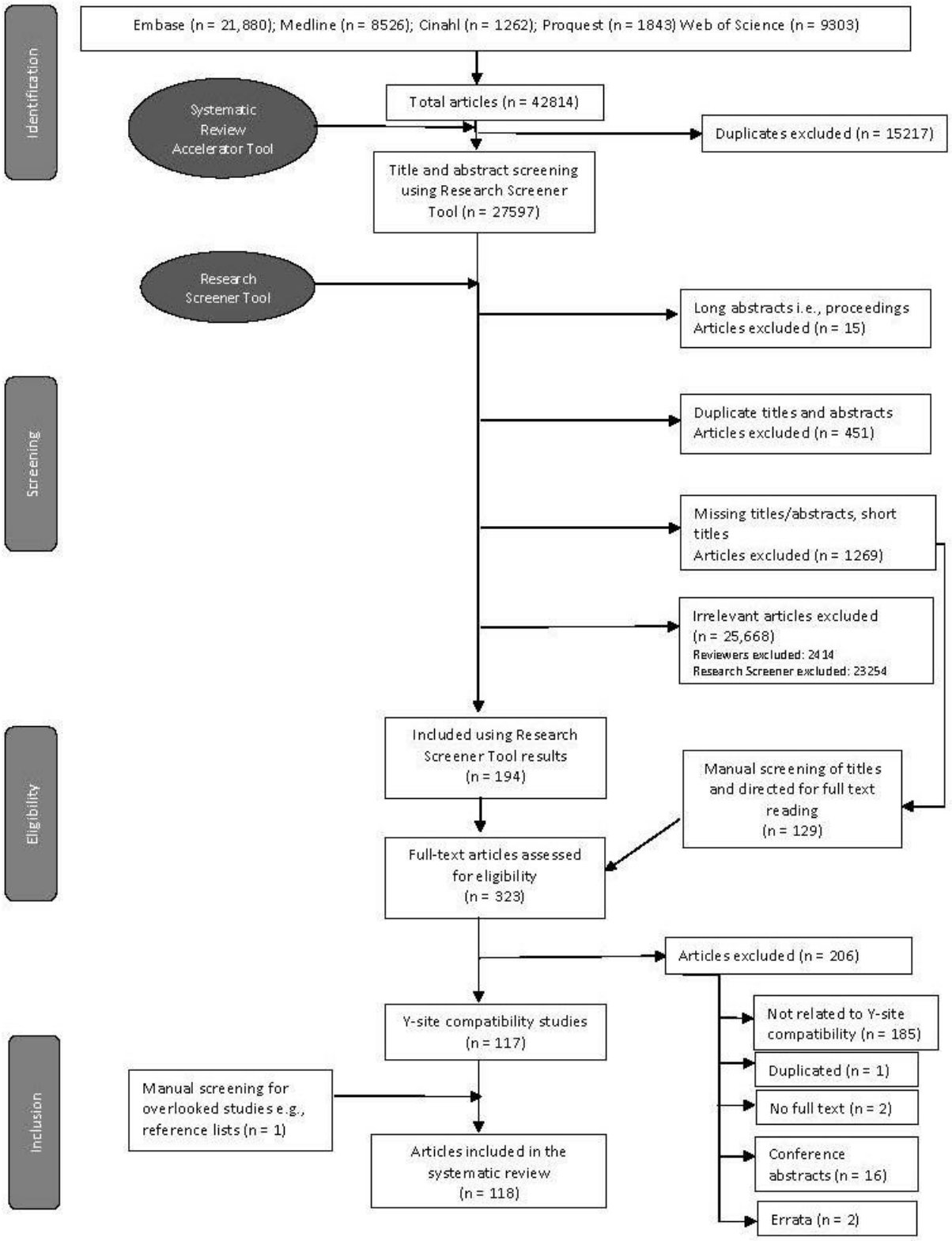


Figure 2.3 PRISMA* flow diagram for the systematic review search, screening and selection process (* PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses)

Reviewer 1 completed 52 cycles of screening via Research Screener, which comprised 10% of the 25,862 references available for screening and concluded after 14 cycles with no abstracts selected (Figure 2.4).

Reviewer 2 completed 35 cycles (6%) of screening and concluded after four cycles with no abstracts selected. As a result, 149 articles were flagged by both reviewers. A further 67 were selected by only one reviewer and classified as conflicts for resolution by the review team, from which 37 were considered potentially eligible and included in the full-text review. Including the eight seed abstracts, a total of 194 articles (0.75%) were directed for full-text consideration at this stage. The kappa coefficient was 0.80, indicating strong agreement.

The 1269 references without titles/abstracts were screened manually by the two reviewers to select potentially eligible reports for full-text read (most included a title and were only missing an abstract) and 129 were selected for progression to full-text review (kappa coefficient 0.78, indicating moderate agreement). Overall, a total of 323 articles were subject to full-text reading, of which 117 were found to fully comply with the inclusion and exclusion criteria and were included in the formal systematic review. Screening of reference lists of the selected articles identified one further study which was not captured in the initial search strategy and was therefore included in the final total of 118 articles for systematic review.

To be included in the review, the studies had to be full text, original research of *in-vitro* experimental studies pertaining to physicochemical compatibility of IV drugs in neonatal setting. Only studies written in the English language and published in peer reviewed journals/forums were included. Studies captured during complementary

search using individual NICU drugs (although in a different setting) at neonatal concentrations were also included to extract compatibility data.

Conference abstracts, other study designs such as observational studies, non-experimental studies, other publication types: reviews, letters, editorials, case studies (unless original compatibility data are reported), studies with irrelevant objectives or outcomes, non-English language, grey literature were excluded. Studies with inadequate data (i.e., which precluded effective data extraction) and studies of drugs/concentrations not applicable to the neonatal setting were excluded from analysis.

Further insights to the value of Research Screener are shown in Figures 2.4 and 2.5. Of the 186 articles which were directed to full-text read (excluding the eight seed abstracts), 55 were eventually selected for inclusion in the systematic review. Reviewer 1 encountered all 55 articles by the 29th cycle of article flagging (1408 papers, 5.4%) and reviewer 2 by the 27th cycle (1304 papers; 5%). Similar results were observed in an acute pain systematic review, where all of the reviewed articles were identified after screening 5% of the search results [194].

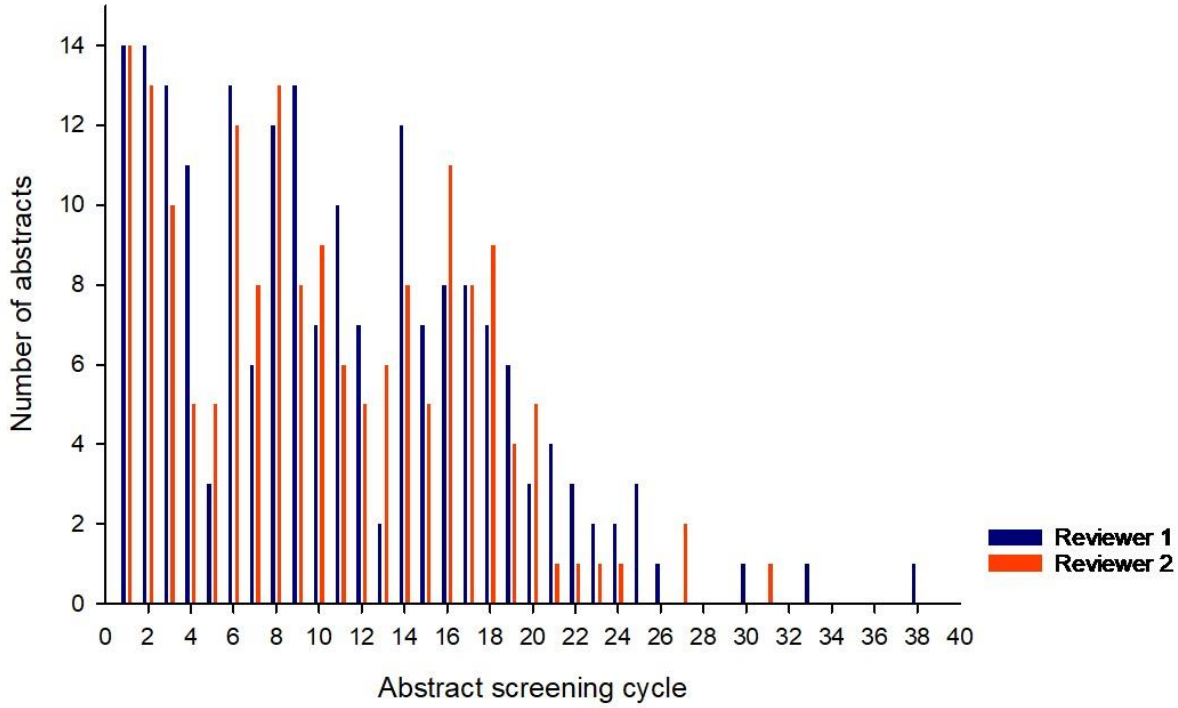


Figure 2.4 Number of abstracts flagged by each reviewer for full-text review in the Research Screener process. Reviewers 1 and 2 completed 52 and 35 cycles, respectively

The cyclical trends in selection of studies for the systematic review (Figure 2.5) demonstrate that Research Screener presented 44% (24/55) of the articles to the reviewers in the first four cycles, ostensibly due to the effective use of the eight seed abstracts. Thereafter, selection rates varied between the two reviewers and became sporadic after 19 cycles.

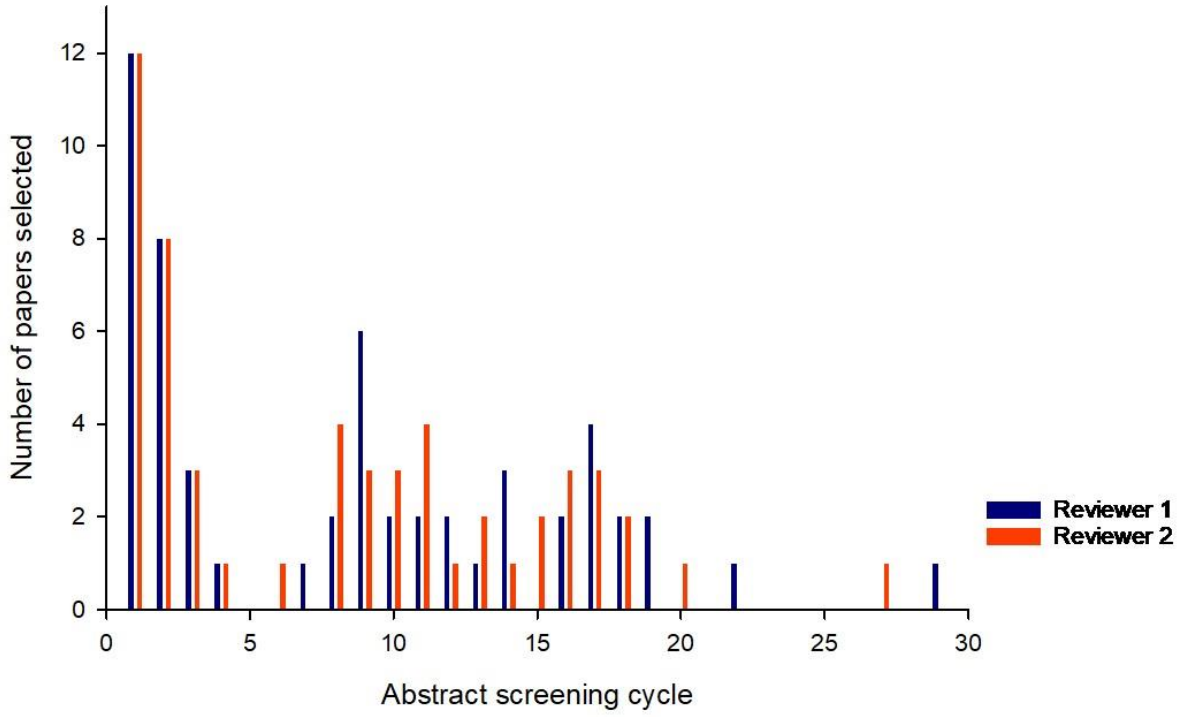


Figure 2.5 Number of papers selected for the review (after full-text read) from each screening cycle

In order to estimate the potential time saved by completing the screening process from <10% of the full search strategy results, screening time data for each reviewer were extracted from Research Screener and analysed. The mean (95% confidence interval; range) time to screen each title/abstract in the final 20% of cycles screened by the two reviewers was 8.4 (6.8-10.1; 2-131) and 15.2 (12.6-17.9;1-244) seconds, respectively. The final 20% of cycles was selected for this analysis because it represented a continuous series of cycles in which relatively few papers were potentially eligible, thus providing a plausible, conservative estimate of the time to screen subsequent cycles, if this had been required. Therefore, based on the >23,250 titles/abstracts that did not require screening, the potential time saving was at least 56 and 98 hours for each reviewer.

2.2 Investigating the physicochemical drug compatibility data pertaining to NICU setting

This section elaborates in detail the extraction, presentation and synthesis of data from the selected studies for the main systematic review, and the quality assessment procedure of the selected studies.

2.2.1 Methods

2.2.1.1 Data Extraction and Presentation

To ensure consistency in data extraction, a standardized data extraction sheet (Appendix 3) was developed, by consensus of the three reviewers, to include study methodology components and physicochemical compatibility data of selected studies. This included:

- Type of compatibility studied (physical/chemical)
- Objective/s of the study
- Drug(s), concentrations tested, and diluents used
- Aspects used to simulate contemporary neonatal setting (incubator temperature, clinically relevant concentrations)
- Method of mixing (static mixing in vessels/ simulation of Y-site tubing/ mixing ratio)
- Test conditions (temperature, dwell time, sampling points)
- Methods to test physical compatibility (visual, viewer, turbidity, pH, particle size analysis), including number of observers/assessors

- Methods to test chemical compatibility (HPLC, other)
- Key results/ conclusions on drug compatibility

To ensure the success of the data extraction sheet, a pilot test was conducted by two independent reviewers using five papers retrieved from the pilot testing of the search strategy (Section 2.1.2.2). Discrepancies were arbitrated by a third independent reviewer.

2.2.1.2 Quality assessment of selected studies

Given that no published, validated quality assessment tools were available for *in-vitro* drug compatibility studies, a separate quality assessment instrument was developed which comprised of the following elements.

- A general tool for all included studies
- A tool for physical compatibility testing
- A tool for chemical compatibility testing

If a single study evaluated both physical and chemical compatibility components, all quality assessment elements were applied. Criteria for the quality assessment were adopted from the model published by Kanji *et al.* [32] and modified accordingly to match the context of the setting of interest (i.e. NICU). The quality assessment instrument was reviewed by at least three independent experts. The developed quality assessment instrument can be found in Appendix 4.

2.2.2 Results

2.2.2.1 Type of compatibility tested and clinical focus of the selected studies

Of the selected articles, 72% (n=85) evaluated only the physical compatibility of drug combinations of interest. Two studies (2%) evaluated chemical compatibility only and 26% (n=31) evaluated combined physical and chemical compatibility. There was a steady trend of conducting combined physical and chemical compatibility testing with time, with the highest number of studies conducted during the period of 2007-2022 (Figure 2.6).

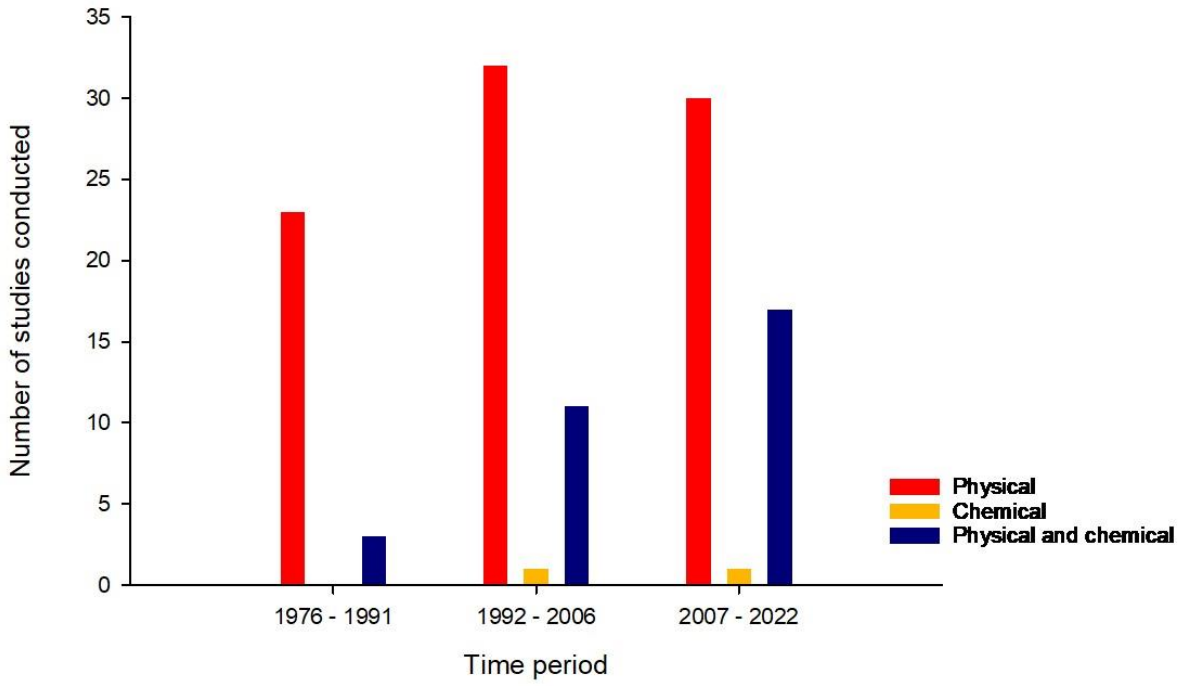


Figure 2.6 Trend in conducting compatibility studies with time

Of the total selected studies, 23 had a focus on neonatal and paediatric setting, 15 focused the adult setting and the remainder (n=80) did not have a specific focus mentioned.

2.2.2.2 Preparation of samples for compatibility testing

Of the selected studies, 19 included filtration of drug samples prior to mixing and 28 specified the order of mixing of drugs, further reversing the order of mixing to test the differences in compatibility based on the order of mixing.

A vast majority (n=113) utilised static mixing of the test drugs as the method to simulate Y-site mixing conditions. Only 12 studies followed actual Y-site mixing of the test drugs. Seven studies followed both static mixing and actual Y-site mixing. Glass or plastic tubes, volumetric flasks, syringes and glass slides were used as vessels for test drug mixing. Most of the studies which followed static mixing of drug combinations, have mixed the binary drug combinations at a 1:1 volume/volume ratio (n=98). A limited number of studies used other volume ratios e.g. mixing to achieve a final concentration of a given drug, 1:6, 1:3, 1:4, 4:1, 1:9 and 9:1.

2.2.2.3 Testing temperatures

Most of the studies (n=100) exposed the test drug combinations to room/ ambient temperature, during the testing period. Five studies have exposed the test samples to higher and lower temperatures e.g., 37°C [94, 206, 207], 32°C [208], 30°C and 17°C [209].

2.2.2.4 Contact (dwelling) time of test drug combinations

In the selected studies, mixture contact times ranged from minutes (5 min; [210]) to hours (1 to 4) [50, 95, 96, 99, 109, 207, 211-219], and several days [106, 110, 206, 209, 220-224]. However, the most followed contact times were 4 hours (n=36) and 24 hours (n=36). Eleven studies have used a contact time of 1 hour for testing. There are

no formal guidelines regarding mixture contact times, although some reports have related the contact time to relevant clinical settings [84, 94].

2.2.2.5 Use of control samples and baseline testing

Approximately 50% (n=58) of the selected studies used negative and/or positive control samples, in parallel to the test samples, for physical and chemical compatibility testing. A majority (n=98) performed baseline (time zero) testing.

2.2.2.6 pH testing

Of the selected studies in the review, 49 tested the pH of drug combinations. pH testing has been used inconsistently, in the selected studies, both as a physical [102, 225-228] and chemical [229, 230] compatibility determinant. Multiple studies have identified varying thresholds to determine physicochemical compatibility e.g. incompatibility was defined as a change in mean pH by >0.5 units from the initial pH [94, 231] , ≥2 pH units after mixing [230], ≥1 pH unit change over the course of the experiment [84, 102, 227, 232], pH variation of more than 0.4 pH units [226] a change of >0.1 pH unit, compared with the baseline reading [233], changes in pH of more than 0.2 units [234], a pH value change >10% over the course of the experiment [235] and pH value outside the optimal range of the drug tested [228, 229].

2.2.2.7 Use of more than one assessor

Only 13 studies (of 116 studies which performed physical compatibility testing) have used more than one assessor to perform the visual observations of the test drug mixtures.

2.2.2.8 Physical compatibility testing methods

All studies which performed physical compatibility testing (n=116), have used macroscopic visual observation by unaided eye, under dark and light backgrounds, for precipitation, haze, turbidity, change in colour and evolutions of gases as physical compatibility determinants. Several studies have further used polarized light [96, 109, 207, 209, 232, 236-239] and high intensity monodirectional light (Tyndall) beams [84, 85, 110, 213-215, 220, 234, 240-245] to aid the visual observation. Twenty-two studies used magnifiers (magnifying lenses and lamps) for this purpose. Subvisual physical compatibility testing components have been used in 52 studies. These methods include turbidity measurement (using laboratory grade turbidimeters or spectrophotometrically), microscopic evaluation of particles (direct observation or observing the filter disks for particles after filtering the solution) and particle counting/ particle size analysing techniques. In addition to the above methods, Yamashita *et al.* [230], have centrifuged drug solutions at 3000 rpm for 10 minutes to concentrate any small particles that had not been visually detected.

Microscopic observation of the filter disks (after filtering the sample mixtures) was performed in five studies [245-249] and the compatibility was determined based on USP chapter <788> Test 2.A criterion for particulate matter in injections: “the amount of particles in aliquot sterile solution is considered physically compatible if it is less than 2 particles/mL measuring ≥25 μm, and less than 12 particles/mL with size ≥10 μm in diameter” [250]. Further, light obscuration particle counting techniques too have been incorporated by several studies [251, 252], which used USP chapter <788> Test 1.B criterion for parenteral solutions: “the preparation is compatible if the average number of particles does not exceed 6000 per container ≥10 μm and does not exceed 600 per container ≥25 μm” [250]. Koller *et al.* [226] used the European Pharmacopoeia

monograph 2.10.19 standard in particle counting tests: “average number of particles does not exceed 25 particles/mL for Particles ≥10 μm; does not exceed 3 particles/mL for particles ≥25 μm” [253].

2.2.2.9 Chemical compatibility testing methods

Of the studies which performed chemical compatibility testing (n=33), a majority (n =27) have used HPLC to evaluate drug concentrations, making it the most widely used drug concentration testing method. In addition, LC-MS [217], immunoassay techniques [254, 255] Thin Layer Chromatography (TLC) [221], capillary electrophoresis [228], spectrophotometry [228, 231] and microbiological bioassays [221, 241] have been used to directly and indirectly evaluate drug concentrations. A majority (n=25) have used an acceptance criterion of 10% change in drug combinations from baseline (time 0) or the nominal drug concentration (of control sample), as a clinically significant incompatibility. A change in drug concentration of 7% [207] and 5% [229, 256] too have been used as a cut-off level for drug concentrations. Several studies have also used statistically significant difference in drug concentration as a determinant of chemical incompatibility [95, 96, 109, 217, 222, 255].

2.2.2.10 Physical and chemical compatibility

The availability of physical and chemical compatibility information for each of the total 95 predetermined list of drugs are illustrated in Table 2.3. A total of 30 drugs did not have chemical compatibility data available in combination with any of the other drugs in the list. Adenosine, erythropoietin, folic acid, glucagon and sodium benzoate had no physical or chemical compatibility data in combination with any drug in the list.

Table 2.3 Number of combinations for which each drug has physical (indicated in green font) and chemical (indicated in blue font) compatibility data available, i.e., Adenosine has no physical compatibility data with any of the other drugs of concern while caffeine base has physical compatibility data with one other drug)

0	1 to 2	3 to 10	11 to 20	>20
Adenosine	Caffeine	Amoxicillin	Amikacin	Aciclovir
Erythropoietin	Clonazepam	Amoxicillin/ clavulanic acid	Atropine	Alprostadil
Folic Acid	Flecainide	Amphotericin B	Azithromycin	Amiodarone
Glucagon	Flumazenil	Amphotericin (liposomal)	Cefepime	Ampicillin
Sodium Benzoate	Neostigmine	Benzylpenicillin	Cefotaxime	Calcium gluconate
	Octreotide	Caffeine citrate	Ceftriaxone	Cefazolin
Adenosine	Omeprazole	Dexmedetomidine	Cefuroxime	Ceftazidime
Amoxicillin/ clavulanic acid	Pyridoxine	Flucloxacillin	Clarithromycin	Ciprofloxacin
Ampicillin	Rifampicin	Ganciclovir	Clindamycin	Cloxacillin
Atropine	Salbutamol	Hydralazine	Clonidine	Dobutamine
Azithromycin	Suxamethonium	Imipenem	Dexamethasone	Dopamine
Cefuroxime		Naloxone	Diazepam	Epinephrine
Clonazepam	Alprostadil	Pantoprazole	Digoxin	Fentanyl
Cloxacillin	Amoxicillin	Propranolol	Doxapram	Fluconazole
Doxapram	Amphotericin (liposomal)	Rocuronium	Erythromycin	Furosemide
Erythropoietin	Benzylpenicillin	Sildenafil	Hydrocortisone	Gentamicin
Flecainide	Caffeine	Vitamin K	Ibuprofen lysine	Heparin
Flumazenil	Caffeine citrate		Indometacin	Ibuprofen
Folic Acid	Cefazolin	Aciclovir	Isoprenaline	Insulin
Ganciclovir	Cefotaxime	Amikacin	Ketamine	Linezolid
Glucagon	Ceftriaxone	Amiodarone	Lidocaine	Magnesium sulfate
Hydralazine	Clindamycin	Amphotericin B	Levetiracetam	Meropenem
Ibuprofen lysine	Dexamethasone	Calcium gluconate	Metronidazole	Midazolam
Lidocaine	Dexmedetomidine	Clarithromycin	Pancuronium	Milrinone
Naloxone	Diazepam	Erythromycin	Paracetamol	Morphine
Neostigmine	Digoxin	Epinephrine	Phenobarbitone	Norepinephrine
Octreotide	Flucloxacillin	Fentanyl	Phenytoin	Pentoxifylline
Omeprazole	Hydrocortisone	Furosemide	Sodium bicarbonate	Piperacillin-tazobactam
Pantoprazole	Ibuprofen	Gentamicin	Sodium Nitroprusside	Potassium chloride
Propranolol	Imipenem	Heparin	Trimethoprim-sulfamethoxazole	Ranitidine
Pyridoxine	Indometacin	Ketamine		Tobramycin
Salbutamol	Isoprenaline	Magnesium sulfate	Cefepime	Vancomycin
Sodium Benzoate	Levetiracetam	Metronidazole	Ceftazidime	Vecuronium
Suxamethonium	Linezolid	Morphine	Ciprofloxacin	Zidovudine
Vitamin K	Meropenem	Norepinephrine	Clonidine	
Zidovudine	Pancuronium	Paracetamol	Dobutamine	Milrinone
	Phenobarbitone	Phenytoin	Dopamine	Pentoxifylline
	Rifampicin	Piperacillin-tazobactam	Fluconazole	Vancomycin
	Rocuronium	Potassium chloride	Insulin	
	Sodium Nitroprusside	Ranitidine	Midazolam	

0	1 to 2	3 to 10	11 to 20	>20
	Trimethoprim-sulfamethoxazole Vecuronium	Sodium bicarbonate Tobramycin Sildenafil		

Physical and chemical drug compatibility summary charts were created with all possible drug combinations of the drugs of interest. On grounds of space, physical and chemical compatibility charts for selected drugs (drugs which have reported physical compatibility data for more than 10 combinations) are illustrated in Figure 2.7 and 2.8 respectively. As per data reported in the selected studies for the systematic review, the boxes in the charts were named with a “C” for compatible combinations, with an “I” for incompatible combinations and with “#” if the compatibility depended on special conditions. The drug combinations with no compatibility data reported in the selected studies, were left unchecked. It was evident from the data compilation that for crucial NICU drugs such as epinephrine, norepinephrine, alprostadil, dopamine, dobutamine, ibuprofen, indometacin and morphine, significant gaps in chemical compatibility data was observed (Figure 2.8), in comparison to physical compatibility data (Figure 2.7). Though caffeine (citrate and base) is a major drug in neonatal therapeutics, it’s not included in the chart as it only had physical compatibility data available for less than 10 combinations (Table 2.3) according to the selected studies in the review.

Drug combinations which were reported as both compatible and incompatible under different testing conditions, for example, concentrations, testing times, diluents, are listed in Table 2.4, and its consistent with boxes specified as “#” in the physical and chemical compatibility charts (Figure 2.7 and 2.8)

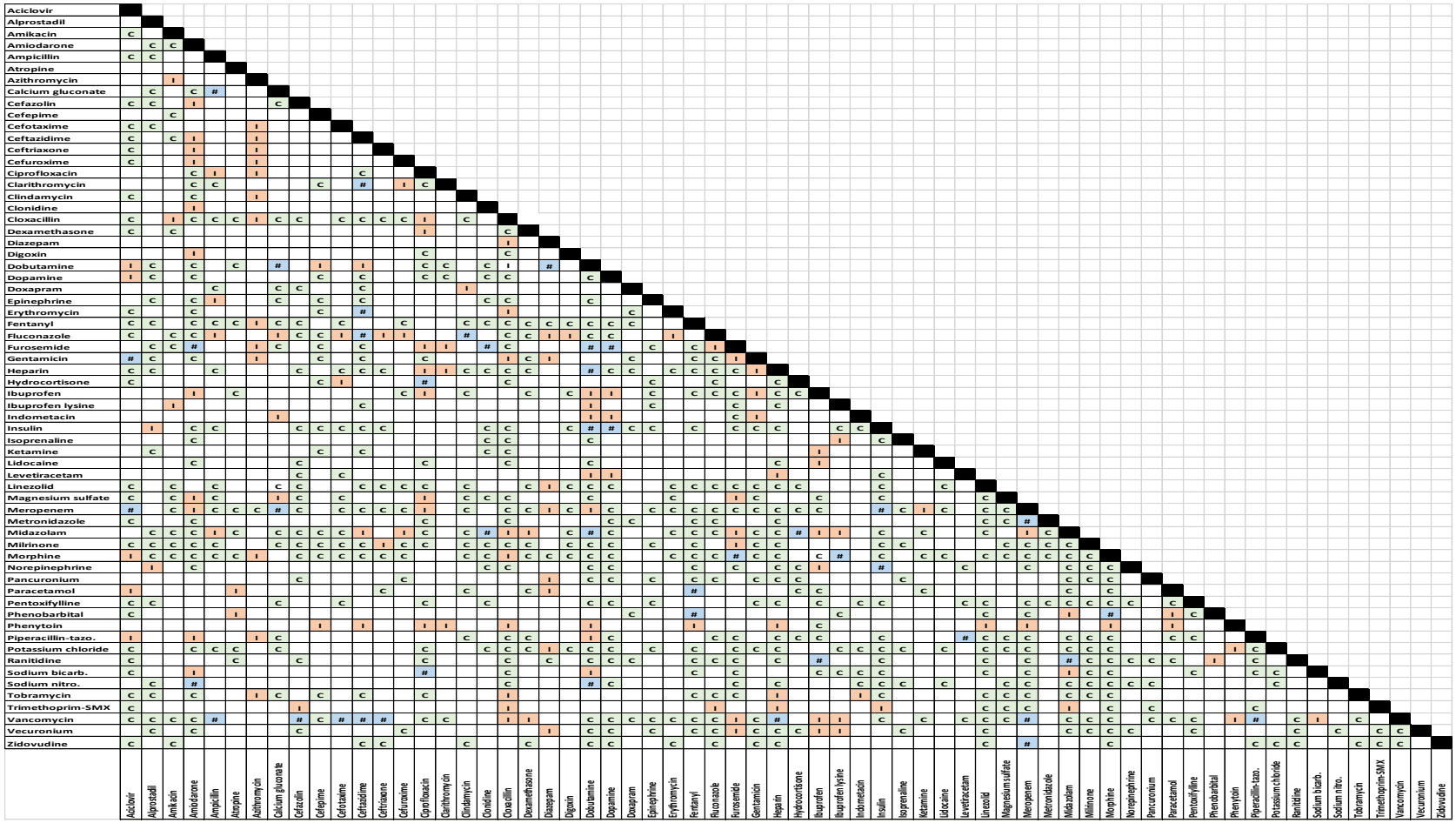


Figure 2.7 Physical compatibility data for selected drug combinations (Piperacillin-tazo.–Piperacillin-tazobactam; Sodium bicarb. – Sodium bicarbonate; Sodium nitro.– Sodium nitroprusside; Trimethoprim-SMX–Trimethoprim-sulfamethoxazole; C– Compatible; I– Incompatible; #– compatible in special situations)

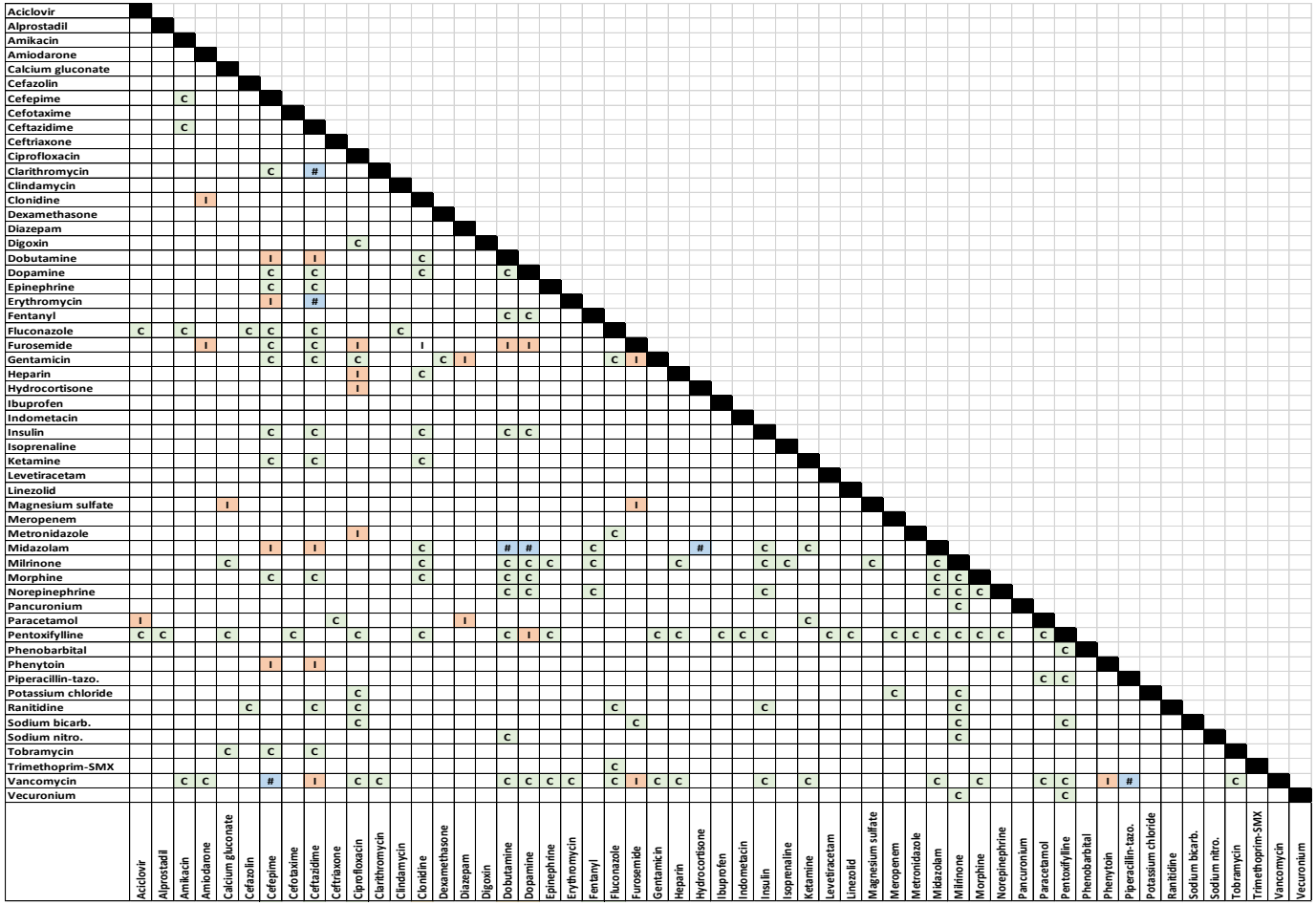


Figure 2.8 Chemical compatibility data for selected drug combinations (Piperacillin-tazo.–Piperacillin-tazobactam; Sodium bicarb.–Sodium bicarbonate; Sodium nitro.–Sodium nitroprusside; Trimethoprim-SMX–Trimethoprim-sulfamethoxazole; C–Compatible; I–Incompatible; #–compatible in special situation

Table 2.4 Drug combinations reported as both compatible and incompatible under different testing conditions by selected studies. All data are related to physical compatibility unless specified. (D5W – glucose 5% w/v; NS – normal saline (0.9% w/v sodium chloride); U – undiluted; v/v – volume/volume ratio; RT – room temperature)

Drug combination	Compatible	Incompatible
Aciclovir and gentamicin	Aciclovir 5 mg/mL (D5W) and gentamicin 1.6 mg/mL (premixed); Y-site mixed at 1:1 v/v, for 4 hrs at RT [257]	Aciclovir 5 mg/mL (NS; infusion rate 100 mL/hr) and gentamicin 30 mg/mL (0.45% sodium chloride; infusion rate 10 mL/30 min) at RT; formation of thick paste [247]
Aciclovir and meropenem	Aciclovir 5 mg/mL (WFI) and meropenem 1 mg/mL (NS); Y-site mixed at 1:1 v/v, for 4 hrs at RT [258]	Aciclovir 5 mg/mL (WFI) and meropenem 50 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [258]
Amiodarone and furosemide	Amiodarone 6 mg/mL (D5W) and furosemide 1 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 24 hrs at RT [259]	Amiodarone 6 mg/mL (D5W) and furosemide 10 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; immediate opaqueness [259] Amiodarone 6 mg/mL (D5W) and furosemide 10 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; immediate turbidity [231]
Amiodarone and sodium nitroprusside	Amiodarone 6 mg/mL (D5W) and sodium nitroprusside 0.4 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 24 hrs at RT [259] Amiodarone 6 & 15 mg/mL (D5W) and sodium nitroprusside 0.3 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 48 hrs at RT [110]	Amiodarone 1.5 mg/mL (D5W) and sodium nitroprusside 0.3 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; cloudiness in 4 hrs [110] Amiodarone 1.5, 6 & 15 mg/mL (D5W) and sodium nitroprusside 1.2 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [110] Amiodarone 1.5, 6 & 15 mg/mL (D5W) and sodium nitroprusside 3 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [110]
Amphotericin B and fluconazole	Amphotericin B 0.1 mg/mL (WFI) and fluconazole 2 mg/mL (U); Y-site mixed at 1:1 v/v, for 72 hrs at RT [222]	Amphotericin B 5 mg/mL and fluconazole 2 mg/mL (U); Y-site mixed at 1:1 v/v, for 24 hrs at RT; delayed precipitate [260]
Ampicillin and vancomycin	Ampicillin 250 mg/mL (U), 50, 10, 1 mg/mL (NS) and vancomycin 2 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261] Ampicillin 50, 10, 1 mg/mL (NS) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Ampicillin 250 mg/mL (U) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT; transient precipitate [261]
Ampicillin and calcium gluconate	Ampicillin 40 mg/mL (NS) and calcium gluconate 4 mg/mL (NS); Y-site mixed at 1:1 v/v, for 3 hrs at RT [50]	Ampicillin 40 mg/mL (NS) and calcium gluconate 4 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; colour change in 1 hr [50]

Drug combination	Compatible	Incompatible
Calcium gluconate and dobutamine	Calcium gluconate 4 mg/mL (NS/D5W) and dobutamine 4 mg/mL (NS/D5W); Y-site mixed at 1:1 v/v, for 3 hrs at RT [50]	Calcium gluconate 50 mg/mL (NS/D5W) and dobutamine 2 mg/ml (D5W/NS); Y-site mixed at 1:1 v/v, at RT; particles at 24 hrs [262]
Calcium gluconate and meropenem	Calcium gluconate 4 mg/mL (WFI) and meropenem 1 mg/mL (NS); Y-site mixed at 1:1 v/v, for 4 hrs at RT [258]	Calcium gluconate 4 mg/mL (WFI) and meropenem 50 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; colour change at 4 hrs [258]
Cefazolin and vancomycin	Cefazolin 200 mg/mL (WFI), 10 & 1 mg/mL (D5W) and vancomycin 2 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Cefazolin 50 mg/mL (D5W) and vancomycin 2 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [261]
	Cefazolin 1 mg/mL (D5W) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Cefazolin 200 mg/mL (WFI), 50 & 10 mg/mL (D5W) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [261]
Cefotaxime and vancomycin	Cefotaxime 200 mg/mL (WFI), 50, 10, 1 mg/mL (D5W) and vancomycin 2 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Cefotaxime 200 mg/mL (WFI), 50 mg/mL (D5W) and vancomycin 20 mg/mL (D5W) Y-site mixed at 1:1 v/v, at RT; immediate precipitation [261]
	Cefotaxime 10, 1 mg/mL (D5W) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	
Ceftazidime and clarythromycin	Ceftazidime 120 mg/mL (flow rate 2mL/h) and clarythromycin 500/50 mg/mL (0.3 hr infusion) physicochemically compatible at RT [238]	Ceftazidime 120 mg/mL (flow rate 2mL/h) and clarythromycin 500/10 mg/mL (0.3 hr infusion) at RT; precipitation [236, 238]
Ceftazidime and erythromycin	Ceftazidime 120 mg/mL (flow rate 2mL/h) and erythromycin 1,000/100 mg/mL (0.3 hr infusion) physicochemically compatible at RT [238]	Ceftazidime 120 mg/mL (flow rate 2mL/h) and erythromycin 1,000/20 mg/mL (0.3 hr infusion) at RT; precipitate [236, 238]
Ceftazidime and fluconazole	Ceftazidime 120 mg/mL (flow rate 2mL/h) and fluconazole 200/100 mg/mL (0.4 hr infusion) physicochemically compatible at RT [238]	Ceftazidime 40 mg/mL (D5W) and fluconazole 2 mg/mL (U) Y-site mixed at 1:1 v/v, at RT for 72 hrs; colour change [222]
		Ceftazidime 20 mg/mL (D5W) and fluconazole 2 mg/mL (U) Y-site mixed at 1:1 v/v, at RT; immediate precipitate [260]
Ceftazidime and vancomycin	Ceftazidime 200 mg/mL (WFI), 50, 10, 1 mg/mL (D5W) and vancomycin 2 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Ceftazidime 200 mg/mL (WFI), 50, 10 mg/mL (D5W) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitate [261]
	Ceftazidime 1 mg/mL (D5W) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Ceftazidime 120 mg/mL (flow rate 2mL/h) and vancomycin 1500/50 mg/mL (30 mg/mL); 1 hr infusion [236]

Drug combination	Compatible	Incompatible
Ceftriaxone and vancomycin	Ceftriaxone 200 mg/mL (WFI), 50, 10, 1 mg/mL (D5W) and vancomycin 2 mg/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [261]	Ceftriaxone 200 mg/mL (WFI), 50, 10, 1 mg/mL (D5W) and vancomycin 20 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [261]
Ciprofloxacin and hydrocortisone	Ciprofloxacin 2 mg/mL (D5W) and hydrocortisone 1 mg/mL (NS); Y-site mixed at 1:1 v/v, for 2 hrs at RT physically compatible (chemically incompatible) [217]	Ciprofloxacin 2 mg/mL (NS/D5W) and hydrocortisone 100 mg/2mL (U); Y-site mixed at 1:1 v/v; immediate precipitation [263]
Ciprofloxacin and sodium bicarbonate	Ciprofloxacin 10 mg/mL (D5W) and sodium bicarbonate 1 meq/mL (D5W); Y-site mixed at 1:1 v/v, for 4 hrs at RT [243]	Ciprofloxacin 10 mg/mL (D5W) and sodium bicarbonate0.1 meq/mL (D5W); Y-site mixed at 1:1 v/v; initial haze and precipitate in 1 hr [243]
Clindamycin and fluconazole	Clindamycin 12 mg/mL (D5W) and fluconazole 2 mg/mL (U); Y-site mixed at 1:1 v/v, for 72 hrs at RT [222]	Clindamycin 24 mg/mL and fluconazole 2 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [260]
Clonidine and furosemide	Clonidine 7.5 µg/mL (NS) and furosemide 2 mg/mL (NS); Y-site mixed at 1:1 v/v, for 24 hrs at RT [229]	Clonidine 4.5 µg/mL (NS) and frusemide 5.34 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; pH change at 6 hrs [226]
	Clonidine 4.5 µg/mL (NS) and frusemide 0.64 mg/mL (NS) Y-site mixed at 1:1 v/v, for 6 hrs at RT [226]	Clonidine 15 µg/mL (NS) with frusemide 0.64 and 5.34 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; pH change at 2 & 4 hrs [226]
Clonidine and midazolam	Clonidine 15 µg/mL (NS) and midazolam 3.6 mg/mL (NS) Y-site mixed at 1:1,1:10;10:1 v/v, for 7 days at RT physicochemically compatible [223]	Clonidine 15 µg/mL and midazolam 5 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; colour change in 24 hours [239]
Diazepam and dobutamine	Diazepam 0.2 mg/mL (NS/D5W) and dobutamine 4 mg/mL (NS/D5W) Y-site mixed at 1:1 v/v for 3 hrs at RT [50]	Diazepam 2.5 mg/mL (NS/D5W) and dobutamine 2 mg/ml (D5W/NS); Y-site mixed at 1:1 v/v, at RT; initial clouding and yellow precipitate in 24 hrs [262]
Dobutamine and furosemide	Dobutamine 4 mg/mL (NS) furosemide 1mg/mL (NS); Y-site mixed at 1:1 v/v for 3 hrs at RT [50]	Dobutamine 4 mg/mL (D5W) furosemide 1mg/mL (D5W); Y-site mixed at 1:1 v/v at RT; precipitation in 1 hr [50]
		Dobutamine 4 mg/mL (D5W) and furosemide 10 mg/mL (U); Y-site mixed at 1:1 v/v at RT; immediate precipitation [264]
		Dobutamine 5 mg/mL (D5W) and furosemide 10 mg/mL (U); Y-site mixed at 1:1 v/v at RT; instant turbidity [231]
		Dobutamine 2 mg/ml (D5W/NS) and furosemide 5 mg/mL (NS/D5W); Y-site mixed at 1:1 v/v at RT; immediate precipitation [262]

Drug combination	Compatible	Incompatible
		Dobutamine 8 mg/mL (NS/ D5W) and furosemide 2 mg/mL (NS); Y-site mixed at 1:1 v/v at RT; immediate particles [229]
Dobutamine and heparin	Dobutamine 4 mg/mL (NS) and heparin 50 units/mL (NS); Y-site mixed at 1:1 v/v for 3 hrs at RT [50]	Dobutamine 4 mg/mL (D5W) and heparin 50 units/mL (D5W); Y-site mixed at 1:1 v/v at RT; immediate precipitation [50]
		Dobutamine 2 mg/mL (D5W/NS) and heparin 50 units/mL (NS/D5W); Y-site mixed at 1:1 v/v at RT [262]
		Dobutamine 2 mg/mL (D5W/NS) and heparin 5000 units/mL (NS/D5W); Y-site mixed at 1:1 v/v at RT; Pink at 6 hr [262]
		Dobutamine 2 mg/mL (D5W) and heparin 100 units/mL (D5W); Y-site mixed at 1:1 v/v at RT; immediate precipitate [265]
		Dobutamine 4 mg/mL (D5W) and heparin 100 units/mL; Y-site mixed at 1:1 v/v at RT; precipitation [230]
Dobutamine and insulin	Dobutamine 8 mg/mL (NS/D5W) and insulin 1 units/mL (NS/D5W); Y-site mixed at 1:1 v/v for 24 hrs at RT [229]	Dobutamine 2 mg/mL (D5W/NS) and insulin 50 units/mL (NS/D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [262]
	Dobutamine 4 mg/mL (D5W) and insulin 1 units/mL (D5W); Y-site mixed at 1:1 v/v for 24 hrs at RT [230]	
Dobutamine and midazolam	Dobutamine 8 mg/mL (D5W) and midazolam 4 mg/mL (D5W); Y-site mixed at 1:1 v/v for 24 hrs at RT; physicochemically compatible [229]	Dobutamine 2 mg/mL (D5W) and midazolam 1mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; crystalline particles at 8 hrs [49]
	Dobutamine 8 mg/mL (NS) and midazolam 4 mg/mL (NS); Y-site mixed at 1:1 v/v for 24 hrs at RT; Physically compatible but chemically incompatible [229]	
	Dobutamine 4 mg/mL (D5W) with midazolam 1 mg/mL (D5W); Y-site mixed at 1:1 v/v for 24 hrs at RT [230]	
Dobutamine and pantoprazole	Dobutamine 1.0 mg/mL (D5W) and pantoprazole 0.16 and 0.4 mg/mL (NS); Y-site mixed at 1:1 v/v for 12 hrs at RT [44]	Dobutamine 1.0 mg/mL (D5W) and pantoprazole 0.8 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; cloudiness [44]
	Dobutamine 2.5 mg/mL (D5W) and pantoprazole 0.16 and 0.4 mg/mL (NS); Y-site mixed at 1:1 v/v for 12 hrs at RT [44]	Dobutamine 2.5 mg/mL (D5W) and pantoprazole 0.8 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; cloudiness [44]

Drug combination	Compatible	Incompatible
		Dobutamine 4 mg/mL (D5W) and pantoprazole 0.16, 0.4 and 0.8 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; cloudiness [44]
Dobutamine and sodium nitroprusside	Dobutamine 5 mg/mL (U) and sodium nitroprusside 0.3 and 1.2 mg/mL (D5W) is physicochemically compatible; Y-site mixed at 1:1; 1:4; 4:1 v/v for 24 hrs at RT [232] Dobutamine 1.5 mg/mL (D5W-0.2% sodium chloride) and Sodium nitroprusside 0.3, 1.2 and 3 mg/mL (D5W); Y-site mixed at 1:1 v/v for 48 at RT [110]	Dobutamine 6 mg/mL (D5W-0.2% sodium chloride) and sodium nitroprusside 1.2 and 3 mg/mL (D5W); Y-site mixed at 1:1 v/v at RT; colour change [110] Dobutamine 12.5 mg/mL (U) and sodium nitroprusside 1.2 and 3 mg/mL (D5W); Y-site mixed at 1:1 v/v at RT; colour change [110]
Dopamine and furosemide	Dopamine 8 mg/mL (NS/D5W) and furosemide 2 mg/mL (NS); Y-site mixed at 1:1 v/v for 24 hrs at RT [229]	Dopamine 3.2 mg/mL (D5W) and furosemide 10 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; turbidity within 4 hrs [231]
Fentanyl and acetaminophen	Fentanyl 50 µg/mL and acetaminophen 10 mg/mL; Y-site mixed at 1:1 v/v for 4 hrs [246]	Fentanyl 50 µg/mL and acetaminophen 10 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; particle formation in 1 hr [245]
Fentanyl and phenobarbitone	Fentanyl 25 µg/mL (D5W) and with phenobarbitone 2mg/mL (D5W); Y-site mixed at 1:1 v/v for 48 hrs at RT [220]	Fentanyl 50 µg/mL and phenobarbitone 50 mg/mL physically incompatible in 15 minutes [65]
Furosemide and morphine	Furosemide 10 mg/mL (U) and morphine 2 mg/mL (D5W); Y-site mixed at 1:1 v/v for 4 hrs at RT [264]	Furosemide 10 mg/mL (U), 2.4,0.8 mg/mL (D5W) and morphine 1 mg/mL; Y-site mixed at 1:1 v/v, at RT; immediate precipitation [266]
Heparin and sildenafil	Heparin 1 units/mL (D5W; final concentration in container) and sildenafil 800 µg/mL; for 30 days at RT [106]	Heparin 100 units/mL (D5W) and sildenafil 800 µg/mL; Y-site mixed at 1:1 v/v, at RT; haze within 24 hrs [94]
Heparin and vancomycin	Heparin 5000 units/mL (NS; final conc 10 mg/mL) vancomycin (NS; final concentration); physicochemically compatible; Y-site mixed at 1:1 v/v for 72 hrs at 37°C [206]	Heparin 1000 units/mL and vancomycin 10 mg/mL; Y-site mixed at 1:1 v/v; precipitation [267] Heparin 100 units/mL (NS/D5W) and vancomycin 10 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; precipitation [248]
Hydrocortisone and midazolam	Hydrocortisone 1 mg/mL (NS) physicochemically compatible with midazolam 1 mg/mL (NS) but chemically incompatible with 2 mg/mL (NS); Y-site mixed at 1:1 v/v for 2hrs at RT [217]	Hydrocortisone 50 mg/mL and midazolam 5 mg/mL (U); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [239]
Ibuprofen and ranitidine	Ibuprofen 4 mg/mL (NS/RL/ D5W) and ranitidine 10 mg/mL; 1:1 mixing on a slide [268]	Ibuprofen 100 mg/mL (U) and ranitidine 10 mg/mL; 1:1 mixing on a slide; precipitation [268]
Ibuprofen lysine and morphine	Ibuprofen lysine 10 mg/mL (U) and morphine 0.5 mg/mL (D5W/NS);	Ibuprofen lysine 10 mg/mL (U) and morphine 50 mg/mL (U); Y-

Drug combination	Compatible	Incompatible
	Y-site mixed at 1:1 v/v for 4hrs at RT [104]	site mixed at 1:1 v/v, at RT; immediate haze [104]
Insulin and meropenem	Insulin 0.2 units/mL (WFI) meropenem 1 mg/mL and 50 mg/mL (NS); Y-site mixed at 1:1 v/v for 4hrs at RT [258]	Insulin 1 IU/mL (NS/D5W) and meropenem 20 mg/mL (NS/D5W); Y-site mixed at 1:1; 1:4; 4:1 v/v; colour change in 2 hrs [269]
Insulin and norepinephrine	Insulin 1 IU/mL (NS/D5W) and norepinephrine 0.32 mg/mL (D5W); Y-site mixed at 1:1 v/v for 24 hrs at RT [229]	Insulin 1 IU/mL (D5W) and norepinephrine 0.064 mg/mL (D5W); Y-site mixed at 1:1 v/v, at RT; immediate precipitation [230]
Levetiracetam and piperacillin/tazobactam	Levetiracetam 20.83 mg/mL (NS) and piperacillin/tazobactam 166/20.83 mg/mL (D5W); Y-site mixed at 1:9; 9:1; 1:1 v/v for 4hr at RT [270]	Levetiracetam 5 mg/mL (NS) and piperacillin/tazobactam 45 mg/mL (NS); Y-site mixed at 1:1 v/v, at RT; turbidity within 30 min [235]
Meropenem and metronidazole	Meropenem 50 mg/mL (WFI) and metronidazole 5 mg/mL; Y-site mixed at 1:1 v/v for 4 hrs at RT [251]	Meropenem 1 and 50 mg/mL (NS) and metronidazole 5 mg/mL (WFI); Y-site mixed at 1:1 v/v, at RT; colour change within 4 hrs [258]
Meropenem and vancomycin	Meropenem 1 and 50 mg/mL (NS) and vancomycin 5 mg/mL (WFI); Y-site mixed at 1:1 v/v for 4 hrs at RT [258]	Meropenem 50 mg/mL (WFI) and vancomycin 50 mg/mL; Y-site mixed at 1:1 v/v, at RT; immediate precipitation [251]
Meropenem and zidovudine	Meropenem 1 mg/mL (NS) and zidovudine 4 mg/mL (WFI); Y-site mixed at 1:1 v/v for 4 hrs at RT [258]	Meropenem 50 mg/mL (NS) and zidovudine 4 mg/mL (WFI); Y-site mixed at 1:1 v/v at RT; colour change in 1 hr [258]
Midazolam and ranitidine	Midazolam 5 mg/mL (U) and ranitidine 0.5 mg/mL; Y-site mixed at 1:1 v/v for 24 hrs at RT [239]	Midazolam 5 mg/mL (U) ranitidine 50 mg/2 mL at RT; immediate precipitation [271]
Morphine and phenobarbitone	Morphine 1 mg/mL (D5W) and phenobarbitone 2mg/mL (D5W); Y-site mixed at 1:1 v/v for 48 hrs at RT [220]	Morphine 15 mg/mL and phenobarbitone 50 mg/mL; precipitation in 15 min [65]
Vancomycin and piperacillin /tazobactam	Vancomycin 2 mg/mL (D5W) and piperacillin /tazobactam 200/25 mg/mL (U), 50/6.5 mg/mL (D5W), 10/1.25 mg/mL (D5W), 1/0.125mg/mL (D5W); Y-site mixed at 1:1 v/v for 4 hrs at RT [261]	Vancomycin 20 mg/mL (D5W) and piperacillin /tazobactam 200/25 mg/mL (U), 50/6.5 mg/mL (D5W), 10/1.25 mg/mL (D5W); Y-site mixed at 1:1 v/v at RT; immediate precipitation [261]
	Vancomycin 20 mg/mL (D5W) and piperacillin /tazobactam 1/0.125mg/mL (D5W); Y-site mixed at 1:1 v/v for 4 hrs at RT [261]	Vancomycin 10 mg/mL (D5W) and piperacillin/tazobactam 40:5 mg/mL (D5W); Y-site mixed at 1:1 v/v at RT; precipitation within 4 hrs [242]
	Vancomycin 5 mg/mL (D5W) and piperacillin/tazobactam 28 mg/mL (D5W) chemically (bioassay) compatible; Y-site mixed at 1:1 v/v for 24 hrs at RT [241]	Vancomycin 10 mg/mL and 15 mg/mL (D5W) and piperacillin/tazobactam 28 mg/mL (D5W) visually incompatible but chemically (bioassay) compatible; Y-site mixed at 1:1 v/v for 24 hrs at RT [241]
Vancomycin and cefepime	Vancomycin 10 mg/mL and cefepime 83.33 mg/mL	Vancomycin 10 mg/mL and cefepime 200 mg/mL chemically

Drug combination	Compatible	Incompatible
	physicochemically compatible; Vancomycin 10 mg/mL and cefepime 200 mg/mL physically compatible; Y-site mixed at 1:1 v/v for 1 hr at RT [207]	incompatible; Y-site mixed at 1:1 v/v for 1 hr at RT [207]

Quality assessment characteristics of the selected studies that evaluated physical and chemical compatibility are presented in Table 2.5. Drug manufacturers were reported in 108 of 118 studies (92%). Of the selected studies, 112 (95%) defined the drug contact times, 104 (88%) defined the study conditions such as temperature and 109 (92%) clearly reported the mixing vessels (tubes/ containers and Y-site tubing). All studies defined the drug concentrations used for compatibility studies. Drug diluents were reported for all tested drugs in 104 (88%) studies, and 110 (93%) have defined sampling times (or completion of Y-site infusion). Baseline level testing performed in 98 (83%) studies and only 58 (49%) have used parallel controls in the experiment. Testing was performed in replicates, in 88 (75%) studies and 112 (95%) defined the mixing ratios of drug combinations. All 118 studies provided implications for future research.

All 116 studies which investigated physical compatibility have evaluated compatibility by one or more of the visually observable changes, for example, precipitate formation, haze, colour change and gas production. Sub-visual physical compatibility has been assessed by 52 (45%) studies, using techniques such as turbidity measurement and microscopic evaluation. Measurement of pH was carried out by 49 (42%) studies, 114 (98%) clearly defined the acceptance criteria for physical compatibility evaluation. More than one assessor involved in visual observation, in 13 (11%) studies.

Of the 33 studies which evaluated chemical compatibility, 30 (91%) studies either described or referenced the analytical method used for chemical testing. Method

validation was reported or referenced in 27 (82%) studies; 23 (70%) studies provided quality assurance (QA) data. All 33 studies defined acceptance criteria for chemical compatibility.

Table 2.5 Quality assessment for physical and chemical compatibility study components

Quality criterion evaluated	Number of studies complied (from a total of 118 studies)
Drug manufacturers listed	108
Drug batches listed	106
Number and frequency of observations defined	107
Study duration (exposure time) defined	112
Study conditions i.e. temperature, defined	104
Mixing vessels mentioned (tubes/ containers/ Y-tubing)	109
Drug concentrations defined	118
Drug diluents reported for all drugs studied	104
Sampling times defined (or completion of infusion if actual Y-site simulation conducted)	110
Base line level testing (t=0) performed	98
Parallel controls used	58
Testing performed in replicates	88
Mixing ratios (or infusion rates if actual Y-site simulation conducted) defined	112
Study provides implications for future research and clinical practice	118
Physical compatibility testing quality criterion	**Number of studies complied (from a total of 116 studies)**
Precipitate formation /haze/ colour change/ gas production evaluated visually	116
Sub-visual physical compatibility i.e. Turbidity measured instrumentally, microscopic evaluation	52
pH testing performed	49
Acceptance/compatibility criteria defined	114
Visual observation done by more than one assessor	13
Chemical compatibility testing quality criterion	**Number of studies complied (from a total of 33 studies)**
Analytical method described or referenced	30
Method validation is reported/ referenced (selective or stability-indicating)	27
Analytical method QA data provided (LOQ, accuracy, precision)	23
Acceptance/compatibility criteria defined	33

2.3 Discussion

The methodology utilized for the systematic review comprised the SPIDER systematic review model, a broad search strategy to capture over 27,000 deduplicated articles and screening via the machine learning tool, Research Screener, to expedite the extraction of eligible articles for a pharmaceutical science systematic review. The literature search and screening process were tested using a pilot study and assessment of inter-reviewer reliability.

It was determined in the pilot study that the search strategy required several generic terms, such as “stability,” “compatib*,” “intravenous*,” and “injection*” (Table 2.1) to ensure that all eligible reports were captured. It was concluded that this requirement to include common terms may be a broader issue for systematic reviews in pharmaceutical sciences and other scientific disciplines. Hence, the iterative process of the pilot study was an important evaluation step in developing the systematic review, to maximize the capture of relevant references, and this course of action is highly encouraged. The value of machine learning screening tools is that large databases from search strategies can be efficiently managed to extract articles for full-text review.

The pilot study indicated that 7.3% (119/1622) of the captured articles could be relevant to the systematic review, which was comparable to 7.5% in a previous study [32], and therefore suggested approximately 2000 articles would be identified as potentially eligible for the systematic review. However, the proportion of articles selected for full-text review was lower than predicted from the pilot study and appeared to be related to at least two factors.

Firstly, many of the selected articles included several drugs from concept 3 of the search strategy (Table 2.1), thus limiting the overall pool of eligible studies. Second,

in retrospect, the pilot study included some IV drugs which are more commonly used in neonatal/paediatric settings than in adult patients, or for which there is a limited body of relevant, published literature (e.g., caffeine, pentoxifylline, indometacin, and sotalol).

It was noted (anecdotally) that some terms, such as stability and IV, are used in a wide range of contexts and a number of abstracts were easily and swiftly excluded. Importantly, due to the machine learning algorithms and user-friendly operation of Research Screener, the overall workload impact in the screening process was modest. Further investigation of the reasons behind the relatively low selection rate from the initial pool of articles was outside the scope and value of the present study, as the goal was to optimize capture of eligible papers.

There was an appreciable time saving associated with Research Screener. Recent reports indicate the time to screen abstracts for systematic reviews ranges from 30 to 60 seconds per abstract and varies according to the experience of the reviewer [194, 197, 272, 273]. In the present study, the two reviewers noted that screening the cycles with a rich source of eligible papers was more time consuming than the latter cycles (after cycle 20), where most abstracts could be rapidly excluded. As a result of the Research Screener ranking and screening process, whereby the average title/abstract screening time from the final 20% of cycles for the two reviewers was 8.4 and 15.2 seconds, respectively, the overall time saving was at least 56 and 98 hours, respectively, if screening the results of the full search strategy was necessary.

One limitation of Research Screener and similar tools is the preclusion of papers which do not contain an abstract. In the present systematic review, 1269 such references were required to be manually screened; however, there was moderate inter-reviewer

agreement, and this was an important pool of articles in the present study, contributing approximately half of the final body of literature for the systematic review.

Overall, the importance of testing the systematic review search strategy process and optimizing the literature captured was well demonstrated. Semi-automated machine learning tools such as Research Screener may then be utilized to efficiently screen the results of the search strategy, providing a manageable workload and confidence in the outcomes and scientific rigor of the systematic review.

In retrospect, the disadvantage of using English language as a limiter during data base searching was evident. There was a likelihood of key-articles pertaining to drug compatibility, published in languages other than English, being overlooked. One such example in the present review was the article published by Audet and colleagues [274], which was published in French.

Another unavoidable limitation is that, if the search data bases do not contain certain journals, articles of that journal may be overlooked. The article published by Mitchell and Gailey (1999) [275], pertaining to caffeine compatibility, was not retrieved by the search strategy of the present review, due to the journal being non-existent in the search databases.

Although the focus of most studies was not neonatal or paediatric setting, they were chosen for the systematic review as they contained compatibility information of drugs commonly used in NICU setting, usually at clinically relevant concentrations.

Amongst the key compatibility information reported in the selected studies, the paucity of combined physicochemical compatibility data was highlighted. Physical drug incompatibilities can lead to adverse outcomes to patients such as catheter obstruction, venous irritation, pulmonary and renal embolism, whereas chemical incompatibility

can potentially lead to therapeutic failure due to changes in drug concentration, and formation of toxic compounds [270]. The most crucial fact is that physical compatibility does not exclude the possibility of chemical incompatibility [276] and visual compatibility data cannot be extrapolated into chemical compatibility of medications [249]. Thus, combined physicochemical compatibility information is imperative for clinical decisions, however, according to the present review, it has been reported in <30% of published literature. Furthermore, the review revealed that a significant number of potential drug combinations do not have compatibility data available. This finding is consistent with the systematic review published by Kanji *et al*. [32], which concluded that the vast majority of compatibility studies are of physical compatibility and almost half of the potential combinations of ICU drugs have never been studied. This relative paucity of compatibility data may result in the use of additional venous access points for multiple drugs, presenting a possibility for infections, mechanical damage, and thrombotic complications. Administration of inappropriate drug combinations due to the absence of reliable compatibility information is reported in literature [277]. Clinicians should be aware of the differences in applicability of physical and chemical compatibility studies and chemical stability should not be assumed from physically compatible drug combinations. Indeed, it may be concluded from the present review that there is a significant need for chemical compatibility data for a wide range of drugs used in NICU settings (Figure 2.8).

An interesting finding of the present review is the trend in studies conducted to evaluate combined physical and chemical compatibility, which most likely reflect the better access to analytical techniques (Figure 2.6).

A considerable degree of heterogeneity with respect to the methodology of compatibility studies conducted, regarding components of preparation of samples prior to testing, temperature exposed, contact time, use of controls and baseline testing, pH testing, involvement of more than one assessor in observations, physical and chemical testing methods, was identified in the review.

Filtration of drug samples using syringe filters prior to testing has been conducted to reduce the background noise of particles [84, 248] , and to remove any potential glass debris from the solution in concerning drugs in glass ampoules [251, 252]. Medications that require reconstitution had also been filtered using 0.2 µm filters, immediately prior to use, to remove potential particles [109].

Reversing the order of mixing has been used to eliminate the sequence of mixing as the cause of any incompatibility. Some studies reported of identical results observed when the order of drug mixing was reversed [85, 110, 278], while another study reported the order of mixing affecting the compatibility of drugs [244]. However, in Y-site administration, an order of mixing does not exist, therefore, in case of incompatibility detection in one of the orders, the Y-site administration of the drug pair should be contraindicated [84, 240].

Although most of the studies have used static mixing to simulate Y-site administration conditions, a study which conducted both dynamic (actual Y-simulation) and static mixing clearly demonstrated a discordance between simulated and actual Y-site evaluation. Vancomycin 5 mg/mL in D5W combined with piperacillin-tazobactam 67.5 mg/mL in D5W via simulated Y-site infusion demonstrated no evidence of physical incompatibility, however, actual Y-site infusion of the 2 drugs at the same concentrations resulted in precipitation suggestive of physical incompatibility. Hence,

the importance of including actual Y-site evaluation as a component of future compatibility studies, was suggested [227]. Similarly, Humbert-Delaloye *et al.* [232], demonstrated that amiodarone, was seen to interact with the administration equipment, hence concluded that while it is more pragmatic and faster to carry out static assays, the dynamic tests have proved to be useful by better simulating the actual clinical administration condition of an ICU.

It was first reported in 1977, that the mixing of the IV fluid in an IV administration set with the secondary additive from the Y-site to the needle tip was found by a dye dilution technique to occur approximately in a 1:1 ratio [66]. Hence a single ratio of 1:1 had been used in most of the Y-site drug compatibility testing methodologies by researchers. However, there are studies which have used different ratios i.e., 1:9, 9:1, 1:4, 4:1 to simulate cases where one of the two drugs is administered faster than the other and will thus reach the Y-site tubing at higher or lower concentrations [223, 232, 237, 269, 270].

The use of control samples is important in the compatibility testing methods to arrive at a comparative evaluation of physicochemical compatibility. Physical compatibility of the test samples could be determined by checking the visual changes compared to the control samples. For chemical compatibility testing, drug concentrations in the test samples (drug combinations) could be compared with that of the control solutions. Baseline (time zero) testing is carried out in studies to determine the changes in physical and chemical compatibility parameters, with time, since the point of mixing.

Although different contact times have been used for testing compatibility, conclusions are made that even in the case of long infusion lines combined with low flow rates and a manifold located distant to the patient, a contact time of more than 8 hours will not

be achieved [223]. Similarly, AlSalman *et al*. [94] concluded that modelling of contact times between drugs at minimal flow rates (0.1 mL/hr) and maximum combined dead-space of tubing, determined maximum contact time to be 8 hours, however, their testing was carried out over 24 hours. A study period of 60 minutes has been justified as a plausible maximum contact time that two drug solutions would be in IV tubing from the Y-site to the tip of a cannula (e.g., the volume of 50 cm of 1 mm internal diameter tubing is approximately 400 µL) [109]. Longer contact times of multiple hours to days will be practically difficult to perform in research settings and the study duration should be chosen based on the clinical application, i.e. if drugs are clinically administered through a Y-site, for a few minutes, mixing drugs for multiple hours and days during the compatibility study is unnecessary. However, extending the time of the study builds up more knowledge for clinical practice which may allow for extensions to IV run times within clinical guidelines, particularly for treatment in extremely low birth weight babies.

Temperatures higher than ambient/room temperature have been used in studies to mimic the environment in the temperature-controlled incubators (humidicribs) in NICU's [94]. However, as most of the Y-site tubing length does not exist in incubator temperature, and incubators do not have a fixed temperature, the use of higher temperatures in IV compatibility testing is uncommon, and the specific requirements and experimental conditions remain unclear.

Kanji *et al*. [32] identified in their review of physicochemical drug compatibility studies, that pH measurement has been inconsistently applied and clinical significance of a pH change over time is unclear. Further, if thresholds were defined, there was no clinical or biochemical justification. Similarly, the present review indicates that pH testing has been used both as physical and chemical compatibility determinant, and

varying threshold limits have been defined to determine physical or chemical compatibility (i.e. pH unit change 0.1 units to 2 units). As changes in pH can contribute to precipitation as well as the presence of chemical reaction [53], and as it further affects drug solubility [237], pH testing could be of value to be used as a complementary test to the main physical and chemical compatibility testing.

Although human resource is a demanding factor in research, the presence of two or more evaluators to perform visual observations of samples has aided in the reduction of potential bias in visual observation [102].

Many of the physical compatibility studies were reported more than two decades ago and several drugs are currently produced by a range of manufacturers. Furthermore, there is a variety of formulations (e.g. solutions, lyophilised powders) available for some drugs (e.g. aciclovir). Different excipients present in different formulations may behave differently during compatibility studies and therefore, formulation details should be an important inclusion of the study.

Given the reported compatibility studies are vastly heterogeneous in terms of methodology, the present review emphasises the importance of methodologic integrity in future compatibility studies, particularly with respect to the NICU setting. Further it is recommended that future studies incorporate items in the quality assessment tool provided such as incorporating multiple reviewers in the observation. This review complements the findings of previous reviews by reporting compatibility information for a vast range of crucial NICU drugs including sildenafil and caffeine, which were not previously reviewed for compatibility data [32].

Although compatibility summary charts are used as information tools to aid clinical decisions in co-administration of drugs at the clinical setting, their utility may be

limited as it does not provide the conditions, for example concentrations and duration of exposure pertaining to the compatibility. Furthermore, a single chart would not be helpful to provide both physical and chemical compatibility information.

Overall, the present systematic review highlighted the strong need of conducting combined physicochemical compatibility studies as the knowledge pertaining to chemical compatibility is limited. Furthermore, given the high clinical relevance, the review warranted future physicochemical compatibility studies of NICU drugs such as sildenafil and caffeine in neonatal Y-site administration conditions.

Chapter 3

Physicochemical compatibility of sildenafil injection with parenteral medications used in neonatal intensive care settings

3.1 Introduction and background

3.1.1 Pharmacology of sildenafil

The nitric oxide/ cyclic guanosine monophosphate (NO/cGMP) signalling pathway mediates relaxation of vascular smooth muscle in pulmonary vasodilatation (continuously) and penile erection. Smooth muscle relaxation is partly mediated via protein kinase G (PKG) activation, subsequent potassium channel opening and reductions in intracellular calcium levels (Figure 3.1) [279].

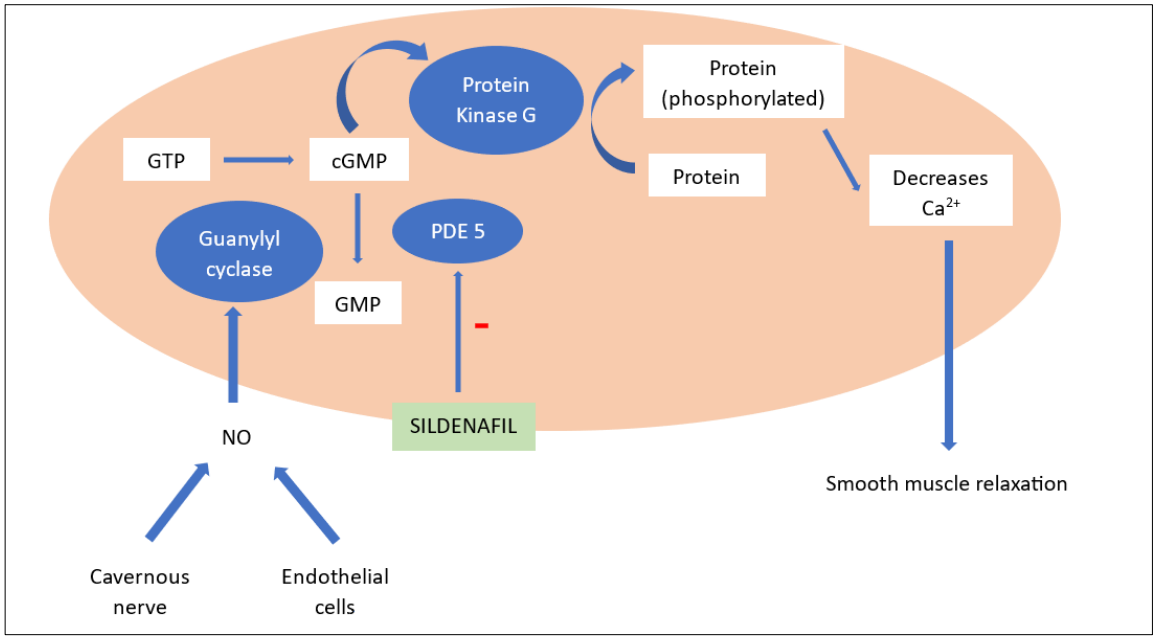


Figure 3.1 Nitric oxide/ cyclic Guanosine monophosphate signalling pathway, illustrating the role of cGMP in decreasing intracellular Ca^{2+} levels and subsequent smooth muscle relaxation

Cyclic nucleotides (i.e., cGMP) are degraded by intracellular phosphodiesterase (PDEs). Five subtypes of PDEs were identified in the mid-1980s, of which, phosphodiesterase type 5 (PDE5), exclusively catalyses the breakdown of cGMP. PDE5 is present in the smooth muscle of the systemic vasculature, and in platelets. In 1986, novel pyrazolopyrimidines were synthesized that were highly potent inhibitors of PDE5 [280]. One compound designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4 methylpiperazine, known as sildenafil, and it's citrate salt (Figure 3.2) is marketed under the trade name Viagra® [280]. The molecular weight of the citrate salt is 666.7 and the base form is 474.6. Sildenafil is an amphoteric molecule with two pKa values at 9.84 (NH-piperazine ring) and 7.10 (NH-amide at pyrazolopyrimidine ring) [281]. Sildenafil demonstrated good potency and excellent selectivity for PDE5 [282].

Sildenafil selectively inhibits cGMP specific PDE5, the enzyme which catalyses hydrolysis of cGMP. This inhibition causes elevation of cellular cGMP, which subsequently decreases intracellular calcium levels, thus leading to smooth muscle relaxation [280, 283].

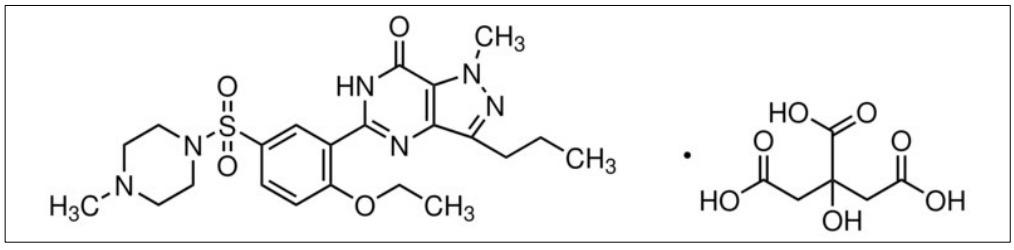


Figure 3.2 Sildenafil citrate chemical formula; molecular weight of citrate salt=666.7; molecular weight of base=474.6

Sildenafil (as Viagra®) was first approved for treatment of erectile dysfunction in 1998 by both the USFDA and the European Medicines Evaluation Agency. Thereafter, other clinical indications of sildenafil emerged. Scientifically supportive findings, for

example, PDE5 gene upregulation in pulmonary hypertension (PH) [284] and PDE5 inhibitors ameliorating pulmonary hypertension in experimental models [285-289], led to a series of preclinical investigations of sildenafil's potential in the management of pulmonary vascular disease.

The placebo-controlled study by Pfizer conducted between 1998 and 2000 demonstrated that IV sildenafil selectively reduced pulmonary pressure and pulmonary vascular resistance in patients with pulmonary arterial hypertension, pulmonary venous hypertension, and pulmonary hypoxic hypertension [280]. It was during this period that significant attention was directed towards the use of sildenafil in pulmonary hypertension.

3.1.2 Effects of sildenafil in pulmonary hypertension

Pulmonary hypertension is a haemodynamic state resulting in a progressive increase in the mean pulmonary artery pressure (≥25mmHg at rest or ≥30mmHg with exercise) [290]. According to the World Health Organization's classification, pulmonary arterial hypertension (PAH) is a subtype of PH with a pulmonary capillary wedge pressure ≤15mmHg and by pulmonary vascular resistance >3 Wood units. PAH can be idiopathic, familial, or secondary to a variety of conditions such as connective tissue disease, haemoglobinopathies, or human immunodeficiency virus infection [291].

Vascular constriction, thrombosis and remodelling of pulmonary arteries are thought to arise due to endothelial dysfunction caused by an imbalance of endogenous vasodilators (i.e. NO) and vasoconstrictors (i.e., endothelin 1) [292, 293]. PDE5 is found in high concentrations in pulmonary arteries. As described above (Figure 3.1), endothelium-derived NO stimulates intracellular guanylate cyclase, increasing cGMP

levels, and leading to smooth muscle relaxation. Sildenafil, by inhibiting PDE5, inhibits breakdown of cGMP and prolongs its action [294].

Trials of sildenafil have demonstrated its ability to cause rapid vasodilatation, resulting in improved haemodynamics [295]. It significantly decreases mean pulmonary arterial pressure and pulmonary vascular resistance with minimal or no effect on mean arterial pressure and improves cardiac output. Sildenafil has demonstrated comparable haemodynamic effects as other PH treatment modalities e.g. inhaled NO, iloprost aerosol [295].

3.1.3 Pulmonary hypertension in the newborn and the use of sildenafil as treatment

Persistent pulmonary hypertension of the newborn (PPHN) is life-threatening and results from poor haemodynamic and respiratory transition to extrauterine life [296]. It is characterized by an increase in pulmonary vascular resistance, right-to-left shunt, and severe hypoxemia [296]. The most common treatment option for PPHN is inhaled nitric oxide [297]. However, NO is an expensive treatment modality and as a considerable proportion (50%) of infants with PPHN do not respond to NO therapy, clinicians opt for alternative treatment options such as PDE5 inhibitors (e.g. sildenafil) [297]. Upon successful use of sildenafil for treatment of adult PH, research interest for the use of sildenafil in paediatric populations subsequently grew. As a result, sildenafil is now an established alternative to NO as a treatment option for PH in infants and children, despite its off-label use [298, 299], and has been shown to significantly increase oxygenation and reduce mortality, with no clinically important side effects, when administered in PPHN [297].

Sildenafil is available as an oral tablet and a suspension. However, drug bioavailability upon oral absorption is only 40% [300] due to hepatic clearance (demethylation) by cytochrome P450 enzymes 2C9 and 3A4 [301]. Conversely, CYP-mediated metabolism is immature in neonates, hence, there is a higher risk of toxicity [298] due to a prolonged exposure to high drug concentrations. Further, the oral route is undesirable in critically ill neonates with very unpredictable gastrointestinal absorption, making the IV administration of sildenafil preferable [302].

3.1.4 Administration of IV sildenafil in NICU settings

The conventional treatment regimen of IV sildenafil for PPHN is a loading dose of 0.4 mg/kg administered over 3 hours, followed by a continuous infusion of 1.6 mg/kg/day for up to 7 days, with typical sildenafil concentrations in the order of 400-800 µg/mL in D5W injection [303]. In preterm infants, a lower loading dose of 0.1 mg/kg administered over 45 minutes and continuous infusion of 0.5 to 1.2 mg/kg/day is recommended, using sildenafil concentrations in the order of 60-100 µg/mL in D5W injection [304].

3.1.5 Development of assays to evaluate stability and compatibility of sildenafil formulations

Provenza *et al*. [305] conducted physicochemical stability studies of two paediatric liquid oral formulations of sildenafil designed for treatment of PPHN. Physicochemical stability parameters, namely appearance, pH, particle size, rheological behaviour and drug content of formulations, were evaluated at three different temperatures for 90 days. Results concluded that one formulation was physicochemically and microbiologically stable for 90 days at 4°C and 25°C, however, at 40°C drug content remained within the acceptable limits for only 60 days. The

alternative formulation was stable for 30 days at 25°C and 40°C. At 4°C, the active drug content remained within acceptable limits (>90%) for less than 15 days. Along with this reduction, a non redispersible sediment was visible at 4°C, suggesting a reduction in sildenafil solubility at low temperature. The pH and the rheological behaviour remained constant in both formulations. Sildenafil concentrations were determined using a UV/visible spectrophotometric method that was conducted at room temperature. The stability of sildenafil was confirmed in all formulated dosage forms [305].

The study of Daraghmeh *et al.* [306], aimed at developing and validating a HPLC method for the assay of sildenafil citrate and degradant products in tablet formulation. The HPLC system comprised a C_{18} column, a mobile phase of ammonium acetate (pH 7.0, 0.2 M) and acetonitrile (1:1 volume ratio) and a flow rate of 1 mL/min. The UV detector was set at 240 nm. The chromatographic method showed good separation of sildenafil and other related substances. Similarly, a validated, stability-indicating HPLC method was developed by Dinesh *et al.* [307], for the quantitation of sildenafil citrate in pure form and in pharmaceutical samples. The HPLC method comprised a C_{18} column, and a mobile phase of water and acetonitrile (48:52 volume ratio). The mobile phase flow rate was 1 mL/min, and the UV detection was set at 245 nm.

Fejos and colleagues [308] developed a validated HPLC method for quantitative screening of sildenafil, vardenafil, tadalafil and their designer analogs. The method comprised a C_{18} column maintained at 25°C, and a gradient elution. One mobile phase (A) was a 200 mM ammonium acetate solution. The other mobile phase (B) consisted of a mixture of equal volumes of methanol and acetonitrile. The gradient program of the method was: 0-9 min 40-50% B; 9-17 min 50-80% B; 17-20 min 80% B; 20-20.5

min 80-40% B; 20.5-25 min 40% B. The flow rate of the mobile phase was 0.5 mL/min, and the detection of wavelength was 290 nm.

Hashem *et al.* [309], established and validated a rapid HPLC method for simultaneous determination of sildenafil citrate, tadalafil, and apomorphine hydrochloride. The method was composed of a calixarene column, and a binary mobile phase of 35% acetonitrile and 65% 50 mM sodium perchlorate (pH 2.5). The mobile phase flow rate was 1 mL/min.

An ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry method was developed and validated by Shi *et al*. [310], for screening, and quantitation of anti-impotence compounds in dietary supplements. The chromatographic separation was performed using a C_{18} column and a binary mobile phase. One mobile phase (A) was 0.1% formic acid aqueous solution, and the other (B) was acetonitrile. The gradient elution programmed was: 0.0-13.0 min (A: 80-60%, B: 20-40%), 13.0-17.0 min (A: 60-20%, B: 40-80%), 17.0-17.5 min (A: 20-10%, B: 80-90%), 17.5-22.0 min (A: 10%, B: 90%), 22.0-22.5 min (A: 10-80%, B: 90-20%), 22.5-26.0 min (A: 80%, B: 20%).

Atipairin *et al.* [311], have developed and validated a HPLC method for analysis of sildenafil citrate in an extemporaneous preparation. The chromatographic conditions used were a C_{18} column with a mobile phase consisting of 50% 0.2 M ammonium acetate buffer (pH 7.0) and 50% acetonitrile. The flow rate used was 1.0 mL/min, and UV detection wavelength was 245 nm. The proposed method was found to be accurate, reliable and stability-indicating.

Due to the unavailability of information on how sildenafil dilutions for continuous IV administration should be prepared and stored, specifically in paediatric settings, Al

Hadithy *et al.* [312], studied the stability of two sildenafil dilutions (0.067 and 0.667 mg/mL) in D5W (diluent of choice in most NICUs). Both dilutions were stored in polypropylene syringes at ambient room temperature (20-25°C) and at 37°C (laboratory incubator) for 24 hours and 7 days. The concentration of sildenafil in both dilutions were determined using a validated HPLC method comprising a C_{18} column and a mobile phase of acetonitrile and ammonium acetate 10 mM (50:50 volume ratio). The diode array detector of the HPLC system was set at 292 nm. Sildenafil concentrations in both dilutions at the end of the incubation periods (1 and 7 days) suggested no marked degradation at the two temperatures studied. All measured concentrations were higher than 95.4% of the original concentration. The peak-purity index was 1.0 in all measurements, confirming the absence of degradation products. These findings have proven the chemical stability of the sildenafil in D5W solutions studied, for up to 1 week at room temperature and at 37°C.

Overall, these studies confirm the stability of sildenafil in a variety of pharmaceutical and dietary products including oral and IV paediatric formulations. Furthermore, HPLC techniques are commonly used to quantify sildenafil and related products in these formulations. C_{18} columns and mobile phases comprising acetate buffers and acetonitrile are commonly incorporated in these HPLC assays. Both isocratic and gradient methods have been used in chromatographic systems with varying UV detection wavelengths.

Overall, this series of research studies confirm;

i) The stability of sildenafil in a variety of pharmaceutical and dietary products including oral and IV paediatric formulations.

ii) HPLC assays are the most commonly used method to quantify sildenafil and related products in these formulations.

iii) C_{18} columns and mobile phases comprising acetate buffers and acetonitrile are commonly incorporated in sildenafil stability assays.

iv) Both isocratic and gradient methods have been used in chromatographic systems with varying UV detection wavelengths.

3.1.6 Investigations of the physicochemical compatibility of sildenafil with commonly used NICU drugs

Physicochemical compatibility of IV sildenafil has been reported in literature for a limited number of drugs. The study of AlSalman *et al*. [94], has established the physical and chemical compatibility of sildenafil with commonly administered infusions in the paediatric and neonatal intensive care setting. This study evaluated the chemical and physical compatibility of binary and multiple combinations of sildenafil (800 µg/mL) with adrenaline (60 µg/mL), noradrenaline (60 µg/mL), milrinone (200 µg/mL), vasopressin (0.4 units/mL) and heparin (100 units/mL). These were tested using three diluents (NS, D5W and D10W). Prior to physicochemical testing of drug combinations, HPLC methods were developed to quantify each selected drug. The chromatographic separation of sildenafil was achieved using a Kinetex 5 µm C_{18} 100 Å (150×4.6 mm) column, a mobile phase of acetonitrile and ammonium acetate buffer (pH 7) (1:1 volume ratio), a flow rate of 1 mL/min and a 20 µL injection volume. The detection wavelength of sildenafil was 280 nm. Individual HPLC assays were

developed to quantify the concentrations of secondary test drugs in the mixtures. Binary and multiple drug mixtures of sildenafil were examined, combining sildenafil with the secondary drug solution to be tested in a 1:1 volume ratio for a contact time of 24 hours. Visual inspection for precipitation, particulate matter, haze, gas formation and change in colour was performed to evaluate physical compatibility of the drug combinations. To complement the visual inspection, turbidity measurements were performed for physical compatibility testing. Chemical compatibility was evaluated by taking pH measurements and performing drug concentration evaluation using the developed HPLC methods. Incompatibility in this study was defined as a change in mean pH by >0.5 units from the initial pH value, or a change in concentration >10% from initial concentration, as per ICH guidelines [161]. All binary or multi drug combinations containing heparin were deemed incompatible. Of those drug combinations not containing heparin, all were deemed compatible apart from the five-drug mix of sildenafil, milrinone, vasopressin, noradrenaline, adrenaline at 37°C, in D10W.

The stability of sildenafil in combination with heparin and dopamine was studied by Luu *et al.* [106], using a stability indicating HPLC assay. The chromatographic separation was performed using a C_{18} column, an isocratic mobile phase of 60% of 0.2 M ammonium acetate (pH 6.8) and 40% acetonitrile at a flow rate of 0.2 mL/min. The column temperature was 40°C. The method was applied to the investigation of sildenafil alone, sildenafil with heparin, sildenafil with dopamine, and sildenafil with heparin and with dopamine, all in D5W injection at room temperature and under refrigeration for 30 days. The mixing ratio was not 1:1 as in AlSalman *et al.* [94], however, the final concentrations of sildenafil, heparin and dopamine in the binary admixtures were 400 µg/mL, 1 unit/mL and 1.6 mg/mL, respectively. The study

concluded that sildenafil prepared in D5W injection alone, with heparin, and with dopamine retained over 90% potency after 30 days of storage at room temperature and under refrigeration. The triple combination of sildenafil, heparin and dopamine had a potency of <90% after 3 days of storage at room temperature and 21 days of storage under refrigeration. The study was undertaken to support the clinical decision of considering the likelihood of IV sildenafil being administered with other common medications (heparin and dopamine) in patients undergoing treatment for PAH.

Against this background, the objective of the present study was to investigate the physicochemical compatibility of sildenafil with a range of NICU drugs, at higher end concentrations, clinically relevant concentrations, and with a selection of 2-in-1 PN solutions.

3.2 Materials and methods

Sildenafil (sildenafil citrate; $C_{22}H_{30}N_6O_4S.C_6H_8O_7$; MW 666.7; Certified Reference Material), was purchased from Sigma-Aldrich Chemicals, St Louis, MO, USA. HPLC grade acetonitrile was from Fisher Scientific, Fair Lawn, NJ, USA. All other laboratory chemicals were of analytical grade. All parenteral medications and solutions were of clinical grade (see Table 3.1 for the list of medications and manufacturers). The composition of the 2-in-1 PN solutions is provided in Table 3.2.

Table 3.1 Manufacturers/ suppliers of injectable products used for compatibility studies

Injectable drug	Manufacturer/ supplier	Lot No
Aciclovir	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	FT1848AA
Alprostadil	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	FD6386
Amoxicillin	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	IC02KH
Amphotericin B - Fungizone	Neon Healthcare Ltd, Mill Studio Business Centre, Ware, UK	28631TB21
Amphotericin B - Liposomal	Gilead Sciences Pty Ltd, St Kilda Road, Melbourne, VIC, Australia	028780
Ampicillin	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	0P03AY
Benzylpenicillin	Seqirus (Australia) Pty Ltd, Melbourne, VIC, Australia	KT4974
Caffeine base*	Perth Children's Hospital, Nedlands, WA, Australia	6018
Caffeine citrate	Phebra Pty Ltd, Lane Cove West, NSW, Australia	14806
Calcium gluconate	Phebra Pty Ltd, Lane Cove West, NSW, Australia	14688
Cefotaxime	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	G105HA0
Ciprofloxacin	Aspen Pharmacare Australia Pty Ltd, St Leonards, NSW, Australia	10597
Clonidine	Medicianz Healthcare Pty Ltd, Melbourne, VIC, Australia	CLA032
Cloxacillin	Xion Pharmaceuticals Pty Ltd, Pune, India	101
Dexmedetomidine	Accord Healthcare Pty Ltd, Melbourne, VIC, Australia	M2110403
Dobutamine	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	FY3034AA
Dopamine	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	A21F3R
Epinephrine	Aspen Pharmacare Australia Pty Ltd, St Leonards, NSW, Australia	AS116A1
Fentanyl citrate	Piramal Critical Care Pty Ltd, Chatswood, NSW, Australia	HC5M
Flucloxacillin	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	0X18HY
Fluconazole	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	B560704
Furosemide	Baxter Health care Pty Ltd, Old Toongabbie, NSW, Australia	B5E0004A
Gentamicin	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	B785
Heparin	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	159049A
Hydrocortisone	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	ER8089
Ibuprofen	Seqirus (Australia) Pty Ltd, Melbourne, VIC, Australia	10098R
Ibuprofen lysine	Prasco Laboratories, Commerce Ct, Mason, United States	B215329
Indometacin	Promedica SRL, Via Palermo, Parma, Italy	22906
Insulin (Actrapid®)	Novo Nordisk Pharmaceuticals Pty Ltd, Baulkham Hills, NSW, Australia	LR79K53
Levetiracetam	Apotex Pty Ltd, Macquarie Park, NSW, Australia	275447
Linezolid	Fresenius Kabi Australia Pty Ltd, Mount Kuring-gai, NSW, Australia	15PDE270
Meropenem	Sun Pharma ANZ Pty Ltd, Macquarie Park, NSW, Australia	DFC2162A
Metronidazole	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	10221
Midazolam	Pharmaco (Australia) Ltd, Gordon, NSW, Australia	F0021F01
Milrinone	Sanofi-Aventis Australia Pty Ltd, Macquarie Park, NSW, Australia	J0496
Morphine hydrochloride	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	A22A18
Morphine sulfate	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	212004
Norepinephrine	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	208599
Paracetamol	B.Braun Australia Pty Ltd, Bella Vista, NSW, Australia	21436451

Injectable drug	Manufacturer/ supplier	Lot No
Phenobarbitone	Aspen Pharmacare Australia Pty Ltd, St Leonards, NSW, Australia	042344
Piperacillin/tazobactam	Sandoz Pty Ltd, Macquarie Park, NSW, Australia	LN1377
Rifampicin	Sanofi-Aventis Australia Pty Ltd, Macquarie Park, NSW, Australia	0J6511
Sildenafil	Viatris Australia Pty Ltd, Sydney, NSW, Australia	B710506
Sodium bicarbonate	Phebra Pty Ltd, Lane Cove West, NSW, Australia	14924a
Vancomycin	Hospira Australia Pty Ltd, Mulgrave, VIC, Australia	J016913AA
Vecuronium	Sun Pharma ANZ Pty Ltd, Macquarie Park, NSW, Australia	HAC2372A

*Caffeine base 10 mg/mL injection comprises caffeine, sodium chloride, hydrochloride acid and Water for Injection; the injection is isotonic and has a pH approximately 4.2 (AUSPMAN/ Perth Children's Hospital)

Table 3.2 Composition of the 2-in-1 PN solutions, manufactured at King Edward Memorial Hospital

	PN 1 Preterm A	PN 2 Preterm B	PN 3 Term	PN 4 Custom 1	PN 5 Custom 2	PN 6 Custom 3
Amino acids (Primine), g/100mL	2.98	2.98	2.3	0.5	3.5	2.3
Glucose, g/100mL	4.96	7.94	12	2	14	8
Sodium, mmol/100mL	3.97	3.97	4	4	4	4
Potassium, mmol/100mL	1.99	1.99	2	2	2	2
Calcium, mmol/100mL	1.49	1.49	0.9	1.5	1.5	1.5
Magnesium, mmol/100mL	0.25	0.25	0.25	0.25	0.25	0.25
Phosphate, mmol/100mL	1.49	1.49	0.9	0.5	1.5	1.5
Chloride, mmol/100mL	2	2	2.54	1.8	2.08	1.97
Acetate, mmol/100mL	1.99	1.99	2.6	1.79	2.08	1.96
Heparin, units/100mL	49.63	49.63	50	50	50	50
Trace elements, mL/100mL	0.73	0.73	0.74	0.74	0.74	0.74

3.2.1 Stability-indicating HPLC assay development and validation

The Agilent 1200 series HPLC system comprised a binary pump with degasser, auto-sampler, thermostated column oven and a dual wavelength UV detector (Agilent Technology, Waldbronn, Germany). Chemstation software (vRev. B.03.01.SR1; Agilent Technology) was used to acquire and process data.

A reversed phase HPLC column (Kinetex, 5µm, C_{18}; 100 × 4.6 mm; Phenomenex, USA) was maintained at 30°C. The mobile phase was an isocratic mixture of 40% v/v acetonitrile and 60% v/v 50 mM potassium dihydrogen orthophosphate buffer (pH 6; HI 5221 pH Meter, Hanna Instruments, Rhode Island, USA). The flow rate and UV detector were 1 mL/min and 240 nm respectively. The injection volume was 5 µL, unless otherwise specified.

The stability indicating HPLC method development was guided by previous studies [94, 106, 311], however, based on previous laboratory experience it was decided to use a phosphate buffer to achieve stable chromatography. The method was validated in accordance with the ICH guidelines on validation of analytical procedures [179]. Validation characteristics selected for investigation were specificity, linearity and range, accuracy, precision, and robustness.

Specificity is the ability to detect the analyte in the presence of other components e.g., impurities, degradants, and matrix. Sildenafil 600 µg/mL was prepared by diluting sildenafil injection (Revatio; Viatris, Australia; Table 3.1) with D5W and exposed to forced degradation experiments with oxidative, acidic, alkali, heat and light stress conditions, to demonstrate the selectivity for sildenafil in the presence of other degradants.

Oxidative stress: Sildenafil 600 µg/mL was mixed 1:1 with 20% v/v hydrogen peroxide (2 mL volume in 4 mL glass vials with impermeable caps, n=3), and stored in a stability chamber at 45°C (Fitoclima 600, Aralab, Rio de Mouro, Portugal). Samples (300 µL) were withdrawn at 0, 1, 2, 4 and 7 days and immediately frozen (-80°C) to arrest further degradation, until assayed. At the time of assay, samples were

thawed at ambient temperature (22°C), vortex mixed, diluted 1-in-50 with water, then analysed by HPLC as described above (injection volume 20 µL).

Acid stress: Sildenafil 600 µg/mL was mixed with 4 M hydrochloric acid (1:1 v/v; 2 mL in 4 mL glass vials with impermeable caps, n=3), and stored at 45°C. Samples (300 µL) were withdrawn at 0, 1, 2, 4 and 7 days, neutralised with 4 M sodium hydroxide solution and immediately frozen (-80°C). At the time of assay, samples were thawed at ambient temperature (22°C), vortex mixed, diluted 1-in-50 with water, then analysed by HPLC as described above (injection volume 20 µL).

Alkali stress: A similar process as described above for acid stress was followed, using 4 M sodium hydroxide solution, and neutralisation with 4 M hydrochloric acid.

Heat stress: Sildenafil 600 µg/mL was mixed with water (1:1 v/v; 2 mL in 4 mL glass vials with impermeable caps, n=3), and stored at 60°C (PURA 4 water bath, Julabo GmbH, Seelbach, Germany). Samples (500 µL) were withdrawn at 0 and 3 days, and immediately frozen (-80°C). At the time of assay, samples were thawed at ambient temperature (22°C), vortex mixed and analysed by HPLC as described above (injection volume 5 µL).

Light stress: Sildenafil 600 µg/mL was mixed with water (1:1 v/v; 2 mL in 4 mL glass vials with impermeable caps, n=3), and exposed to light (laboratory fluorescent lighting 24/7 and normal daylight (indirect sunlight) for approximately 12 hours per day) at room temperature (22°C). Samples (500 µL) were withdrawn at 0 and 7 days, and frozen (-80°C). At the time of assay, samples were thawed, vortex mixed and analysed by HPLC as described above (injection volume 5 µL).

To establish linearity and range for the HPLC assay, a calibration curve was constructed using sildenafil solutions at concentrations of 3, 10, 30, 100, 300 and 800

µg/mL (n=3). Calibration curve and analyte concentration data were analysed using Microsoft Excel (Version 2309 Build 16.0.16827.20166). The LOD and LLOQ were estimated as described in Chapter 1 (Section 1.2.4.3), where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve [179]. LLOQ was confirmed by precision data.

Accuracy and precision of the HPLC assay was evaluated at sildenafil concentrations of 600, 100, 10 and 2.9 (LLOQ) µg/mL (n=5) using the sildenafil reference standard and the commercial sildenafil injection diluted with D5W. The concentrations of the two series were compared (expressed as a fraction of the nominal concentration). Intra-assay and inter-assay precision were determined by calculating %RSD for the same sildenafil concentrations.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To evaluate robustness, sildenafil 100 µg/mL samples (from sildenafil standard and commercial injection; n=5) were tested using minor changes to the standard method. Changes with respect to standard method parameters included flow rate (0.8 mL/min) and mobile phase composition (acetonitrile: buffer ratio 45:55). The accuracy of the modified methods was compared with the standard method.

3.2.2 Preparation of samples for physical and chemical compatibility testing

Sildenafil injection (800 µg/mL) was diluted using D5W to achieve clinically relevant concentrations of 60 and 600 µg/mL. The higher sildenafil concentration is consistent with a ‘high-end’ dosage regimen for infants ≥37 weeks gestational age, and the lower sildenafil concentration is consistent with a ‘low-end’ dosage regimen for pre-term

infants <37 weeks gestational age [39]. Secondary test drugs and 2-in-1 PN solutions were prepared/diluted in accordance with the manufacturer's instructions or standard local neonatal clinical protocols at King Edward Memorial Hospital (KEMH) [39]. Drug concentrations were based on the recommendations for a patient weighing 2 kg. Medications that were originally contained in glass ampoules or required reconstitution were filtered immediately prior to mixing (33 mm × 0.22 µm Polyethersulfone (PES) membrane, Millex GP, Merck Millipore Ltd, Carrigtwohill, Co. Cork, Ireland).

A panel of 45 drugs and 6 PN were selected and endorsed by clinical experts from KEMH. Five drugs were included in the study as positive (compatible; epinephrine, norepinephrine, milrinone and dopamine) and negative (incompatible; heparin) controls, and the remaining 40 drugs were previously untested against sildenafil. Epinephrine, norepinephrine, milrinone and dopamine were tested in the present study at different concentrations to previous reports [94, 106].

Drug combinations (sildenafil and the test drug or PN solution) were mixed at 1:1 (v/v) ratio, to simulate y-site administration, consistent with established methods [66, 94, 246, 254, 313]. Drug preparation, mixing and testing were carried out at room temperature (22°C).

The first stage of compatibility tests comprised a combination of sildenafil 600 µg/mL and the secondary drug at clinically relevant 'high-end' concentrations, consistent with the standard NICU protocols and expert advice. If incompatibility was detected, the drug combination was then tested using sildenafil 600 µg/mL and the secondary drug at a 'low-end' clinically relevant concentration, if applicable. The third and fourth stages of tests comprised sildenafil 60 µg/mL and the secondary drug at high- and low-

end concentrations, respectively, as applicable. The ‘up to four-way’ combination design optimised the scope for clinically relevant information on incompatible combinations.

Twelve 2 mL clear glass HPLC vials with impermeable screw cap lids were used for each binary combination of drugs/fluids and the respective control solutions. Sildenafil and secondary drug combinations, and the control samples were prepared as described below:

- Set 1 – Sildenafil injection solution (0.4 mL of 60 or 600 µg/mL) and secondary test drug solution/fluid (0.4 mL); n=4.
- Set 2 – Sildenafil injection solution (0.4 mL of 60 or 600 µg/mL) diluted with 0.4 mL of the diluent of the secondary test drug (n=4) as the reference control solution for the purpose of visual comparison and HPLC assay of sildenafil concentration. For PN solutions, the diluent was D5W.
- Set 3 – The test drug solution/fluid (0.4 mL) was diluted with 0.4 mL of D5W (n=4) for the purpose of visual comparison.

3.2.3 Physical compatibility testing

All vials were gently mixed and inspected with an unaided eye against a black and white background for any change in colour, haze, or precipitation. The observations were carried out at time 0 (immediately after mixing), 5, 15, 60 and 120 minutes. Samples were also observed under a polarized light viewer (Apollo I Liquid Viewer with a LED light source and 1.7 × Magnifier, Adelphi Manufacturing Company Ltd, United Kingdom) for any visible precipitation or particulate matter. Physical compatibility was based on the visual appearance of the drug combination (set 1) in comparison to control solutions (set 2 and 3). Any inconclusive observation was

confirmed by a second independent observer and all physical incompatibilities were photographed. If precipitation or particles were observed in the drug combination vials, an aliquot was examined under light microscopy (Leica MC190HD, 40 × magnification, Leica Microsystems (Switzerland) Ltd, CH – 9435, Heerbrugg, Switzerland).

3.2.4 Chemical compatibility testing

The HPLC assay was used to evaluate chemical compatibility if the combination was physically compatible. If any physical incompatibility was observed, such combinations were not chemically tested, to avoid contamination of the HPLC system. At 2 hours after mixing, the sildenafil concentration in the four vials of sildenafil plus test drug (set 1) was measured by HPLC and compared to the four sildenafil reference solution vials (set 2). The ratio of the mean peak areas was determined, and the 95% CI of the ratio was calculated using the confidence limits from a two-sided t-test (α = 0.05; SigmaPlot V.15; Inpixon GmbH, Düsseldorf, Germany). Consistent with previous studies, incompatibility of sildenafil:drug combinations was defined as a ratio of the mean peak area outside the range of 90-110% [94, 106, 223, 226, 314].

3.2.5 Physical compatibility testing of sildenafil with a lipid emulsion

The IV lipid emulsion tested was SMOFlipid 20% (Fresenius Kabi Australia Pty Ltd, North Ryde, NSW, Australia) which comprises soybean oil 6%, medium chain triglycerides 6%, olive oil 5% and fish oil 3%. The emulsion (0.5 mL) was combined with sildenafil injection (600 and 60 µg/mL prepared in D5W) separately, at 1:1 volume ratio, to simulate Y-site injection.

Mixing was carried out in 2 mL clear glass vials with screw cap lids, n=3, at room temperature (22°C). The vials were gently mixed and visually inspected at 0, 1 and 2 hours for phase separation, change in colour, gas production or other visually observable changes. The droplet diameter of the lipid emulsion and emulsion/ fluid mixtures was determined at 0 and 2 hours after mixing using the Mastersizer 3000 instrument (Malvern Instruments, Worcestershire, UK). Data capture included the volume-weighted MDD, the mass median diameter (MMD; Dv50 or d0.5) and the percentage of droplets in the following diameter bands: <0.01, 0.01-0.05, 0.05-0.1, 0.1-0.2, 0.2-0.5, 0.5-1, 1-5, 5-30 and >30 μm. The formal criterion for compatible lipid/drug mixtures was MDD <0.5 μm (n≥3) [53, 93, 122, 315]. Data were analysed using Microsoft Excel (Version 2309 Build 16.0.16827.20166) and expressed as mean ± SD unless otherwise indicated.

Glucose 5% w/v was mixed with lipid emulsion using the same experimental procedure as described above, as negative controls.

The Mastersizer's particle size analysing method parameters were adopted from previously reported method [316]. As the refractive index of soybean oil, medium chain triglycerides and olive oil is 1.47, 1.45 and 1.46, respectively, a refractive index of 1.46 and a density of 0.95 was used. The dispersant was deionised water, sonicated to eliminate air bubbles. The stirrer speed in the wet dispersion unit was 1000 rpm and the validated absorption index was 0.003.

3.2.6 Evaluation of absorption/adsorption loss of sildenafil by syringe filters

The compatibility of sildenafil injection with conventional syringe filters has not previously been reported, but is clinically relevant information, and was required for subsequent tests in the present study. Six types of syringe filters and two inline filters composed of different filter membranes (cellulose esters, nylon, polyvinylidene fluoride, polyethersulfone, polypropylene; Table 3.3) were tested to evaluate the absorption/adsorption loss of sildenafil during the process of filtration.

Table 3.3 Syringe filter types tested, the membrane and mesh size description.

Abbreviation	Description	Manufacturer/ Supplier
RC	Regenerated Cellulose, 15 mm diameter, 0.2 µm membrane, non-sterile	Phenomenex Australia Pty Ltd, 2 Chaplin Dr, Lane Cove West NSW 2066
NY	Nylon, 15 mm diameter, 0.2 µm membrane, non-sterile	Phenomenex Australia Pty Ltd, 2 Chaplin Dr, Lane Cove West NSW 2066
PVDF	Polyvinylidene Fluoride, 15 mm diameter, 0.2 µm membrane, non-sterile	Phenomenex Australia Pty Ltd, 2 Chaplin Dr, Lane Cove West NSW 2066
PES	Millex-GP, Polyethersulfone, 33 mm, 0.22 µm membrane, sterile	Merck Millipore Ltd., Tullagreen, Carrigtwohill, County Cork, Ireland
MCE	Millex-GS, Mixed cellulose esters, 33 mm, 0.22 µm membrane, sterile	Merck Millipore Ltd., Tullagreen, Carrigtwohill, County Cork, Ireland
GHP	Hydrophilic polypropylene 13 mm, 0.2 µm membrane, non-sterile	Pall Life Sciences 1 - 2 Wandarri Court, Cheltenham 3192, Melbourne, Australia
Inline	Polyethersulfone, 0.2 µm membrane, sterile	Pall Medical, Avenue de Tivoli 3, CH-1700 Fribourg, Switzerland
Inline lipid	Lipid filter, 1.2 µm membrane, sterile	Codan US Corporation, USA

Sildenafil 60 µg/mL and 600 µg/mL solutions were used for filter testing and the drug recovery in the filtrate was determined by HPLC assay. The peak area values obtained with and without filtration were compared and data were reported as percent recovery according to the following formula:

Recovery of sildenafil (%)

$$= \frac{\text{sildenafil concentration (filtered; peak area of the chromatogram)}}{\text{sildenafil concentration of the unfiltered solution}} \times 100$$

A pilot test was carried out using the eight filter types (Table 3.3) and the two concentrations of sildenafil solution in D5W. Filtrate was collected as five separate, consecutive one-millilitre portions of solution to examine the influence of the volume of filtrate on the drug recovery. Testing was carried out in triplicate and a new filter unit was used for each sample.

Based on the pilot study results, four filters were selected for further testing, due to the recovery data and/or clinical relevance of the filters: Nylon (NY, 15 mm × 0.2 µm); Millex-GP (PES, polyethersulfone, 33 mm × 0.22 µm); Millex-GS (MCE, mixed cellulose esters, 33 mm × 0.22 µm); Inline filter (polyethersulfone 25 mm × 0.2 µm). Sildenafil commercial injection solution (60 and 600 µg/mL in D5W) was tested in a similar manner using a test volume of 4 mL (n=3).

3.3 Results

3.3.1 HPLC method validation

The HPLC chromatograms revealed the sildenafil peak was well resolved from the solvent and degradation product peaks in all stress conditions tested (Figure 3.3-3.7). Sildenafil eluted at approximately 4.2 minutes whereas all degradation products eluted at less than 3 minutes. Oxidation of sildenafil resulted in the most extensive degradation profile, with a loss of 14.9% at the 7th day of exposure. Degradation products were detected at 1.5, 1.7 and 2.9 min (Figure 3.3). Alkali degradation of sildenafil was found to be 11.4% at the 7th day of exposure, with one degradation

product detected at 0.9 min (Figure 3.4). Exposure of sildenafil to acid (Figure 3.5), heat (Figure 3.6) and light (Figure 3.7) showed no detectable degradation peaks, with post exposure sildenafil drug concentrations of 98.5%, 103.6% and 99.2% respectively.

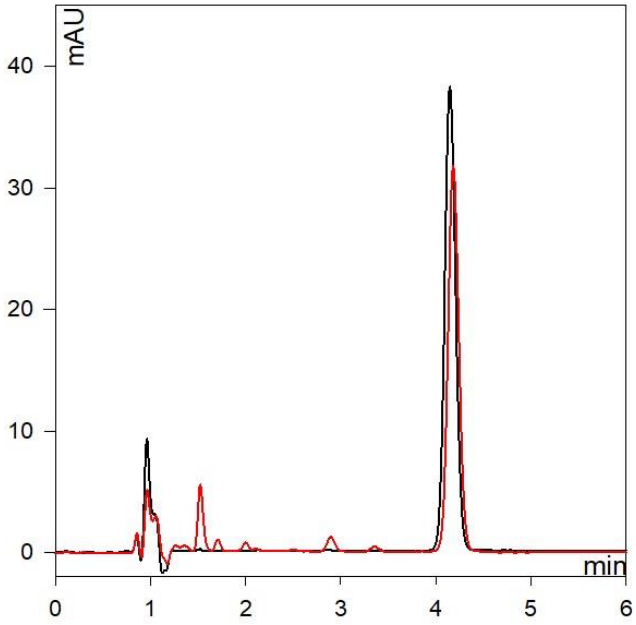


Figure 3.3 Sildenafil 600 µg/mL exposure to 20% v/v hydrogen peroxide (1:1 v/v), stored at 45°C; Sample diluted 1-in-50 with water at the point of assay; injection volume 20 µL. (− Time 0; − Day 7); Degradation products were detected at 1.5, 1.7 and 2.9 min, at Day 7

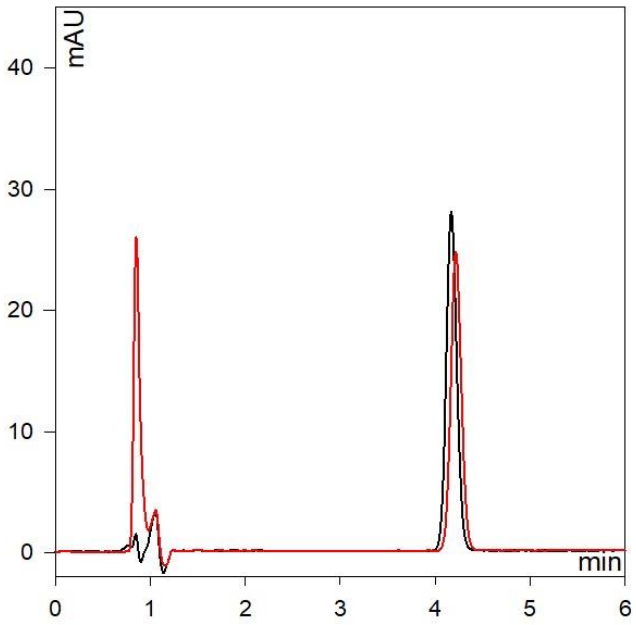


Figure 3.4 Sildenafil 600 µg/mL exposure to 4 M NaOH (1:1 v/v), stored at 45°C; Sample neutralized with 4 M HCl and diluted 1-in-50 with water at the point of assay; Injection volume 20 µL. (− Time 0; − Day 7); A degradation product was detected 0.9 min, at Day 7

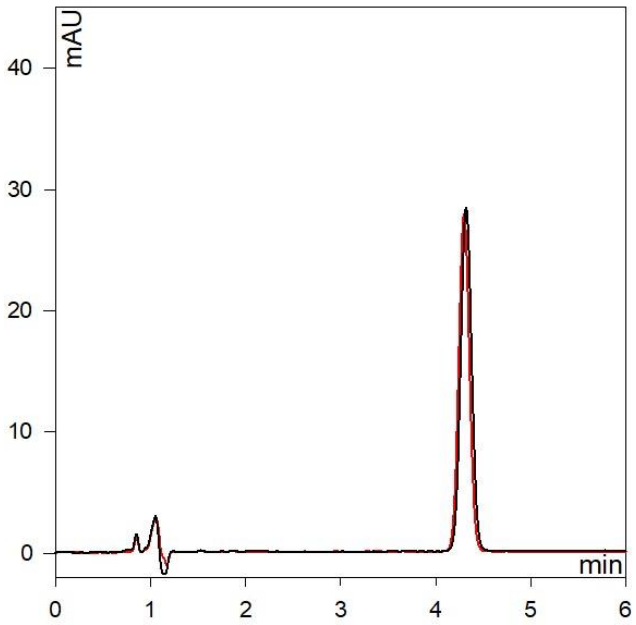


Figure 3.5 Sildenafil 600 µg/mL exposure to 4 M HCl (1:1 v/v), stored at 45°C; Sample neutralized with 4 M NaOH and diluted 1-in-50 with water at the point of assay; Injection volume 20 µL. (− Time 0; − Day 7); No degradation products observed

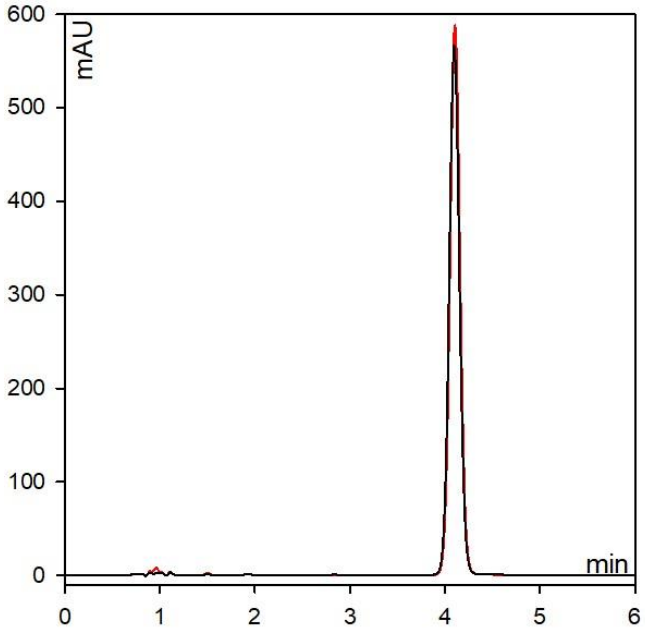


Figure 3.6 Sildenafil 600 µg/mL in water (1:1 v/v) exposure to heat at a temperature of 60°C in a water bath; Injection volume 5 µL (− Time 0; − Day 3); No degradation products observed

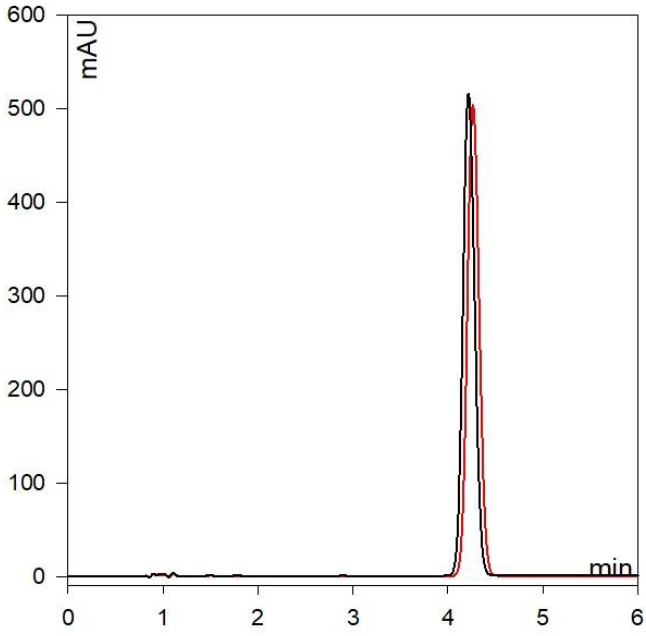


Figure 3.7 Sildenafil 600 µg/mL in water (1:1 v/v) exposure to laboratory fluorescent lighting 24/7 and normal daylight (indirect sunlight) for approximately 12 hours per day at 22°C (room temperature); Injection volume 5 µL; (− Time 0; − Day 7); No degradation products observed

The assay was linear for sildenafil in aqueous solution (n=3) within the concentration range 3-800 µg/mL ($r^2 > 0.999$) (Figure 3.8). The LOD and LLOQ for sildenafil were 0.96 and 2.9 µg/mL respectively.

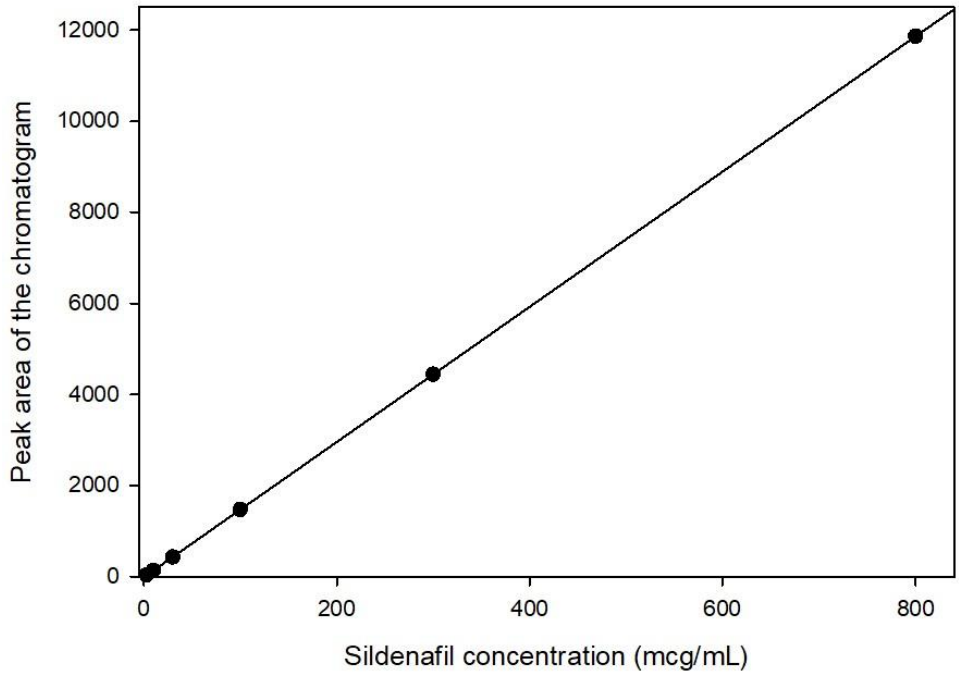


Figure 3.8 Linearity curve for sildenafil solution in aqueous solution within the concentration range 3-800 µg/mL (n=3); Correlation coefficient (r^2) >0.999; Regression equation Y = 14.86x – 10.036

The HPLC method was accurate and precise according to standard definitions [179], with accuracy 100-105% for all samples and precision <4.2% for inter- and intra-assay samples (Table 3.4).

Table 3.4 Accuracy, intra-assay and inter-assay precision data for selected sildenafil concentrations

Sildenafil concentration (µg/mL); n = 5	Sildenafil concentration as a % of nominal concentration (Mean ± SD, n=5)	Intra assay-precision (% RSD)	Inter-assay precision (% RSD pooled)
LLOQ (2.9)	100.2 ± 2.5	2.2	4.2
10	104.0 ± 0.8	3.1	2.0
100	104.6 ± 0.5	0.7	0.6
600	104.0 ± 0.4	0.3	0.3

The percentage concentrations of sildenafil in robustness testing experiment revealed that the method was robust despite small deliberate changes in method parameters (Table 3.5).

Table 3.5 Robustness test results for deliberate changes in method parameters

Parameters	Conditions	Sildenafil concentration as a % of nominal concentration (Mean ± SD, n=5)
Flow rate	1 mL/min	105.1 ± 1.0
	0.8 mL/min	105.1 ± 1.1
Mobile phase composition (Acetonitrile: Buffer)	40:60	105.1 ± 1.0
	45:55	105.2 ± 1.0

3.3.2 Sildenafil compatibility

3.3.2.1 Sildenafil 600 µg/mL

Sildenafil 600 µg/mL was physically and chemically compatible with 29 of the 45 drugs tested at 'high-end' clinical concentrations in the present study: alprostadil, liposomal amphotericin, benzylpenicillin, caffeine (base), caffeine citrate, cefotaxime, ciprofloxacin, clonidine, cloxacillin, dexmedetomidine, dobutamine, dopamine, epinephrine, fentanyl, fluconazole, gentamicin, insulin, levetiracetam, linezolid, metronidazole, midazolam, milrinone, morphine hydrochloride, morphine sulfate, norepinephrine, paracetamol, piperacillin/tazobactam, vancomycin and vecuronium (Table 3.6). However, sildenafil 600 µg/mL was physically incompatible with 16 drugs and all six 2-in-1 PN solutions, with precipitates and haziness occurring almost immediately (Table 3.6). In the first series of re-testing sildenafil 600 µg/mL with secondary drugs at lower, clinically relevant concentrations, three of the combinations were found to be compatible (calcium gluconate 50 mg/mL; heparin 2 units/mL; hydrocortisone 1 mg/mL; Table 3.6). Amoxicillin (100 mg/mL and 50 mg/mL), ampicillin (100 mg/mL and 50 mg/mL) and meropenem (50 mg/mL and 25 mg/mL) were incompatible with sildenafil 600 µg/mL (Table 3.6). All physical incompatibilities were visible to the naked eye, except for the combination with calcium gluconate (100 mg/mL) which required polarized light for clear visualisation. Photographs of incompatible drug combinations and their corresponding photomicrographs can be found in Appendix 5.

Table 3.6 Physicochemical compatibility of sildenafil 600 µg/mL with secondary drugs and 2-in-1 PN solutions (see Table 3.2)

Secondary drug	Test concentration	Diluent	P/C	SIL ratio	95% CI of ratio
Aciclovir	5 mg/mL	D5W	I[a]	-	-
Alprostadil	20 µg/mL	NS	C	99.9	99.4 - 100.4
Amoxicillin	100 mg/mL	WFI	I[b]	-	-
Amoxicillin	50 mg/mL	WFI	I[b]	-	-
Amphotericin (Fungizone)	100 µg/mL	D5W	I[b]	-	-
Amphotericin liposomal	2 mg/mL	D5W	C	99.9	99.0 - 100.8
Ampicillin	100 mg/mL	WFI	I[b]	-	-
Ampicillin	50 mg/mL	WFI	I[b]	-	-
Benzylpenicillin	100 mg/mL	WFI	C	101.2	99.7 - 102.7
Caffeine (base)	10 mg/mL	U	C	101.0	100.1 - 101.9
Caffeine citrate	20 mg/mL	U	C	100.4	99.6 - 101.2
Calcium gluconate	100 mg/mL	U	I[c]	-	-
Calcium gluconate	50 mg/mL	NS	C	100.0	99.1 - 100.8
Cefotaxime	100 mg/mL	WFI	C	102.1	99.9 - 104.3
Ciprofloxacin	2 mg/mL	U	C	101.3	100.3 - 102.2
Clonidine	2 µg/mL	NS	C	99.7	99.1 – 100.4
Cloxacillin	100 mg/mL	WFI	C	101.1	100.2 - 102.0
Dexmedetomidine	1 µg/mL	NS	C	100.0	98.9 - 101.1
Dobutamine hydrochloride	7.2 mg/mL	NS	C	99.9	99.1 - 100.7
Dobutamine hydrochloride	7.2 mg/mL	D5W	C	100.4	99.5 - 101.3
Dopamine	7.2 mg/mL	NS	C	100.5	99.9 - 101.0
Dopamine	7.2 mg/mL	D5W	C	100.7	100.2 - 101.3
Epinephrine	64 µg/mL	D5W	C	99.9	99.3 - 100.5
Fentanyl	50 µg/mL	U	C	98.2	95.4 -100.9
Flucloxacillin	50 mg/mL	D5W	I[d]	-	-
Fluconazole	2 mg/mL	U	C	100.2	99.4 - 100.9
Furosemide	1 mg/mL	D5W	I[b]	-	-
Furosemide	0.2 mg/mL	D5W	I[b]	-	-
Gentamicin	10 mg/mL	WFI	C	101.9	101.3 - 102.5
Gentamicin	10 mg/mL	NS	C	102.2	100.4 - 104.0
Heparin	100 units/mL	NS	I[d]	-	-
Heparin	2 units/mL	NS	C	99.1	98.3 - 100.0
Hydrocortisone	10 mg/mL	NS	I[a]	-	-
Hydrocortisone	1 mg/mL	NS	C	99.7	93.2 – 106.1
Ibuprofen	5 mg/mL	NS	I[e]	-	-
Ibuprofen lysine	4 mg/mL	NS	I[e]	-	-
Indometacin	200 µg/mL	NS	I[e]	-	-
Insulin	0.2 units/mL	NS	C	100.5	98.6 - 102.4
Levetiracetam	5 mg/mL	NS	C	99.7	98.8 - 100.6
Linezolid	2 mg/mL	U	C	98.8	97.8 - 99.8
Meropenem	50 mg/mL	NS	I[b]	-	-
Meropenem	25 mg/mL	NS	I[b]	-	-
Metronidazole	5 mg/mL	U	C	99.2	98.3 - 100.1
Midazolam	1 mg/mL	U	C	100.3	99.9 - 100.8
Midazolam	120 µg/mL	NS	C	99.9	98.6 - 101.2
Midazolam	120 µg/mL	D5W	C	100.5	99.6 - 101.4
Midazolam	500 µg/mL	NS	C	100.5	98.4 – 102.7
Milrinone	400 µg/mL	D5W	C	100.5	99.5 - 101.4
Morphine hydrochloride	200 µg/mL	D5W	C	100.4	99.6 - 101.2
Morphine sulfate	200 µg/mL	D5W	C	99.9	99.4 - 100.3
Norepinephrine	64 µg/mL	D5W	C	100.1	99.2 - 101.0
Paracetamol	10 mg/mL	U	C	100.0	99.4 - 100.6
Phenobarbitone	20 mg/mL	WFI	I[b]	-	-
Piperacillin/tazobactam	200 mg/mL	WFI	C	101.6	101.0 - 102.2
Rifampicin	6 mg/mL	NS	I[f]	-	-
Sodium bicarbonate	4.2% w/v	WFI	I[b]	-	-

Secondary drug	Test concentration	Diluent	P/C	SIL ratio	95% CI of ratio
Vancomycin	10 mg/mL	D5W	C	100.4	99.4 - 101.4
Vecuronium	1 mg/mL	WFI	C	101.4	100.7 - 102.1
Parenteral nutrition PN 1	-	-	I[a]	-	-
Parenteral nutrition PN 2	-	-	I[a]	-	-
Parenteral nutrition PN 3	-	-	I[a]	-	-
Parenteral nutrition PN 4	-	-	I[a]	-	-
Parenteral nutrition PN 5	-	-	I[a]	-	-
Parenteral nutrition PN 6	-	-	I[a]	-	-

(P/C – Physicochemical compatibility; SIL – Sildenafil; C – Compatible; I – Incompatible; D5W – Glucose 5%; WFI – Water for Injection; NS – Normal Saline/ 0.9% Sodium chloride; U – Undiluted). a – A white precipitate appeared 5 – 10 minutes after mixing; b – A white precipitate appeared immediately after mixing; c – Particles observed under polarized light; d – A haze developed after mixing; e – A milky turbidity appeared immediately after mixing; f – A heavy precipitate appeared immediately after mixing – the colour couldn't be determined as the solution is coloured.

3.3.2.2 Sildenafil 60 µg/mL

All drug and PN fluid combinations tested against sildenafil 60 µg/mL were physically compatible, except furosemide, meropenem and sodium bicarbonate (Table 3.7). The only combination shown to be physically compatible and chemically incompatible was ibuprofen. By contrast, sildenafil 60 µg/mL was compatible with ibuprofen lysine.

Thirteen drug combinations with sildenafil 60 µg/mL, including the six PN solutions, resulted in sildenafil ratios >102% (Table 3.7). These combinations were re-tested, after filtering the combinations and control samples using nylon filters (Table 3.8). Apart from aciclovir and rifampicin (which were classified as compatible), all re-tested combinations of sildenafil with secondary drugs and PN solutions produced a significantly lower sildenafil ratio after filtration. The sildenafil ratio (filtered) was in the range of 90-110% for amoxicillin, ampicillin, phenobarbitone and three PN solutions; hence these combinations also were classified as compatible (Table 3.8). However, as the sildenafil ratio (filtered) was <90% for amphotericin, flucloxacillin and three PN solutions, possibly due to a sub-visible precipitate being filtered by the

nylon filters (personal communication, C Locher & EKY Tang), these combinations were classified as incompatible (Table 3.8).

Table 3.7 Physicochemical compatibility of secondary drugs and 2-in-1 PN solutions tested with sildenafil 60 µg/mL, their concentrations, and diluents.

Secondary drug	Test concentration	Diluent	P/C	SIL ratio	95% CI of ratio
Aciclovir	5 mg/mL	D5W	R	105.8	105.2 - 106.4
Amoxicillin	100 mg/mL	WFI	R	105.9	105.4 - 106.4
Amphotericin (Fungizone)	100 µg/mL	D5W	R	104.2	102.8 - 105.7
Ampicillin	100 mg/mL	WFI	R	105.8	105.0 - 106.5
Flucloxacillin	50 mg/mL	D5W	R	105.7	104.9 - 106.5
Furosemide	1 mg/mL	D5W	I[a]	-	-
Furosemide	0.2 mg/mL	D5W	I[b]	-	-
Heparin	100 units/mL	NS	C	99.3	98.7 - 99.9
Hydrocortisone	10 mg/mL	NS	C	99.8	99.5 - 100.0
Hydrocortisone	1 mg/mL	NS	C	99.8	99.3 - 100.3
Ibuprofen	5 mg/mL	NS	I	**74.0**	72.9 - 75.1
Ibuprofen lysine	4 mg/mL	NS	C	99.4	98.9 - 99.9
Indometacin	200 µg/mL	NS	C	99.1	98.7 - 99.5
Meropenem	50 mg/mL	NS	I[b]	-	-
Meropenem	25 mg/mL	NS	I[b]	-	-
Phenobarbitone	20 mg/mL	WFI	R	104.3	103.1 - 105.6
Rifampicin	6 mg/mL	NS	R	102.4	101.5 - 103.3
Sodium bicarbonate	4.2% w/v	WFI	I[c]	-	-
Sodium bicarbonate	4.2% w/v	NS	I[c]	-	-
Sodium bicarbonate	4.2% w/v	D5W	I[c]	-	-
Parenteral nutrition PN 1	-	-	R	103.9	103.3 - 104.6
Parenteral nutrition PN 2	-	-	R	105.4	104.2 - 106.6
Parenteral nutrition PN 3	-	-	R	105.7	104.9 - 106.4
Parenteral nutrition PN 4	-	-	R	104.8	103.8 - 105.9
Parenteral nutrition PN 5	-	-	R	105.5	104.8 - 106.2
Parenteral nutrition PN 6	-	-	R	106.6	105.3 - 107.8

(P/C – Physicochemical compatibility; SIL – Sildenafil; C – Compatible; I – Incompatible; R - Re-test by filtration (see Table 3); D5W – Glucose 5%; WFI – Water for Injection; NS – Normal Saline/ 0.9% Sodium chloride; U - Undiluted). Bold SIL ratio shows chemical incompatibility. a – A white precipitate appeared 1 hour after mixing; b - Particles observed under polarized light; c - A haze developed after mixing

Table 3.8 Re-testing of drug combinations with sildenafil 60 µg/mL in which the SIL ratio (Table 3.7) was > 102%. Combinations were considered compatible if the sildenafil filtered ratio was in the range of 90-110% (nylon filters; see Methods for further details).

Secondary drug	Test concentration	SIL ratio (Unfiltered)	95% CI of ratio (Unfiltered)	SIL ratio (Filtered)	95% CI of ratio (Filtered)	P/C
Aciclovir	5 mg/mL	107.1	106.3 - 108.0	106.1	104.2 - 108.0	C
Amoxicillin	100 mg/mL	105.9	103.6 - 108.1	98.3	95.4 - 101.3	C
Amphotericin (Fungizone)	100 µg/mL	105.8	104.8 - 106.8	78.3	75.1 - 81.5	I
Ampicillin	100 mg/mL	102.6	100.0 - 105.2	94.4	92.2 - 96.5	C
Flucloxacillin	50 mg/mL	106.1	104.4 - 107.9	84.9	82.0 - 87.8	I
Phenobarbitone	20 mg/mL	102.8	100.8 - 104.8	95.5	92.7 - 98.3	C
Rifampicin	6 mg/mL	102.7	100.5 - 104.8	108.6	106.0 - 111.2	C
Parenteral nutrition PN 1	-	107.0	106.0 - 108.1	87.5	86.5 - 88.6	I
Parenteral nutrition PN 2	-	105.5	104.6 - 106.3	91.2	89.3 - 93.2	C
Parenteral nutrition PN 3	-	105.9	105.1 - 106.7	94.1	92.0 - 96.1	C
Parenteral nutrition PN 4	-	106.9	106.1 - 107.6	77.9	72.4 - 83.5	I
Parenteral nutrition PN 5	-	105.8	105.1 - 106.6	94.0	92.5 - 95.5	C
Parenteral nutrition PN 6	-	106.2	105.1 - 107.4	88.9	87.2 - 90.6	I

(SIL – Sildenafil; P/C - Physicochemical compatibility; C – Compatible; I – Incompatible)

3.3.3 Physical compatibility of sildenafil with lipid emulsion

Sildenafil (600 and 60 μg/mL) were compatible with the lipid emulsion, with the MDDs of the combinations being, 0.313 and 0.311 μm respectively (Table 3.9), for 2 hours since mixing.

Table 3.9 Mean and median droplet diameter data (MDD and Dv50, respectively) at 0 and 2 hours after mixing combinations of lipid emulsion and IV drugs/fluids

Fluid/Drug	Concentration	MDD 0 hours (μm)	MDD 2 hours (μm)	Dv50 0 hours (μm)	Dv50 2 hours (μm)
SMOFlipid 20%	-	0.304 ± 0.007	-	0.290 ± 0.008	-
Glucose	5% w/v	0.310 ± 0.005	0.312 ± 0.001	0.296 ± 0.006	0.299 ± 0.001
Sildenafil	600 μg/mL	0.312 ± 0.004	0.313 ± 0.001	0.298 ± 0.004	0.299 ± 0.001
Sildenafil	60 μg/mL	0.312 ± 0.002	0.311 ± 0.002	0.298 ± 0.002	0.298 ± 0.002

Data are mean ± SD of n=3

There was no visual evidence of incompatibility, and no particles of > 1 μm were detected in the size distribution plots of volume density (%) against droplet (particle) size (μm) of each of the combinations of sildenafil (600 and 60 μg/mL) with SMOFlipid (20%), both immediately and 2 hours after mixing (size distribution plots can be found in Appendix 6).

3.3.4 Absorption/adsorption loss of sildenafil by filter material

The pilot study using eight filters and 5 mL sildenafil solution showed the lowest drug recovery was in the first millilitre of the filtrate in all filters studied. For sildenafil 600 µg/mL solution, the first millilitre had a drug recovery >90% in all filters tested (Figure 3.9). In the second to fifth millilitres, drug recovery was >98%. However, for sildenafil 60 µg/mL solution, only the nylon, polypropylene and inline 'lipid' filters showed a drug recovery of >90% in the first millilitre of the filtrate. All filter types showed a drug recovery >94% in the remainder of the sildenafil 60 µg/mL filtrate (Figure 3.10).

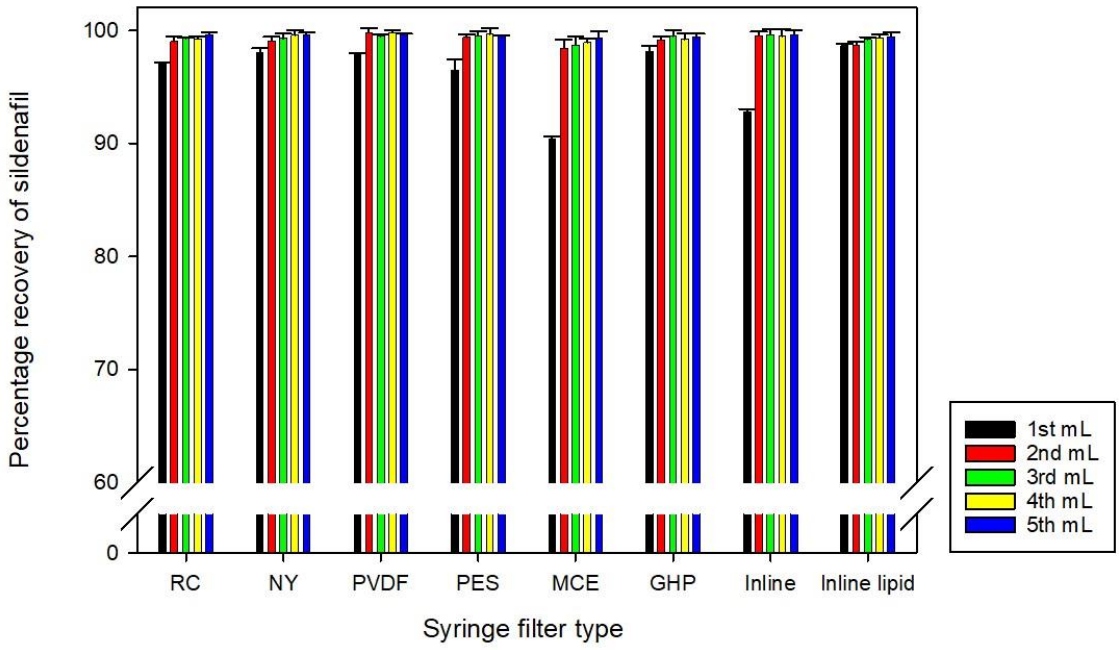


Figure 3.9 Recovery (%) of sildenafil by different filters using the 600 µg/mL solution (n=3); The coloured bars represent the recovery in five separate, consecutive millilitre portions – pilot study

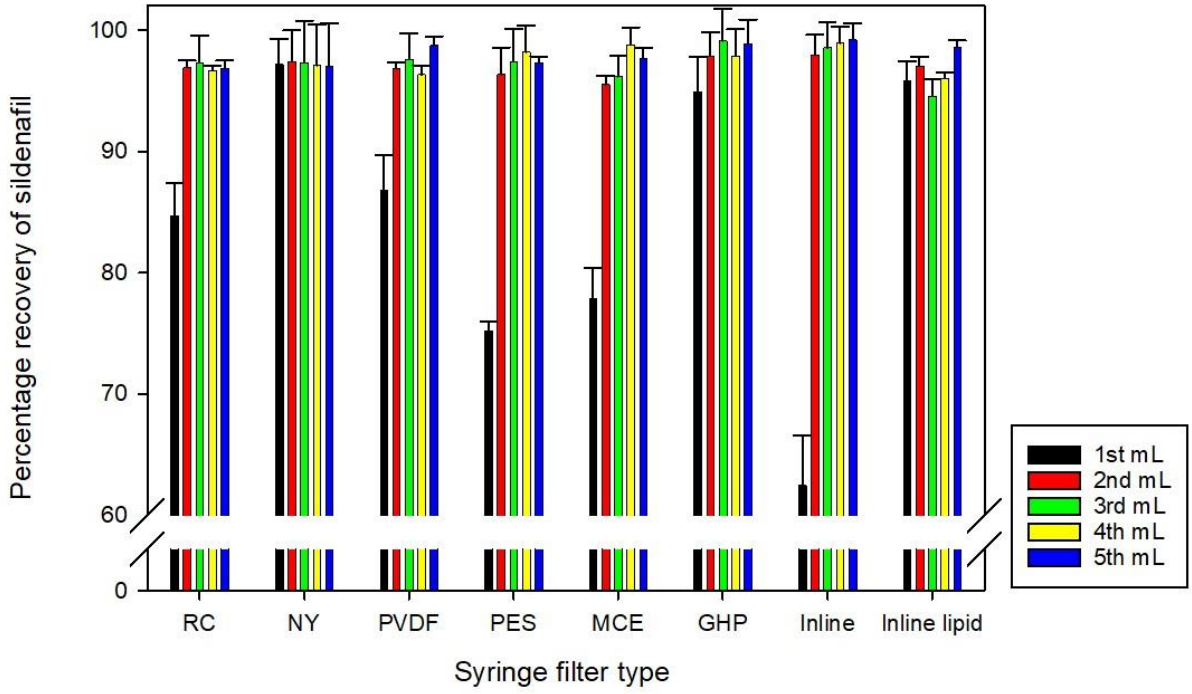


Figure 3.10 Recovery (%) of sildenafil by different filters using the 60 µg/mL solution (n=3); The coloured bars represent the recovery in five separate, consecutive millilitre portions – pilot study

The filter test results obtained using the sildenafil commercial injection solution (600 µg/mL) revealed that all filter types tested (NY, PES, MCE and Inline PES) showed a drug recovery >90% in the first millilitre of the filtrate (Figure 3.11). One way ANOVA results showed a statistically significant difference in drug recovery in the first millilitre compared to the remainder of the filtrate ($P < 0.05$).

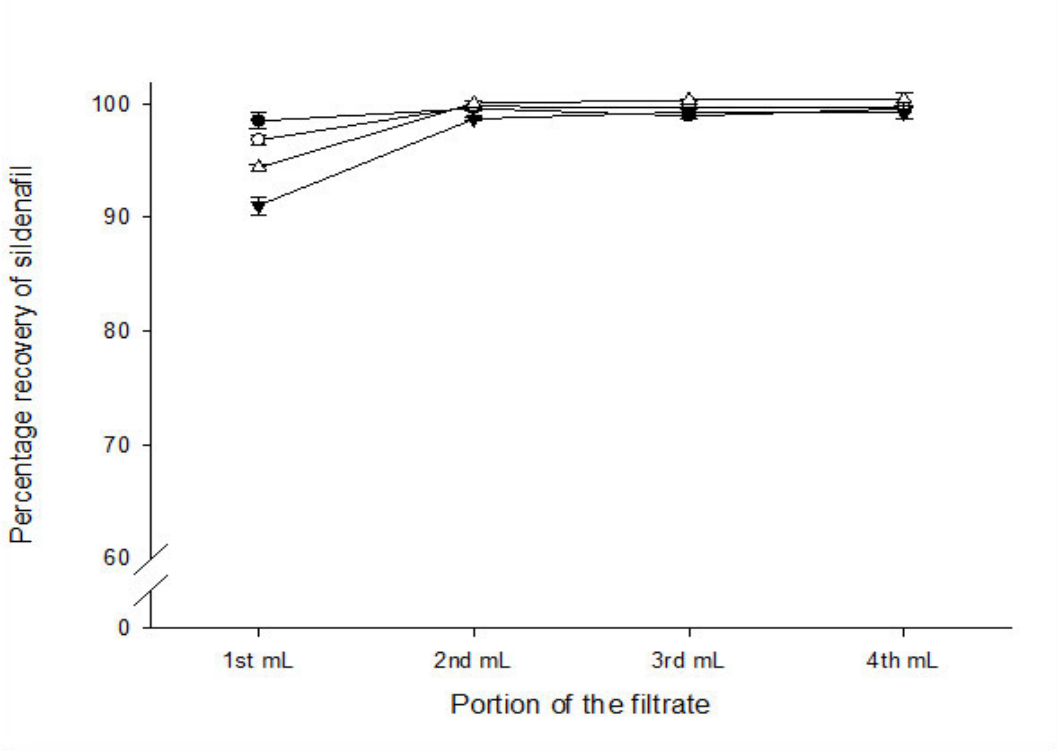


Figure 3.11 Recovery (%) of sildenafil 600 µg/mL injection solution from sterilising filters. Sildenafil concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ polyethersulfone; ▼ mixed cellulose esters; △ inline polyethersulfone; see Table 3.3 for further details). Data are mean ± SD (n=3).

However, in the sildenafil 60 µg/mL solution PES, MCE and Inline filters showed a drug recovery <80% in the first millilitre of the filtrate (Figure 3.12). The drug recovery was >97% in all millilitre portions of the filtrate when the nylon filters were used and no statistically significant difference in drug recovery was observed between any millilitre portions. The first millilitre of the filtrate had a statistically significantly lower drug recovery (P <0.05) than the remaining filtrate in all other filters used.

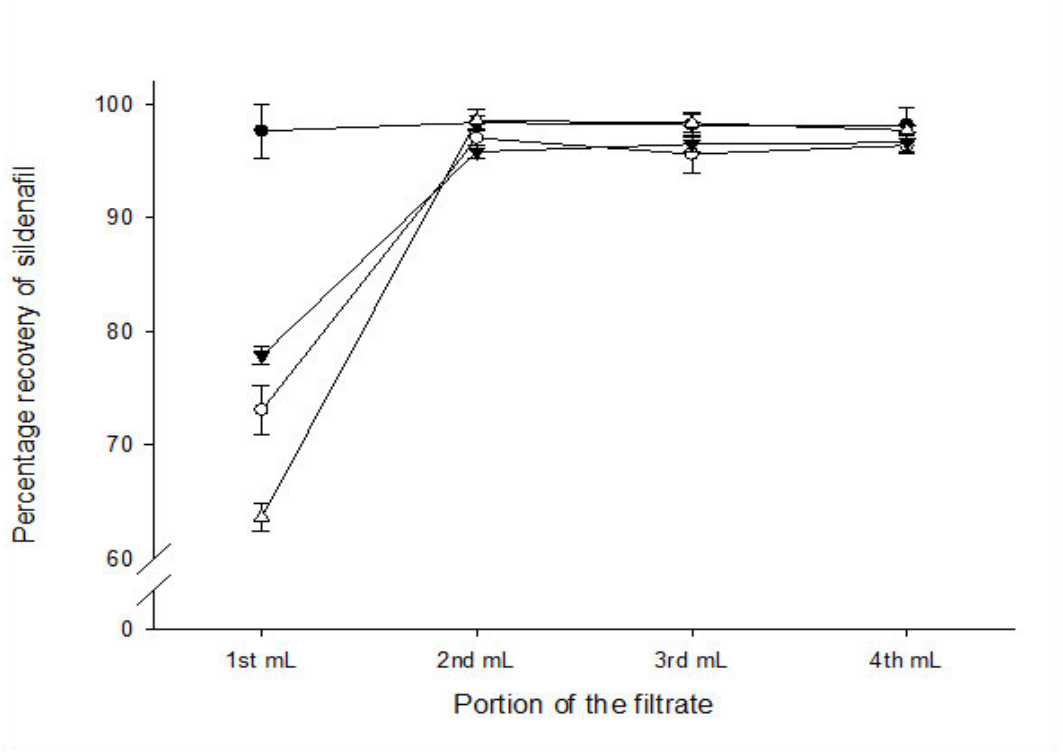


Figure 3.12 Recovery (%) of sildenafil 60 µg/mL injection solution from sterilising filters. Sildenafil concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ polyethersulfone; ▼ mixed cellulose esters; △ inline polyethersulfone; see Table 3.3 for further details). Data are mean ± SD (n=3)

3.4 Discussion

The degradation experiments of sildenafil by exposure to peroxide, alkali, acid, heat and light conditions demonstrated that the most extensive degradation profile, with a loss of 14.9% of sildenafil was upon exposure to peroxide for 7 days. Similarly, a previous report has shown that oxidation of sildenafil using 3% hydrogen peroxide resulted in the most remarkable degradation profile, however, the drug loss was reported to be higher than that observed in the present study (with a loss of 14% after 24 hours and a total drug loss of 49% after five days of sampling) [106]. Exposure of sildenafil to sodium hydroxide (0.1 M) resulted in a decrease in concentration of 11% within the first 24 hours, and minimal subsequent degradation. The sildenafil concentration was 14% lower than the initial concentration after five days of exposure. Exposure of sildenafil to 0.1 M perchloric acid resulted in minimal degradation within the first 24 hours (1%) and has not exceeded 10% loss until six days of exposure had passed [106].

In the present study, the percentage reduction of sildenafil upon exposure to alkali was 11.4% at the 7th day of sampling, however, acid, heat and light resulted in minimal or no degradation of sildenafil. The results of these forced-degradation experiments align with those reported in literature, with sildenafil being relatively stable in acidic and basic environments but resulting in comparatively higher degradation by oxidation [106, 311].

The present study has demonstrated that 29 IV drugs at ‘high-end’ clinically relevant concentrations for NICU settings were physically and chemically compatible with sildenafil 600 µg/mL injection (Table 3.6). None of these drugs were tested at lower

concentrations or against sildenafil 60 µg/mL in the present study; rather, it was concluded that lower drug concentrations also would be compatible.

Sixteen of the secondary drugs (at their standard or high-end clinically relevant concentration) and all six 2-in-1 PN solutions were physically incompatible with sildenafil 600 µg/mL (Table 3.6). Nine of these 16 drugs were evaluated at only one relevant concentration and subsequently tested against sildenafil 60 µg/mL. A further four were evaluated at lower, clinically relevant concentrations and found to be physically incompatible (amoxicillin, ampicillin, furosemide, meropenem); hence, 13 of the 45 IV drugs were deemed incompatible with sildenafil 600 µg/mL at concentrations relevant to NICU settings. However, three drugs were found to be compatible with sildenafil 600 µg/mL at low concentrations (calcium gluconate 50 mg/mL, heparin 2 units/mL and hydrocortisone 1 mg/mL; Table 3.6) and could be co-administered at these lower, clinically relevant concentrations if required. The results for heparin align with previous data indicating that heparin was incompatible at higher concentration (100 units/mL) [94] and compatible at a lower concentration (1 unit/mL) [106]. Furthermore, the calcium gluconate concentration used for urgent correction of hypocalcaemia is 50 mg/mL [39] and this concentration was found to be physicochemically compatible with sildenafil 600 µg/mL. Hence, calcium gluconate was not tested with sildenafil 60 µg/mL.

Fifteen drugs and the six 2-in-1 PN solutions were tested against the lower sildenafil concentration of 60 µg/mL, which is used in preterm infants [304]. Four drugs showed physical and chemical compatibility, three were physically incompatible and one (ibuprofen) was chemically incompatible (Table 3.7). The remaining seven drugs and the PN solutions were found to have sildenafil ratios >102%. Although there was no

visible or microscopic evidence of precipitation (including Tyndall beam and magnified polarised light observation), unpublished data suggesting sub-visible precipitates for other drug combinations (personal communication, C Locher & EKY Tang) were available. Therefore, a series of filter validation studies were conducted, which identified 0.2 µm nylon filters as the most suitable, and these combinations were investigated before and after filtering (Table 3.8). Based on pre-determined criteria for the 90-110% sildenafil ratio (filtered), it was concluded that aciclovir, amoxicillin, ampicillin, phenobarbitone and rifampicin were compatible with sildenafil 60 µg/mL, but amphotericin and flucloxacillin were incompatible. Three of the PN solutions also were classified as compatible; however, there were no notable features of these three formulations (#2, #3 and #5) compared to the incompatible formulations and further investigation of this finding was beyond the scope of the present study.

Physical incompatibilities in the present study ranged from florid precipitation to hazy fluids and potential sub-visible precipitation (Appendix 5). The former were generally visible to the naked eye, where the limit of detection is approximately 100 µm for discrete particles and 10 µm for hazy or cloudy fluids [317], the observation of which may be enhanced by polarised light [96] or Tyndall beam [220]. Sub-visible particles in the order of 1-2 µm also may be detected by the visual enhancement techniques or light microscopy; however, it has been postulated that incompatible drug combinations could cause nano- or micro-precipitation, ostensibly <1 µm (personal communication, C Locher & EKY Tang). In the present study, sub-detectable precipitation may explain the substantially lower sildenafil ratio after 0.2 µm filtration for amphotericin, flucloxacillin and three PN solutions. Although the clinical impact of injection of particulate matter <1 µm is unclear, the pre-determined criteria for the sildenafil ratio

(outside the range of 90-110%) was applied in the present study to define incompatible drug combinations and recommend avoidance in NICU clinical settings.

Based on the visual observation and particle size analysis (MDD data), sildenafil 600 and 60 µg/mL were physically compatible with the lipid emulsion tested.

The present study included some limitations that are consistent with previous investigations of physicochemical compatibility. For example, due to the resource constraints and unclear interpretation or clinical significance of pH changes [32], determination of pH was not performed (the volume of drug solutions required for pH determination would be >5 mL). Further, introducing a wet pH probe to the consecutive samples may reduce the drug concentration and produce false results. As pH changes may contribute to chemical reaction [53] or altered drug solubility [237], the use of HPLC analysis in the present physicochemical study would likely counter the need for pH analysis. Another potential issue was conducting HPLC analysis only for the primary drug (sildenafil). This is consistent with previous IV physicochemical compatibility studies where a large number of secondary drugs have been tested [217, 222, 236, 238]. However, there are some reports where both the primary and secondary drugs have been assayed, typically in studies where a modest range of secondary drugs have been tested [94, 223, 246]. HPLC analysis of both the primary and secondary IV drugs would have significant cost and complexity implications, to ensure validated HPLC assays were developed for each secondary drug. Consequently, it was assumed that physicochemical incompatibility would cause a decline in the concentration of both IV drugs and be detected by HPLC assay of the primary drug. Nevertheless, there may be situations where quantifying the secondary drug concentration is of potential

value, if chemical incompatibility is suspected or inconclusive results require further investigation.

A potential limitation related to clinical interpretation of the present study was the drug combination contact time of 2 hours, which was based on a previous report that 60 minutes was a plausible maximum contact time for two drug solutions in the IV tubing from the Y-site to the tip of a cannula in NICU settings [109]. By comparison, a 4-hour study duration is commonly used for drug compatibility studies and may be applicable to other clinical settings [66, 84, 246, 254, 264, 313, 318-320]. A further clinical consideration is that the present study and most IV compatibility re-search has been conducted at room temperature [84, 212, 264, 318, 319], which is comparable to the ambient temperature in the majority of clinical settings, including NICU. However, whilst the IV drugs in syringes (or other delivery devices) and a proportion of the IV tubing in NICU will most likely be at room temperature, part of the IV tubing may be inside a humidicrib up to 37°C and some recent IV compatibility studies have been conducted at elevated temperature to simulate the humidicrib environment [94, 116].

It should be emphasised that the compatibility data generated are specific to the formulations and batches tested. Results may vary upon different formulations and excipients used by manufacturers.

3.5. Conclusion

Sildenafil 600 µg/mL was physicochemically compatible with approximately 70% of the 45 clinically relevant IV drugs used in NICU settings that were tested in the present study. A further seven drugs were compatible with sildenafil 60 µg/mL. Six drugs (amphotericin, flucloxacillin, furosemide, ibuprofen, meropenem and sodium

bicarbonate) were incompatible with sildenafil and should not be co-administered via Y-site infusions. Six 2-in-1 PN solutions were incompatible with sildenafil 600 µg/mL; however, three appeared to be compatible with sildenafil 60 µg/mL and three were deemed incompatible. Sildenafil solution was compatible with nylon syringe filters; however, absorption/adsorption loss from the first millilitre of filtrate occurred with polyethersulfone and cellulose ester filters, which should be avoided for small volumes and/or low concentrations of sildenafil solution.

Chapter 4

Physicochemical compatibility of caffeine citrate and caffeine base injections with parenteral medications used in neonatal intensive care settings

4.1 Introduction and background

4.1.1 Methylxanthines and caffeine

Caffeine is a purine alkaloid and is among the most widely consumed psychostimulant drugs in the world [321]. Structurally, caffeine (1,3,7-trimethylxanthine) is closely related to the other natural xanthines, theobromine, theophylline, and paraxanthine and to many crucial endogenous molecules such as the purine bases, adenine and guanine (Figure 4.1).

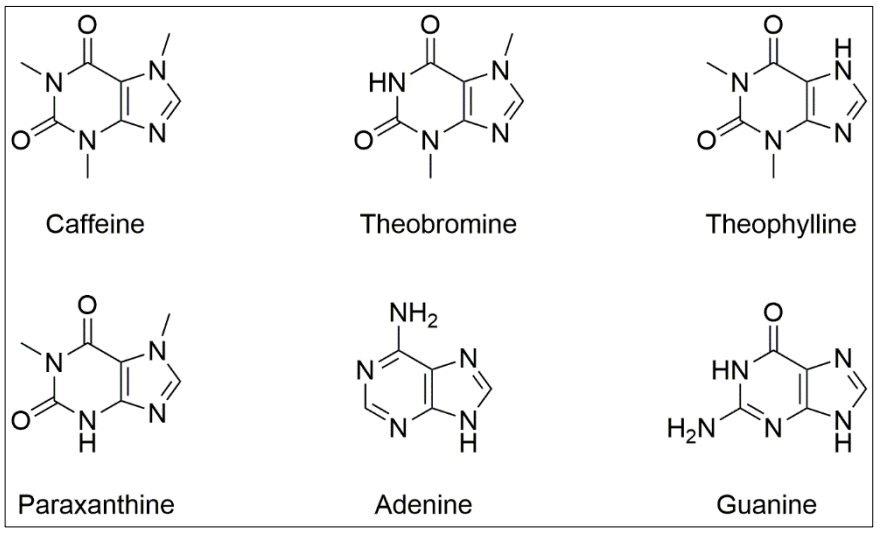


Figure 4.1 Chemical structure of caffeine and other xanthines: theobromine; theophylline; paraxanthine; adenine and guanine.

4.1.2 Mechanism of action of caffeine and other methylxanthines in neonatal apnoea

By definition, apnoea of prematurity (AOP), is a transient cessation of breathing which may be accompanied by bradycardia and oxygen desaturation and is common in premature infants [322]. The central pathophysiology of AOP, is thought to be the inability of the respiratory control mechanisms to respond to changes in partial pressures of oxygen and carbon dioxide, secondary to the immaturity of the central nervous system in neonates [323]. The American Academy of Paediatrics Task Force on Prolonged Infantile Apnoea defines pathological AOP as cessation of breathing for at least 20 seconds, or as a briefer episode of apnoea accompanied by bradycardia, cyanosis, and pallor [324]. The physiologic consequences of apnoea include effects on haemodynamics, ventilation, and oxygenation, which might be harmful to the infant. Prolonged apnoea (>20 seconds' duration) can lead to long hypoxaemia which may adversely affect neurodevelopment, leading to brain lesions, and even sudden death if untreated [325, 326].

Since the 1970s to date, methylxanthines have been the mainstay of treatment of AOP. The two most used agents are caffeine and theophylline [327].

Methylxanthines act both centrally and peripherally to stimulate medullary respiratory centres, increase carbon dioxide sensitivity, induce bronchodilation, and enhance diaphragmatic function. These actions improve ventilation and reduce hypoxic respiratory depression [327, 328]. Theophylline is known to increase tidal volume by increasing the inspiratory drive in preterm infants [328].

Studies have proven that methylxanthines (including caffeine and theophylline) reduced both the number of apnoea events and the necessity of mechanical ventilation

in premature infants. Further, caffeine was associated with improved long-term clinical outcomes and minimal toxicity due its pharmacokinetic properties [329-332].

4.1.3 Pharmacokinetics of caffeine

In humans, caffeine and related methylxanthines cross all biological membranes and distribute in body fluids without accumulating in tissues and organs. In adults, caffeine is extensively metabolized by the liver to dimethylxanthines, paraxanthine, theophylline, and theobromine [333-335], and less than 2% of caffeine is excreted unchanged in urine [336]. However, in newborns, 85% of caffeine administered is excreted unchanged in the urine, hence, plasma clearance is extremely slow, and half-life is prolonged in the preterm newborn [337]. Theophylline has a prolonged plasma elimination in preterm newborns (t½-30 h) [338], however for caffeine, it's even longer (t½-102 h) [339].

Caffeine has a wide therapeutic index in preterm newborns, therefore, plasma caffeine monitoring is not necessary for standard dosing regimens but may be useful if caffeine exceeds standard doses [340]. The therapeutic range is reported to be 5-20 mg/L, and toxicity is relatively rare and typically reversible at plasma caffeine concentrations below 50 mg/L [341]. Furthermore, available evidence suggests that plasma caffeine concentrations as high as 90 mg/L were tolerated by preterm newborns with only transient adverse effects [339, 342-344]. In contrast, theophylline has a narrow therapeutic index in newborns, with a therapeutic plasma level of (5-12 mg/L) and toxicity beginning at (13-15 mg/L) [345].

4.1.4 Oral and IV administration of caffeine

Caffeine is rapidly absorbed when given orally with complete bioavailability following oral dosing and switching between parenteral and oral administration requires no dose adjustments [340]. Caffeine in its pure anhydrous form is extremely bitter and is not as tolerable as the citrate salt when given orally. Caffeine citrate contains anhydrous citric acid and 50% anhydrous caffeine base; therefore, the dose of caffeine base is approximately half that of caffeine citrate.

The routine dose for caffeine citrate for AOP, comprises a loading dose of 20 mg/kg (10 mg/kg of caffeine base) followed by a daily maintenance dose of 5 mg/kg [346]. However, higher doses have been used by different neonatal centres and alternative options of loading doses up to 80 mg/kg (caffeine citrate) and maintenance doses of up to 20 mg/kg (once daily) for neonates up to 34 weeks post menstrual age, are recommended [347].

4.1.5 Use of caffeine in neonatal intensive care settings, and its co-administration with other drugs

Clinical benefits of caffeine such as reduced plasma concentration monitoring and dosing frequency, proven efficacy and safety by clinical studies, therapeutic superiority in comparison to theophylline [329, 348], have led to its worldwide popularity for treatment of neonatal apnoea. Caffeine's beneficial effects on neonatal morbidities [349] and improved long-term outcomes [350-354] have been well demonstrated, and hence, caffeine has established its place as 'the silver bullet' in neonatal therapeutics [354].

As caffeine is one of the most prescribed drugs in the NICU settings, it may be co-administered with other neonatal IV medications. Therefore, physicochemical compatibility of caffeine with other NICU medications is an important clinical consideration, as outlined in Chapter 1; section 1.1.

4.1.6 Physical and chemical compatibility of caffeine with other NICU drugs

Compatibility information available in the literature, for caffeine citrate, is mostly limited to physical compatibility, where conclusions are made depending on visually observable changes and particle count testing [274, 275]. Mitchell and Gailey, in 1999 [275], have evaluated the visual compatibility of caffeine citrate (20 mg/mL) with 29 NICU medications, given intravenously. During testing, the secondary test drug was mixed with caffeine citrate at a 1:1 volume ratio, to simulate Y-site injection. Each combination was tested in duplicate. Upon mixing, the combination was gently swirled, and visually inspected under both normal fluorescent and high intensity lighting conditions, against a black and white background, with the aid of a magnifying glass, at 25°C. The observations were carried out by two independent observers to confirm reproducibility of results. The combination samples were visually observed immediately after mixing and at time intervals of 5, 15, 30 minutes, 1 hour and 4 hours, for any evidence of haze, precipitation, change in colour and gas production. Of the tested secondary medications, 24 were physically compatible with caffeine citrate, with no visually observable changes during the testing period of 4 hours. However, aciclovir, furosemide, lorazepam, oxacillin, and nitroglycerine were physically incompatible, at the concentrations tested. Aciclovir and furosemide gave an immediate precipitate, lorazepam an immediate haziness, and oxacillin and nitroglycerine gave an immediate cloudy appearance, upon mixing with caffeine

citrate. The authors concluded that as the concentration of the five medications found to be visually incompatible with caffeine citrate exceeded normal neonatal concentrations, incompatibilities may not have been visualized at lower clinically acceptable concentrations. Further, flushing with at least twice with the priming volume between infusions of the incompatible medications was recommended. In addition, the authors have highlighted that visual compatibility observed for drug combinations is not evidence of full potency or chemical stability, hence, further studies are required to determine the chemical stability of caffeine citrate in such drug combinations.

In 2016, the study conducted by Audet *et al.* [274], (published in French) evaluated the physical compatibility of caffeine citrate injection with 99 other drugs, when administered in Y-sites. In this study, caffeine citrate (20 mg/mL) was combined at a 1:1 volume ratio with 99 undiluted injectable drugs at room temperature. Each combination was carried out twice. The first was visually observed and a light obscuration particle count test was carried out immediately after mixing. These tests were repeated four hours after mixing using the second sample. To be considered physically compatible, the drug combination mixtures had to show no visually observable changes (i.e., precipitation, turbidity, crystals, gas formation and colour change) and had to meet the USP <788> 1.B specification ("injectable solutions supplied in a nominal volume of less than 100 mL pass the compatibility test if the number of particles present in the units tested per container does not exceed 6000 particles larger than 10 µm, 600 particles over 25 µm") [250] at both time zero and four hours after mixing. All drugs including caffeine citrate, were tested at their maximum initial or original concentration, to simulate the maximum level of risk of potential incompatibility. Of the 99 secondary drugs tested, 80 were found to be

compatible both visually with the unaided eye and according to the USP <788> requirements. Incompatibilities were observed in 19 drugs, and they were reported to be clearly visible. Amiodarone, ciprofloxacin, diazepam, dobutamine, dopamine, droperidone, erythromycin, hydroxyzine, lorazepam, midazolam, potassium phosphate, sodium phosphate and vancomycin were among the incompatible secondary drugs. Although 80 drug combinations were compatible, based on physical criteria, the authors did not guarantee the clinical efficacy of these combinations as no chemical compatibility testing was conducted.

Chemical compatibility for caffeine citrate is limited to a few drugs as package insert stability data. These include, dopamine, fentanyl, heparin and calcium gluconate [27].

Although no formal studies have been conducted to investigate physical or chemical compatibility of caffeine base injection with other drugs, stability of caffeine base injection in PN solutions, IV fluids and admixtures has been reported in literature. Nahata *et al.*, in 1989 [355], investigated the stability of caffeine base (10 mg/mL) in multiple IV admixtures and PN solution, at room temperature for 24 hours. The IV admixtures included D5W; D5W with sodium chloride (NaCl) 0.2% w/v injection; D5W with NaCl 0.2% w/v and 20 mEq potassium chloride (KCl) injection; D10W injection; D10W with NaCl 0.2% w/v and 5 mEq KCl injection. The PN solutions studied were 1.1% amino acids with electrolytes; 2.2% amino acids with electrolytes; and 4.25% amino acids with electrolytes. Caffeine base injection was mixed with these admixtures and PN solutions in a 1:1 volume ratio. From each mixture, aliquots were removed at 0 (immediately after mixing), 2, 4, 8, and 24 hours after mixing and the caffeine concentrations were determined in each of the aliquots, using a stability indicating HPLC method. Caffeine concentrations did not change substantially in the

presence of IV admixtures and PN solutions, compared to the initial concentrations, hence caffeine base was considered stable in those solutions for 24 hours after mixing.

Physical compatibility of caffeine citrate and caffeine base injection with an IV lipid emulsion used in NICU setting has been previously studied by Senarathna *et al*. [316]. The IV lipid emulsion studied was SMOFlipid 20% (Fresenius Kabi Australia Pty Ltd, North Ryde, NSW, Australia), which comprises soybean oil 6%, medium chain triglycerides 6%, olive oil 5% and fish oil 3%. Lipid emulsion and drug solutions were combined 1:1 in glass vials (to simulate Y-site injection), and visually inspected for physical incompatibility at 0, 1 and 2 hours, and assessed on the basis of lipid droplet size at 0 and 2 hours after mixing. The droplet diameter of the lipid emulsion and emulsion–fluid mixtures was measured by laser diffraction. Compatibility was concluded using the formal criterion of MDD<0.5 μm ($n \geq 3$) for compatible lipid/drug mixtures [53, 93, 315]. The study concluded that caffeine base injection (10 mg/mL) was physically compatible with lipid emulsion, however, caffeine citrate (20 mg/mL) was incompatible [316].

Against this backdrop of evidence, the present study aimed to investigate the physicochemical compatibility of caffeine citrate and caffeine base injection with a range of NICU drugs, at higher end, clinically relevant concentrations, and with selected PN solutions. It would complement the currently available physical compatibility information for caffeine citrate injection.

Further, to complement the physical compatibility findings of caffeine and lipid emulsions by Senarathna *et al.* [316], the present study aimed to test caffeine citrate injection at its lower clinically relevant concentration, for compatibility with the lipid emulsion.

In addition to the above objectives pertaining to compatibility, the study attempted to evaluate absorption/adsorption loss of caffeine by syringe filters. As this has not previously been reported, the findings would support the use of different types of filters in the clinical setting, prior to IV administration, as required.

4.2 Materials and methods

Caffeine (caffeine; $C_8H_{10}N_4O_2$; MW 194.19; certified reference material), was purchased from Sigma-Aldrich Chemicals, St Louis, MO63103, USA. HPLC grade acetonitrile was from Fisher Scientific, Fair Lawn, NJ, USA. All other laboratory chemicals were of analytical grade.

Caffeine citrate undiluted injection (20 mg/mL; equivalent to 10 mg/mL of caffeine base) and caffeine base injection (10 mg/mL) were tested with the secondary drugs. Secondary drugs were prepared as per NICU drug administration guidelines of KEMH, using preferred diluents. Drug concentrations were based on the current standard infusion concentrations for a patient weighing 2 kg. In cases of drug incompatibility with caffeine citrate undiluted injection (20 mg/mL), compatibility was tested with caffeine citrate 10 mg/mL solution (diluted in Water for Injection; WFI), which is the recommended concentration for maintenance doses of caffeine [39].

Caffeine citrate, caffeine base injection and all parenteral medications and solutions were of clinical grade (see Table 4.1 for the list of medications, manufacturers, and lot numbers). The composition of the PN solutions is provided in Table 4.2.

Table 4.1 Manufacturers/ suppliers of injectable products used for compatibility studies.

Injectable drug	Manufacturer/ supplier	Lot No
Aciclovir	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	FT1848AA
Alprostadil	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	GE6112
Amoxicillin	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	1C02KH
Amphotericin B - Fungizone	XGen Pharmaceuticals DJB, NY 14814, United States	AQ6316B
Amphotericin B - Liposomal	Gilead Sciences Pty Ltd, St Kilda Road, Melbourne, VIC, Australia	028780
Ampicillin	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	2G05AH
Benzylpenicillin	Seqirus (Australia) Pty Ltd, Melbourne, VIC, Australia	KT4974
Caffeine base*	Perth Children's Hospital, Nedlands, WA, Australia	6599
Caffeine citrate	Phebra Pty Ltd, Lane Cove West, NSW, Australia	15570
Calcium gluconate	Phebra Pty Ltd, Lane Cove West, NSW, Australia	15652
Cefotaxime	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	G101HA1
Ciprofloxacin	Aspen Pharmacare Australia Pty Ltd, Leonards, NSW, Australia	20599
Clonidine	Medicianz Healthcare Pty Ltd, Melbourne, VIC, Australia	CLA032
Cloxacillin	SteriMax Inc, Oakville, ON L6H6R4, Canada	2220042CA
Dexmedetomidine	Accord Healthcare Pty Ltd, Melbourne, VIC, Australia	M2110403
Dobutamine	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	FY3034AA
Dopamine	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	A23M1M
Epinephrine	Aspen Pharmacare Australia Pty Ltd, Leonards, NSW, Australia	AS211A1
Fentanyl citrate	Piramal Critical Care Pty Ltd, Chatswood, NSW, Australia	2307459
Flucloxacillin	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	0X18HY
Fluconazole	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	B631209
Furosemide	Baxter Health care Pty Ltd, Old Toongabbie, NSW, Australia	B5E0004A
Gentamicin	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	C297
Heparin	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	240014
Hydrocortisone	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	ER8089
Ibuprofen	Seqirus (Australia) Pty Ltd, Melbourne, VIC, Australia	10098R
Ibuprofen lysine	Prasco Laboratories, Commerce Ct, Mason, United States	B225248
Indometacin	Promedica SRL, Via Palermo, Parma, Italy	22904
Insulin	Novo Nordisk Pharmaceuticals Pty Ltd, Baulkham Hills, NSW, Australia	LR79K53
Levetiracetam	Apotex Pty Ltd, Macquarie Park, NSW, Australia	275447
Linezolid	Fresenius Kabi Australia Pty Ltd, Mount Kuring-gai, NSW, Australia	15RIA220
Meropenem	Sun Pharma ANZ Pty Ltd, Macquarie Park, NSW, Australia	DFD4194A
Metronidazole	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	10221
Midazolam	Pharmaco (Australia) Ltd, Gordon, NSW, Australia	F0021F01
Milrinone	Generic Health Pty Ltd, Box Hill, VIC, Australia	F2061-01
Morphine hydrochloride	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	A22A18
Morphine sulfate	Pfizer Australia Pty Ltd, Sydney, NSW, Australia	212004
Norepinephrine	Juno Pharmaceuticals Pty Ltd, Cremorne, VIC, Australia	208599
Paracetamol	B.Braun Australia Pty Ltd, Bella Vista, NSW, Australia	22106450
Phenobarbitone	Aspen Pharmacare Australia Pty Ltd, Leonards, NSW, Australia	042344

Injectable drug	Manufacturer/ supplier	Lot No
Piperacillin/tazobactam	Sandoz Pty Ltd, Macquarie Perk, NSW, Australia	MX5674
Rifampicin	Sanofi-Aventis Australia Pty Ltd, Macquarie Park, NSW, Australia	0J05V1
Sodium bicarbonate	Phebra Pty Ltd, Lane Cove West, NSW, Australia	14924a
Vancomycin	Alphapharm Pty Ltd, Carole Park, QLD, Australia	236393
Vecuronium	Sun Pharma ANZ Pty Ltd, Macquarie Park, NSW, Australia	HAD2861A

*Caffeine base 10 mg/mL injection comprises caffeine, sodium chloride, hydrochloride acid and Water for Injection; the injection is isotonic and has a pH approximately 4.2 (AUSPMAN/ Perth Children's Hospital)

Table 4.2 Composition of the 2-in-1 PN solutions, manufactured at King Edward Memorial Hospital

	PN 1 Preterm A	PN 2 Preterm B	PN 3 Term	PN 4 Custom 1	PN 5 Custom 2	PN 6 Custom 3
Amino acid g/100mL	2.7	2.7	2.3	0.5	3.5	2.3
Glucose, g/100mL	5	8	12	2	14	8
Sodium, mmol/100mL	4	4	4	4	4	4
Potassium, mmol/100mL	2	2	2	2	2	2
Calcium, mmol/100mL	1.5	1.5	0.9	1.5	1.5	1.5
Phosphate, mmol/100mL	1.5	1.5	0.9	1.5	1.5	1.5
Magnesium, mmol/100mL	0.25	0.25	0.25	0.25	0.25	0.25
Acetate, mmol/100mL	2	2	2.56	1.79	2.08	1.96
Chloride, mmol/100mL	2.01	2.01	2.57	1.8	2.08	1.97
Trace elements, mL/100mL	-	-	0.74	0.74	0.74	0.74
Heparin, units/100mL	50	50	50	50	50	50

4.2.1 Stability indicating HPLC assay for chemical compatibility testing of caffeine

The stability-indicating HPLC assay method developed by Oliphant *et al.* [356] was adapted for use in determination of caffeine concentration. An Apollo C_{18} HPLC column (150×4.6 mm, 5 μm; Hichrom Ltd, Berkshire, England) was used for chromatographic separation. The isocratic mobile phase comprised 85% water and 15% acetonitrile v/v, pumped at a flow rate of 0.9 mL/min. The column oven temperature was maintained at 30°C and the injection volume was 1 μL. The UV detection wavelength was 273 nm.

The Agilent 1200 series HPLC system comprised a binary pump with degasser, auto-sampler, thermostated column oven and a dual wavelength UV detector (Agilent Technology, Waldbronn, Germany). Chemstation software (vRev. B.03.01.SR1; Agilent Technology) was used to acquire and process data.

The HPLC method was validated in accordance with the ICH guidelines [179] for the validation characteristics of linearity, accuracy, intra- and inter- assay precision, and robustness.

To establish linearity and range for the HPLC assay, a calibration curve was constructed using caffeine solutions at concentrations of 1, 2, 3, 4, and 5 mg/mL (n=3). Calibration curve and analyte concentration data were analysed using Microsoft Excel (Version 2309 Build 16.0.16827.20166). The LOD and the LLOQ were estimated as previously described (Chapter 3; section 3.2.1). LLOQ was confirmed by precision data.

Accuracy and precision of the HPLC assay was evaluated at caffeine concentrations of 5, 3, 1 and 0.2 (LLOQ) mg/mL (n=5) using the caffeine reference standard and the commercial caffeine citrate injection/ caffeine base injection diluted with water. The concentrations of the two series were compared (expressed as a fraction of the nominal concentration). Intra-run and inter-run precision were determined by calculating %RSD for the same caffeine concentrations.

To evaluate robustness, caffeine 5 mg/mL (as caffeine base) samples (from caffeine standard, caffeine citrate commercial injection and caffeine base injection; n=5) were tested using slightly modified methods. Changes with respect to standard method parameters included flow rate (1.0 mL/min) and mobile phase composition (water:

acetonitrile 80:20). The accuracy of the modified methods was compared with the standard method.

4.2.2 Preparation of samples for physicochemical compatibility testing

Caffeine citrate and caffeine base injections were used undiluted (20 and 10 mg/mL concentrations respectively). Medications originally contained in glass ampoules and medications requiring reconstitution were filtered with a 0.22 µm syringe filter, before mixing (33 mm×0.22 µm Polyethersulfone (PES) membrane, Millex-GP, Merck Millipore Ltd, Tullagreen, Carrigtwohill, Co. Cork, Ireland).

A total of 43 drugs and 6 PN solutions were selected and endorsed by clinical experts from KEMH. These included drugs which were previously tested for physical compatibility, as compatible/incompatible controls.

Drug combinations were mixed at 1:1 (volume/volume) ratio, to simulate Y-site administration, consistent with previously established methods reported in literature [66, 246, 254, 274, 275, 313]. Drug preparation, mixing and testing were carried out at room temperature (22°C) and fluorescent laboratory lighting conditions.

The first stage of compatibility testing comprised a combination of caffeine citrate 20 mg/mL and caffeine base injection 10 mg/mL with the secondary drug at clinically relevant ‘high-end’ concentration consistent with NICU protocols and expert advice. If incompatibility was detected, the drug combination then tested using caffeine citrate 10 mg/mL with the secondary drug at its ‘high-end’ concentration. If this combination also was also incompatible, the next set of testing comprised of caffeine citrate 20 mg/mL with the secondary drug at its ‘low end’ concentration (if clinically applicable). Finally, the lower end caffeine concentration (caffeine citrate 10 mg/mL) was tested

with the secondary drug ‘lower-end’ concentration, if previous results indicated this could be relevant.

Nine, 2 mL clear glass HPLC vials with impermeable screw cap lids were used for each binary combination of drugs/fluids and the respective control solutions. Caffeine citrate and secondary drug combinations, and the control samples were prepared as described below.

Set 1 – Caffeine citrate injection solution (0.4 mL of 20 mg/mL) and secondary test drug solution (0.4 mL); n=3.

Set 2 – Caffeine citrate injection solution (0.4 mL of 20 mg/mL) diluted with 0.4 mL of the diluent of the secondary test drug (n=3) as the reference control solution for the purpose of visual comparison and HPLC assay of caffeine concentration. For PN solutions, the diluent was D5W.

Set 3 – The test drug solution (0.4 mL) was diluted with 0.4 mL of WFI (n=3) for the purpose of visual comparison.

The same experimental procedure (described above) was followed for caffeine base injection (10 mg/mL) and conducted as a parallel experiment.

4.2.3 Physical compatibility testing

All vials with the above combinations were observed with an unaided eye against a black and white background for any change in colour, haze, precipitation, and evolution of gases. The observations were carried out at time 0 (immediately after mixing), 5, 15, 60 and 120 minutes. Further, at time 0 and after 2 hours, the samples were observed under a polarized light viewer (Apollo I Liquid Viewer with a LED

light source and 1.7× Magnifier, Adelphi Manufacturing Company Ltd, West Sussex, United Kingdom) for any visible precipitation or particulate matter.

Physical incompatibility was based on the visual appearance in comparison to control solutions (set 2 and 3). Inconclusive observations were confirmed by a second independent observer and all physically incompatible combinations were photographed. If precipitation or particles were observed in the drug combination vials, an aliquot was examined under light microscopy (Leica MC190HD, 40 × magnification, Leica Microsystems (Switzerland) Ltd, CH-9435, Heerbrugg, Switzerland).

4.2.4 Chemical compatibility testing

If any physical incompatibility was observed (precipitates), such combinations were not subject to chemical compatibility testing, to avoid contamination of the HPLC system. Samples from set 1 and 2 of caffeine citrate compatibility experiment (at a nominal concentration of 10 mg/mL) and caffeine base injection compatibility experiment (at a nominal concentration of 5 mg/mL) were analysed by the validated HPLC after 2 hours of observation.

The ratio of the mean peak areas was determined, and the 95% CI of the ratio was calculated using the confidence limits from a two-sided t-test (α=0.05; SigmaPlot V.15; Inpixon GmbH, Düsseldorf, Germany). Consistent with previous studies, incompatibility of caffeine:drug combinations was defined as a ratio of the mean peak area outside the range of 90-110% [223, 226, 314].

4.2.5 Physical compatibility testing of caffeine citrate and caffeine base injection with a lipid emulsion

The IV lipid emulsion tested was SMOFlipid 20% (Fresenius Kabi Australia Pty Ltd, North Ryde, NSW, Australia), stated previously (Chapter 3; section 3.2.5). The emulsion (0.5 mL) was combined with caffeine citrate injection (20 & 10 mg/mL), and caffeine base injection (10 mg/mL) separately, at 1:1 volume ratio, to simulate Y-site injection.

Mixing was carried out in 2 mL clear glass vials with screw cap lids, n=5, at room temperature (22°C). The vials were gently mixed and visually inspected at 0, 1 and 2 hours for phase separation, change in colour, gas production or other visually observable changes. The droplet diameter of the lipid emulsion and emulsion/ fluid mixtures was determined at 0 and 2 hours after mixing using the Mastersizer 3000 instrument (Malvern Instruments, Worcestershire, UK). Data capture included the MDD, the MMD (Dv50 or d0.5) and the percentage of droplets in the following diameter bands: <0.01, 0.01-0.05, 0.05-0.1, 0.1-0.2, 0.2-0.5, 0.5-1, 1-5, 5-30 and >30 μm. The formal criterion for compatible lipid/drug mixtures was MDD <0.5 μm ($n \geq 3$) [53, 93, 122, 315]. Data were analysed using Microsoft Excel (Version 2309 Build 16.0.16827.20166) and expressed as mean ± SD unless otherwise indicated.

WFI and NS were mixed with lipid emulsion using the same experimental procedure as described above, as negative controls. Gentamicin 2 mg/mL and 10 mg/mL, which were previously reported to be incompatible with lipid emulsions [92], were used as positive controls.

The Mastersizer's particle size analysing method parameters were adopted from previously reported method [316]. As the refractive index of soybean oil, medium chain triglycerides and olive oil is 1.47, 1.45 and 1.46, respectively, a refractive index

of 1.46 and a density of 0.95 was used. The dispersant was deionised water, sonicated to eliminate air bubbles. The stirrer speed in the wet dispersion unit was 1000 rpm and the absorption index was 0.003.

4.2.6 Evaluation of absorption/adsorption loss of caffeine by syringe filters

Three types of syringe filters and one inline filter composed of different filter membranes (nylon, regenerated cellulose, and polyethersulfone; Table 4.3) were tested to evaluate the absorption/adsorption loss of caffeine during the process of filtration.

Table 4.3 Syringe filter types tested, the membrane, mesh size description and manufacturer details

Abbreviation	Description	Manufacturer/ Supplier
NY	Nylon, 15 mm diameter, 0.2 µm membrane, non-sterile	Phenomenex Australia Pty Ltd, 2 Chaplin Dr, Lane Cove West NSW 2066
RC	Regenerated Cellulose, 15 mm diameter, 0.2 µm membrane, non-sterile	Phenomenex Australia Pty Ltd, 2 Chaplin Dr, Lane Cove West NSW 2066
PES	Millex-GP, Polyethersulfone, 33 mm, 0.22 µm membrane, sterile	Merck Millipore Ltd., Tullagreen, Carrigtwohill, County Cork, Ireland
Inline	Polyethersulfone, 0.2 µm membrane, sterile	Pall Medical, Avenue de Tivoli 3, CH-1700 Fribourg, Switzerland

Caffeine 10 mg/mL and 5 mg/mL solutions were used for filter testing and the drug recovery in the filtrate was determined by the validated HPLC assay. The peak area values obtained with and without filtration were compared and data were reported as percent caffeine recovery according to the formula previously described (Chapter 3, section 3.2.6). Filtrate was collected as five separate, consecutive one-millilitre portions of solution to examine the influence of the volume of filtrate on the drug recovery. Testing was carried out in triplicate and a new filter unit was used for each sample.

4.3 Results

4.3.1 HPLC method validation

Caffeine was detected by the HPLC method at 273 nm with a retention time of approximately 5.8 minutes (Figure 4.2).

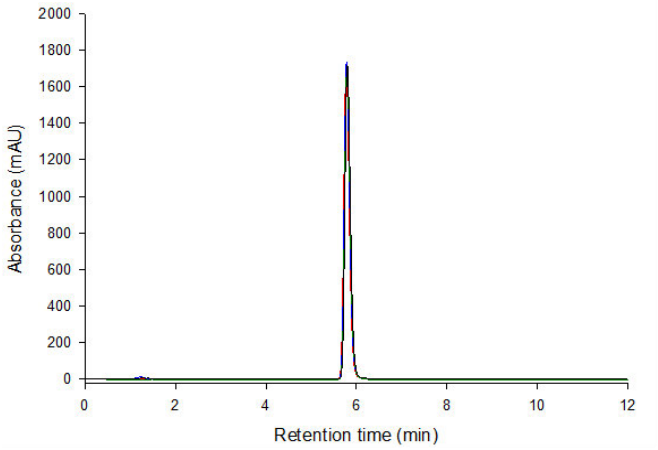


Figure 4.2 Chromatograms of caffeine standard 5 mg/mL (—), caffeine citrate 10 mg/mL (—), caffeine base injection 5 mg/mL (—), a mixture of caffeine standard, caffeine citrate and caffeine base injection in aqueous solution (—); caffeine retention time approximately 5.8 minutes

The assay was linear for caffeine in aqueous solution (n=3) within the concentration range 1-5 mg/mL ($r^2 > 0.999$) (Figure 4.3).

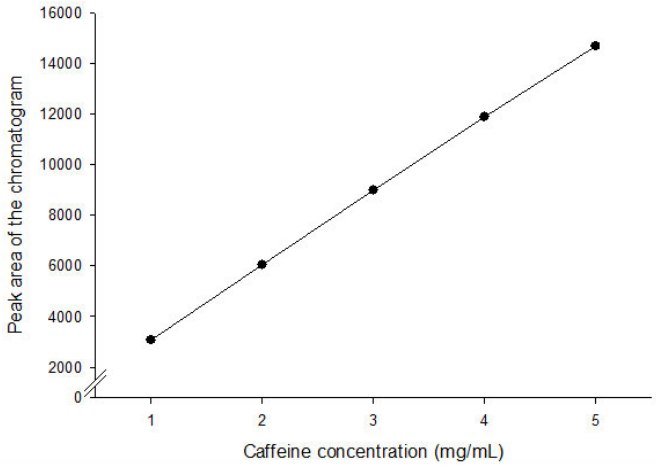


Figure 4.3 Linearity curve for caffeine solution in aqueous solution within the concentration range 1-5 mg/mL (n=3); correlation coefficient (r^2) > 0.999; regression equation Y=2907x+217.13

The LOD and LLOQ for caffeine were 0.07 and 0.2 mg/mL respectively. The HPLC method was accurate and precise according to the standard definitions [179], with accuracy between 98-101.1% for all concentration levels and precision (as %RSD) ≤ 1% for inter- and intra-assay samples (Table 4.4).

Table 4.4 Accuracy, intra-assay, and inter-assay precision data for selected caffeine concentrations (caffeine citrate and caffeine base injection)

Concentration (mg/mL); n=5	Caffeine concentration as a % of nominal concentration (Mean ± SD, n=5)		Intra assay-precision (% RSD)		Inter-assay precision (% RSD pooled)	
	Caffeine citrate	Caffeine base	Caffeine citrate	Caffeine base	Caffeine citrate	Caffeine base
LLOQ	99.3 ± 0.7	99.2 ± 0.1	0.5	0.4	0.4	0.5
1	98.0 ± 0.8	101.1 ± 1.0	0.5	1.0	0.7	0.6
3	98.0 ± 0.8	100.0 ± 1.5	0.4	0.3	0.4	0.4
5	98.3 ± 0.4	99.4 ± 0.6	0.4	0.4	0.2	0.3

The percentage concentrations of caffeine in robustness testing experiment revealed that the method was robust despite deliberate small changes in method parameters (Table 4.5).

Table 4.5 Robustness test results for deliberate changes in method parameters

Parameters	Conditions	Caffeine concentration as a % of nominal concentration, in caffeine citrate injection (Mean ± SD, n=5)	Caffeine concentration as a % of nominal concentration, in caffeine base injection (Mean ± SD, n=5)
Flow rate	0.9 mL/min	98.3 ± 0.4	99.4 ± 0.6
	1.0 mL/min	98.9 ± 0.7	99.7 ± 0.4
Mobile phase composition (water: acetonitrile)	85:15	98.3 ± 0.4	99.4 ± 0.6
	80:20	99.2 ± 0.3	99.6 ± 0.6

4.3.2 Physicochemical compatibility testing

Six of the 43 secondary drugs tested (aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine) were physically incompatible with caffeine citrate undiluted injection, at their high end clinically relevant concentrations. Two of the incompatible drugs were also tested at 'low-end' clinically relevant concentrations: hydrocortisone (1 mg/mL) was physicochemically compatible with caffeine citrate; however, furosemide (0.2 mg/mL) was physically incompatible (Table 4.6). Photographs of physical incompatibilities can be found in Appendix 7.

Table 4.6 Physicochemical compatibility of caffeine citrate 20 mg/mL (10 mg/mL caffeine base) with secondary drugs 2-in-1 PN solutions (see Table 4.2 for details)

Secondary drug	Test concentration	Diluent	P/C	CAF ratio	95% CI of ratio
Aciclovir	5 mg/mL	D5W	I[a]	-	-
Alprostadil	20 µg/mL	NS	C	99.9	98.1 - 101.7
Amoxicillin	100 mg/mL	WFI	C	99.9	99.1 - 100.8
Amphotericin (Fungizone)	100 µg/mL	D5W	C	98.8	97.1 - 100.6
Amphotericin liposomal	2 mg/mL	D5W	I[b]	-	-
Ampicillin	100 mg/mL	WFI	C	99.8	98.9 - 100.7
Benzylpenicillin	100 mg/mL	WFI	C	100.6	99.4 - 101.7
Calcium gluconate	100 mg/mL	U	C	99.5	98.2 - 100.9
Cefotaxime	100 mg/mL	WFI	C	100.2	99.3 - 101.2
Ciprofloxacin*	2 mg/mL	U	C	100.2	99.5 - 100.9
Clonidine	2 µg/mL	NS	C	99.6	98.2 - 101.0
Cloxacillin	100 mg/mL	WFI	C	100.1	98.2 - 101.9
Dobutamine	7.2 mg/mL	NS	C	100.1	99.3 - 100.9
Dobutamine	7.2 mg/mL	D5W	C	100.0	98.8 - 101.3
Dopamine	7.2 mg/mL	NS	C	100.0	99.4 - 100.6
Dopamine	7.2 mg/mL	D5W	C	100.6	99.3 - 101.8
Dexmedetomidine	1 µg/mL	NS	C	99.7	98.4 - 101.1
Epinephrine	64 µg/mL	D5W	C	100.1	99.5 - 100.7
Fentanyl	50 µg/mL	U	C	99.8	98.7 - 101.0
Flucloxacillin	50 mg/mL	D5W	C	99.2	96.9 - 101.5
Fluconazole	2 mg/mL	U	C	99.5	98.6 - 100.5
Furosemide	1 mg/mL	D5W	I[a]	-	-
Furosemide	0.2 mg/mL	D5W	I[c]	-	-
Gentamicin	10 mg/mL	NS	C	99.6	99.1 - 100.1
Heparin	100 units/mL	NS	C	99.7	98.5 - 100.8
Hydrocortisone	10 mg/mL	NS	I[a]	-	-
Hydrocortisone	1 mg/mL	NS	C	99.6	97.5 - 101.7
Indometacin	200 µg/mL	NS	C	99.5	98.7 - 100.3
Ibuprofen	5 mg/mL	NS	I[d]	-	-
Ibuprofen lysine	4 mg/mL	NS	I[d]	-	-
Insulin	0.2 units/mL	NS	C	99.7	98.8 - 100.6
Levetiracetam	5 mg/mL	NS	C	99.5	99.0 - 100.0
Linezolid	2 mg/mL	U	C	99.9	98.8 - 100.9
Meropenem	50 mg/mL	NS	C	98.9	97.1 - 100.8
Metronidazole	5 mg/mL	U	C	99.9	98.9 - 101.0
Midazolam	1 mg/mL	U	C	99.5	97.8 - 101.3
Milrinone	400 µg/mL	D5W	C	100.2	99.7 - 100.8
Morphine hydrochloride	200 µg/mL	D5W	C	99.8	98.4 - 101.2
Morphine sulfate	200 µg/mL	D5W	C	100.6	99.5 - 101.8
Norepinephrine	64 µg/mL	D5W	C	100.3	99.7 - 101.0
Paracetamol	10 mg/mL	U	C	100.2	99.3 - 101.1
Phenobarbitone	20 mg/mL	WFI	C	99.8	99.0 - 100.6
Piperacillin/tazobactam	200 mg/mL	WFI	C	99.8	98.8 - 100.7
Rifampicin	6 mg/mL	NS	C	100.4	98.6 - 102.1
Sodium bicarbonate	4.2% w/v	D5W	C	99.4	98.9 - 99.8
Vancomycin	10 mg/mL	D5W	C	99.3	97.8 - 100.8
Vecuronium	1 mg/mL	WFI	C	99.7	99.4 - 100.1
Parenteral nutrition PN 1	-	-	C	99.2	97.7 - 100.8
Parenteral nutrition PN 2	-	-	C	100.5	99.1 - 102.0
Parenteral nutrition PN 3	-	-	C	99.6	98.9 - 100.2
Parenteral nutrition PN 4	-	-	C	99.9	98.9 - 101.0
Parenteral nutrition PN 5	-	-	C	100.5	99.4 - 101.6
Parenteral nutrition PN 6	-	-	C	99.7	98.8 - 100.6

P/C – Physical compatibility; CAF - Caffeine; C – Compatible; I – Incompatible; D5W – Glucose 5%; WFI – Water for Injection; NS – Normal Saline/ 0.9% Sodium chloride; U – Undiluted; a – A white precipitate appeared 10-15 minutes after mixing; b – A higher opacity observed in the combination samples in comparison to controls; c – Particles observed under polarized light after 30 minutes of mixing; d – a milky turbidity appeared immediately after mixing. * Ciprofloxacin was also tested at 4 hours to obtain a caffeine ratio of 99.6% and a 95% CI of ratio 98.1-101.1%.

All drugs which showed physical incompatibility with caffeine citrate undiluted injection, were also physically incompatible with caffeine citrate 10 mg/mL solution (Table 4.7).

Table 4.7 Physicochemical compatibility of caffeine citrate 10 mg/mL (5 mg/mL caffeine base) with secondary drugs

Secondary drug	Test concentration	Diluent	P/C
Aciclovir	5 mg/mL	D5W	I[a]
Amphotericin liposomal	2 mg/mL	D5W	I[b]
Furosemide	1 mg/mL	D5W	I[a]
Furosemide	0.2 mg/mL	D5W	I[c]
Hydrocortisone	10 mg/mL	NS	I[a]
Ibuprofen	5 mg/mL	NS	I[d]
Ibuprofen lysine	4 mg/mL	NS	I[d]

P/C – Physical compatibility; I – Incompatible; D5W – Glucose 5%; NS – Normal Saline/ 0.9% Sodium chloride; a – A white precipitate appeared 10-15 minutes after mixing; b – A higher opacity observed in the combination samples in comparison to controls; c – Particles observed under polarized light after 30 minutes of mixing; d – A milky turbidity appeared immediately after mixing

All physical incompatibilities were visible to the unaided eye, except the combinations with furosemide 0.2 mg/mL, which required observation under polarized light. As amphotericin (liposomal) was originally a pale-yellow opaque solution, the incompatibility observed was an increase in the opacity in comparison to the control solutions (Photographs and photomicrographs of physically incompatible combinations can be found in Appendix 7).

Further investigation of the incompatibility findings was conducted by mixing the six secondary drugs (separately, as described in section 4.2.2) with citrate buffer pH 4.5 (citric acid monohydrate 5 mg/mL and sodium citrate dihydrate 8.3 mg/mL in water). The same physical incompatibility characteristics (precipitation/haze) were observed

with all six secondary drugs, therefore indicating the citrate buffer was the cause of the incompatibility with caffeine citrate injection. Photographs of physical incompatibilities of citrate butter and secondary drugs can be found in Appendix 8.

In contrast to the caffeine citrate data, all 43 secondary drugs and 6 PN solutions tested were physicochemically compatible with caffeine base injection (Table 4.8), with no visually observable changes and caffeine ratios between 99.3-101.4% for all caffeine-drug/PN combinations tested.

To complement the above results, osmolality of the caffeine citrate 20 mg/mL and caffeine base 10 mg/mL injections was tested and found to be 142 and 269 mOsm/kg, respectively (Osmomat 030 Cryoscopic Osmometer; Gonotec GmbH, Berlin, Germany). By comparison, a recent report indicated that caffeine citrate 20 mg/mL oral solution had an osmolality of 150 mOsm/kg [357].

4.3.3 Physical compatibility of caffeine with lipid emulsion

Caffeine citrate (20 & 10 mg/mL) and caffeine base were compatible with the lipid emulsion, with the MDDs of the combinations being, 0.310, 0.309 and 0.308 μm respectively (Table 4.9), for 2 hours since mixing.

Table 4.8 Physicochemical compatibility of caffeine base injection 10 mg/mL with secondary drugs 2-in-1 PN solutions

Secondary drug	Test concentration	Diluent	P/C	CAF ratio	95% CI of ratio
Aciclovir	5 mg/mL	D5W	C	99.9	98.4 - 101.4
Alprostadil	20 µg/mL	NS	C	99.4	97.8 - 101.1
Amoxicillin	100 mg/mL	WFI	C	100.3	99.6 - 100.9
Amphotericin (Fungizone)	100 µg/mL	D5W	C	101.2	98.9 - 103.5
Amphotericin liposomal	2 mg/mL	D5W	C	100.2	98.8 - 101.6
Ampicillin	100 mg/mL	WFI	C	100.3	98.5 - 102.1
Benzylpenicillin	100 mg/mL	WFI	C	100.2	98.7 - 101.6
Calcium gluconate	100 mg/mL	U	C	100.3	99.1 - 101.5
Cefotaxime	100 mg/mL	WFI	C	99.4	96.9 - 101.8
Ciprofloxacin	2 mg/mL	U	C	99.7	99.1 - 100.3
Clonidine	2 µg/mL	NS	C	99.6	98.2 - 101.1
Cloxacillin	100 mg/mL	WFI	C	100.7	99.6 - 101.7
Dobutamine	7.2 mg/mL	NS	C	100.8	98.4 - 103.2
Dobutamine	7.2 mg/mL	D5W	C	100.0	99.2 - 100.8
Dopamine	7.2 mg/mL	NS	C	100.6	99.6 - 101.5
Dopamine	7.2 mg/mL	D5W	C	100.7	99.3 - 102.1
Dexmedetomidine	1 µg/mL	NS	C	100.9	99.8 - 102.0
Epinephrine	64 µg/mL	D5W	C	99.9	99.3 - 100.4
Fentanyl	50 µg/mL	U	C	100.0	98.7 - 101.4
Flucloxacillin	50 mg/mL	D5W	C	99.8	98.4 - 101.3
Fluconazole	2 mg/mL	U	C	99.6	98.6 - 100.6
Furosemide	1 mg/mL	D5W	C	100.4	99.2 - 101.7
Gentamicin	10 mg/mL	NS	C	99.8	98.9 - 100.7
Heparin	100 units/mL	NS	C	100.1	99.4 - 100.8
Hydrocortisone	10 mg/mL	NS	C	100.6	99.2 - 101.9
Indometacin	200 µg/mL	NS	C	100.4	99.6 - 101.2
Ibuprofen	5 mg/mL	NS	C	99.8	98.5 - 101.2
Ibuprofen lysine	4 mg/mL	NS	C	99.9	98.7 - 101.0
Insulin	0.2 units/mL	NS	C	101.2	98.3 - 104.2
Levetiracetam	5 mg/mL	NS	C	101.1	100.2 - 102.0
Linezolid	2 mg/mL	U	C	100.4	97.8 - 103.0
Meropenem	50 mg/mL	NS	C	99.8	98.8 - 100.7
Metronidazole	5 mg/mL	U	C	100.4	99.5 - 101.3
Midazolam	1 mg/mL	U	C	99.5	98.8 - 100.2
Milrinone	400 µg/mL	D5W	C	99.4	98.2 - 100.5
Morphine hydrochloride	200 µg/mL	D5W	C	99.9	98.8 - 101.0
Morphine sulfate	200 µg/mL	D5W	C	99.6	98.3 - 101.0
Norepinephrine	64 µg/mL	D5W	C	99.7	99.1 - 100.4
Paracetamol	10 mg/mL	U	C	100.0	99.6 - 100.5
Phenobarbitone	20 mg/mL	WFI	C	100.5	98.9 - 102.0
Piperacillin/tazobactam	200 mg/mL	WFI	C	100.0	99.0 - 101.0
Rifampicin	6 mg/mL	NS	C	101.4	99.1 - 103.6
Sodium bicarbonate	4.2% w/v	D5W	C	99.3	98.4 - 100.3
Vancomycin	10 mg/mL	D5W	C	99.9	98.1 - 101.6
Vecuronium	1 mg/mL	WFI	C	100.3	98.6 - 102.0
Parenteral nutrition PN 1	-	-	C	100.1	99.1 - 101.1
Parenteral nutrition PN 2	-	-	C	100.3	98.2 - 102.4
Parenteral nutrition PN 3	-	-	C	99.3	98.0 - 100.7
Parenteral nutrition PN 4	-	-	C	99.7	98.2 - 101.2
Parenteral nutrition PN 5	-	-	C	100.6	99.3 - 101.9
Parenteral nutrition PN 6	-	-	C	99.3	97.6 - 101.1

P/C - Physical compatibility; CAF - Caffeine; C – Compatible; D5W – Glucose 5%; WFI – Water for Injection; NS – Normal Saline/ 0.9% Sodium chloride; U – Undiluted.

Table 4.9 Mean and median droplet diameter data (MDD and Dv50, respectively) at 0 and 2 hours after mixing combinations of lipid emulsion and IV drugs

Fluid/Drug	Concentration	MDD 0 hours (µm)	MDD 2 hours (µm)	Dv50 0 hours (µm)	Dv50 2 hours (µm)
SMOFlipid 20%	-	0.277 ± 0.004	-	0.257 ± 0.005	-
Water for Injection	-	0.310 ± 0.002	0.309 ± 0.001	0.294 ± 0.002	0.294 ± 0.002
Sodium Chloride	0.9% w/v	0.307 ± 0.002	0.309 ± 0.003	0.291 ± 0.002	0.293 ± 0.004
Caffeine citrate	20 mg/mL	0.309 ± 0.001	0.310 ± 0.003	0.293 ± 0.001	0.294 ± 0.003
Caffeine citrate	10 mg/mL	0.300 ± 0.010	0.309 ± 0.001	0.281 ± 0.012	0.293 ± 0.001
Caffeine (base)	10 mg/mL	0.307 ± 0.002	0.308 ± 0.000	0.291 ± 0.002	0.292 ± 0.001
Gentamicin	2 mg/mL	0.391 ± 0.014	0.339 ± 0.029	0.255 ± 0.003	0.243 ± 0.009
Gentamicin	10 mg/mL	**10.8 ± 9.7**	**6.1 ± 8.5**	0.564 ± 0.192	0.523 ± 0.237

Data are mean ± SD of n=5 unless otherwise indicated. Results in bold indicate an incompatible combination.

There was no visual evidence of incompatibility, and no particles of >1 µm were detected in the size distribution plots of volume density (%) against droplet (particle) size (µm) of each of the combinations of caffeine citrate (20 and 10 mg/mL) and caffeine base (10 mg/mL) injection with SMOFlipid (20%), both immediately and 2 hours after mixing (size distribution plots can be found in Appendix 9; Figures 1 to 6).

The MDD of the combination of gentamicin 2 mg/mL with the lipid emulsion was 0.391 µm, immediately after mixing and 0.339 µm 2 hours after mixing (Table 4.9). However, droplets of >1 µm were detected in the particle size distribution (Appendix 9; Figures 7 and 8).

The combination of gentamicin 10 mg/mL with the lipid emulsion had a MDD of 10.793 µm immediately after mixing and 6.114 µm, 2 hours after mixing (Table 4.9). Furthermore, particle size distribution showed particles or aggregates of particles between sizes (Appendix 9; Figures 9 and 10). Visually, the combination demonstrated phase separation after 2 hours of mixing.

WFI and NS were compatible with the lipid emulsion based on the MDD (Table 4.9) and particle size distribution (Appendix 9; Figures 11 - 14).

4.3.4 Evaluation of absorption/adsorption loss of caffeine by syringe filters

All filter types tested, showed a caffeine recovery of 99% in all millilitre portions of the filtrate, at both concentrations (10 and 5 mg/mL) (Figure 4.4 and 4.5).

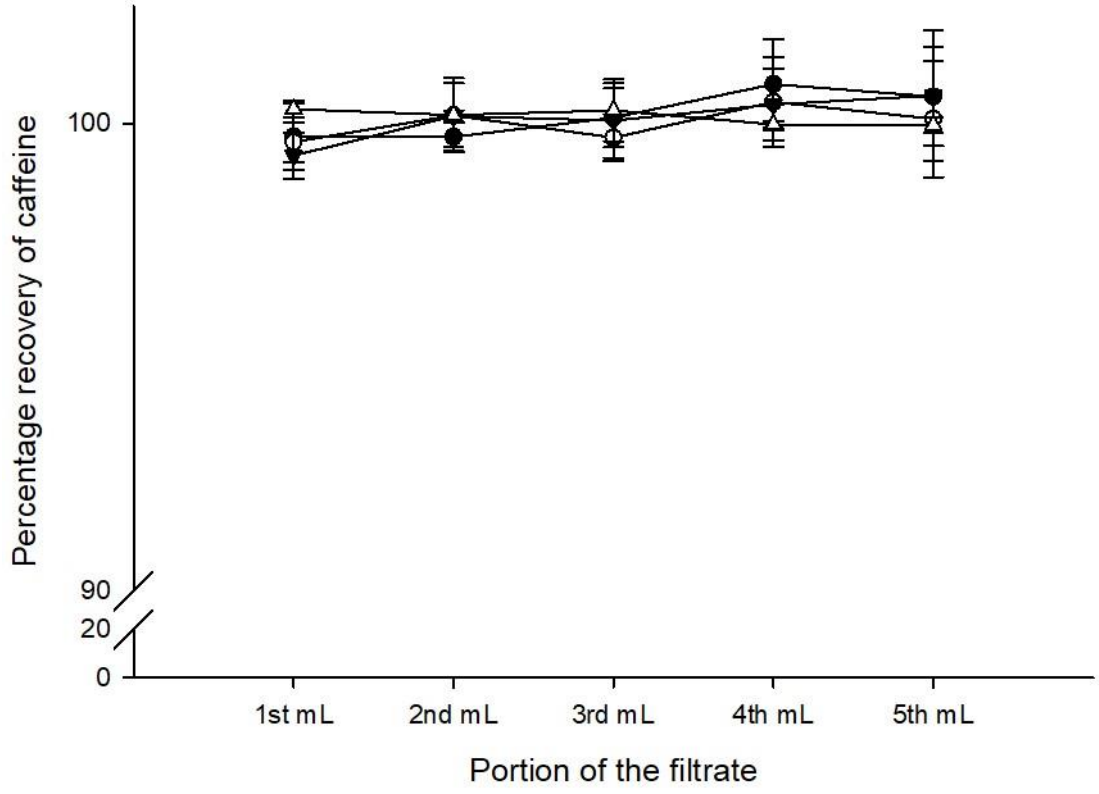


Figure 4.4 Recovery (%) of caffeine 10 mg/mL solution from syringe filters. Caffeine concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ regenerated cellulose; ▼ polyether sulfone; △ inline polyether sulfone;). Data are mean ± SD (n=3).

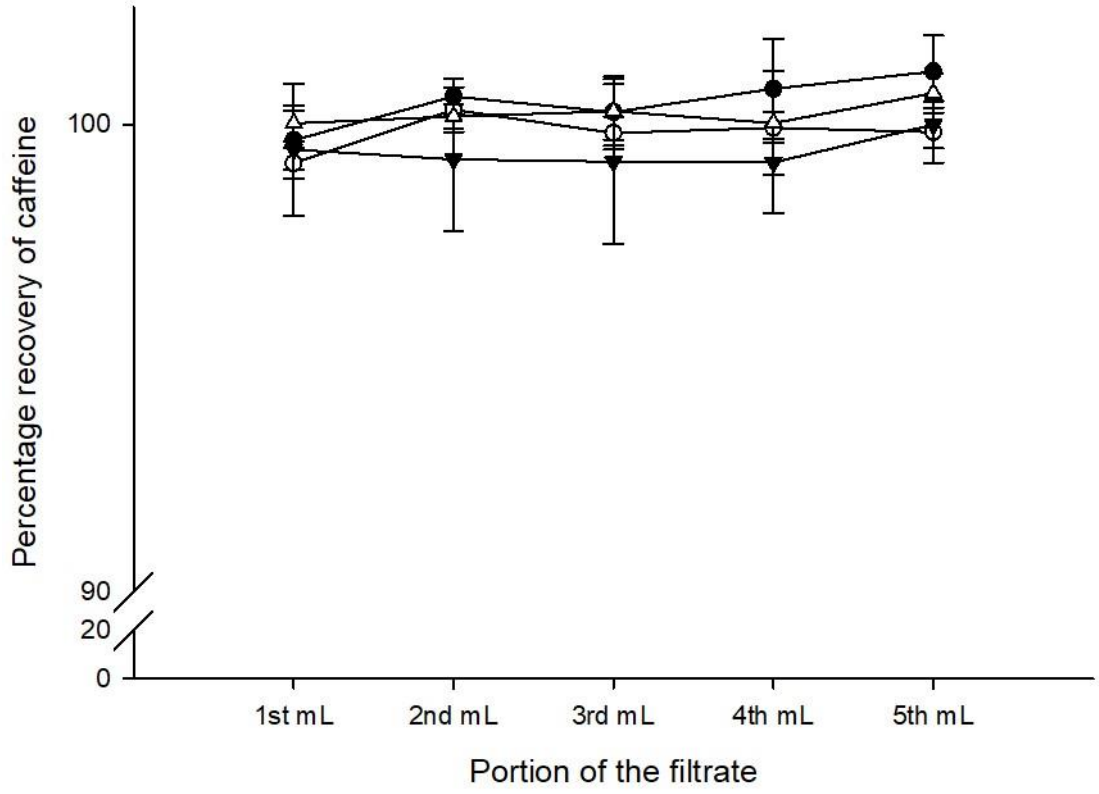


Figure 4.5 Recovery (%) of caffeine 5 mg/mL solution from syringe filters. Caffeine concentration was determined from each of four successive millilitres of solution passed through the filters (● nylon; ○ regenerated cellulose; ▼ polyether sulfone; △ inline polyether sulfone;). Data are mean ± SD (n=3)

4.4 Discussion

The present study has shown that 37 IV drugs tested in a simulated Y-site study design at 'high-end', clinically relevant concentrations for NICU settings were physically and chemically compatible with caffeine citrate 20 mg/mL injection (Table 4.6). The apparent cause of the incompatibility of caffeine citrate injection with aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine injections was found to be the citrate buffer component. By comparison, all 43 drugs were compatible with caffeine base 10 mg/mL injection (Table 4.8). Caffeine citrate and base injections were also compatible with six 2-in-1 PN solutions.

Although physical compatibility information for caffeine citrate with a range of IV drugs has been reported, a modest compilation of chemical compatibility data from manufacturers' package inserts (for dopamine, fentanyl, heparin and calcium gluconate) is available in contemporary guidelines [27]. Consistent with these data, the present study demonstrated physicochemical compatibility of caffeine citrate injection with calcium gluconate, dopamine, fentanyl and heparin, albeit at different concentrations and/or experimental conditions. For example, a mixture of caffeine citrate 20 mg/mL and calcium gluconate 100 mg/mL was found to be physically compatible for 4 [274] and 24 hours [27] at room temperature, and chemically stable for 24 hours at room temperature [27]. These findings provide useful confirmation of our results that caffeine citrate and calcium gluconate injections were physicochemically compatible for 2 hours at room temperature.

Heparin has previously been investigated at 1 unit/mL in D5W, 10 units/mL and 1000 units/mL in combination with caffeine citrate and shown to be physically compatible [27, 274, 275]. The present study complements these reports by demonstrating that heparin 100 units/mL was physicochemically compatible with caffeine citrate, for 2 hours at room temperature (Table 4.6).

Fentanyl 10 µg/mL (in D5W) was reported to be compatible and stable with caffeine citrate for 24 hours at room temperature [27] and two studies have confirmed that fentanyl 50 µg/mL was physically compatible for 4 hours at room temperature [274, 275]. Furthermore, meropenem 50 mg/mL was recently found to be physically compatible with caffeine citrate injection for 4 hours [251]. Hence, these results also provide assurance for the present study, whereby fentanyl 50 µg/mL and meropenem 50 mg/mL separately were found to be physicochemically compatible with caffeine citrate injection (Table 4.6).

The present study also provides evidence of incompatibility between caffeine citrate injection (10 mg/mL and 20 mg/mL) and both ibuprofen (5 mg/mL) and ibuprofen lysine (4 mg/mL), the combinations of which resulted in turbidity immediately after mixing (Appendix 7). Although ibuprofen has not been studied previously for physicochemical compatibility, ibuprofen lysine 20 mg/mL was found to be physically incompatible due to milky white precipitation upon mixing [104].

A range of inconsistent caffeine citrate compatibility data have been reported, some of which may be concentration-dependent or related to the experimental procedures (e.g., duration of admixture or physical methods used to determine compatibility), or the composition of the IV drug formulation [274]. For example, dopamine 0.6 mg/mL (in D5W) was reported to be compatible and stable with caffeine citrate for 24 hours at room temperature [27], and a higher concentration (80 mg/mL) was found to be visually compatible for 4 hours at 25°C [275]. By contrast, Audet and colleagues [274] reported that dopamine 3.2 mg/mL was physically incompatible with caffeine citrate, due to a "yellowish tint" colour change immediately after mixing. However, in the present study, dopamine 7.2 mg/mL (in both D5W and NS) was physically and chemically compatible with caffeine citrate for 2 hours after mixing (Table 4.6). Furthermore, for direct comparison with the previous report [274], combinations of caffeine citrate 20 mg/mL injection with dopamine 3.2 and 1.2 mg/mL (in NS) were investigated and found no evidence of physicochemical incompatibility (physically compatible with no observed colour change and caffeine ratios of 99.4% and 99.1%, respectively).

Conflicting data regarding the compatibility of caffeine citrate with furosemide 10 mg/mL and aciclovir 50 mg/mL (separately) also have been reported, with one study finding the combinations were physically compatible [274], and an earlier study

indicating they were physically incompatible, due to immediate precipitation [275]. By comparison, the present study has shown that lower, clinically relevant concentrations of these drugs (furosemide 1 and 0.2 mg/mL, and aciclovir 5 mg/mL) were physically incompatible with caffeine citrate, as the combinations produced a white precipitate within 15 minutes of mixing (Table 4.6 and appendix 7). These results may indicate concentration-dependent physical incompatibility for mixtures of caffeine citrate and furosemide or aciclovir, which may be evaluated in clinical settings, on the basis of presence/absence of a visible white precipitate.

In regard to amphotericin (liposomal) and hydrocortisone, at concentrations of 4 mg/mL and 250 mg/mL respectively, Audet *et al*. [274] found these two drugs were physically compatible with caffeine citrate for 4 hours at room temperature. By contrast, results in the present study showed that amphotericin (liposomal) and hydrocortisone, at lower clinically relevant NICU concentrations (2 mg/mL and 10 mg/mL respectively) were physically incompatible with caffeine citrate at 10 mg/mL (Table 4.7) and 20 mg/mL (Table 4.6). However, hydrocortisone at a concentration of only 1 mg/mL was physicochemically compatible with caffeine citrate 20 mg/mL (Table 4.6). This finding suggests the lower hydrocortisone IV infusion concentration (1 mg/mL) used in NICU settings may be safely co-administered with caffeine citrate through Y-sites, where required.

Audet *et al*. [274] also reported that midazolam 5 mg/mL was physically incompatible with caffeine citrate, due to formation of a white precipitate at the time of mixing; however, the present study showed that a lower concentration (1 mg/mL) was physicochemically compatible with caffeine citrate (Table 4.6).

Further contradictory studies regarding vancomycin 50 mg/mL or dobutamine 12.5 mg/mL mixed (separately) with caffeine citrate have reported the combinations to be physically compatible [275] and physically incompatible [274], resulting in white precipitate and colour change, respectively, at the time of mixing in the latter study. By comparison, the present study found that vancomycin and dobutamine, at the lower concentrations of 10 mg/mL and 7.2 mg/mL, respectively (in both D5W and NS), were physicochemically compatible with caffeine citrate 20 mg/mL.

One directly conflicting result from the present study relates to the recent report that ciprofloxacin 2 mg/mL was physically incompatible with caffeine citrate 20 mg/mL due to crystal formation at 4 hours of mixing [274]. By contrast, data of the present study indicate the combination is physicochemically compatible for 2 hours at the same concentrations. Hence, to clarify this discrepancy and formally compare the present study with the previous report [274], the combination was tested at 4 hours of mixing and confirmed its physicochemical compatibility in the laboratory, with no physical evidence of precipitate or crystal formation and a caffeine concentration ratio (by HPLC) of 99.6% (Table 4.6). As outlined above, similar inexplicable discrepancies are evident in specific studies [274] and compendia [27], and may require prudent clinical judgement to avoid adverse clinical outcomes.

Compared to the studies of caffeine citrate compatibility with IV drugs, there are no previous comprehensive physical or chemical compatibility studies of caffeine base injection with other drugs. However, the stability of caffeine base in a range of sodium chloride, potassium chloride and glucose IV solutions and PN fluids for up to 24 hours has been reported by Nahata *et al*. [355].

Furthermore, a study carried out to investigate physicochemical compatibility of pentoxifylline has confirmed that its physicochemically compatible with caffeine base injection [109].

Against this background, the present study found that caffeine base injection was physicochemically compatible with all 43 secondary drugs and the six PN solutions tested (Table 4.8). Hence, in the absence of commercial preparations, a locally prepared caffeine base injection may be a useful alternative to caffeine citrate injection for Y-site co-administration with otherwise incompatible IV drugs.

Contradictory to the currently available evidence, caffeine citrate (at 20 mg/mL and 10 mg/mL) was found to be compatible with the lipid emulsion for 2 hours after mixing (Table 4.9), based on the MDD & particle size distribution data [316]. Caffeine base was compatible with the lipid emulsion. WFI and NS were compatible, and gentamicin (at 2 mg/mL and 10 mg/mL) were found to be incompatible. These were consistent with the available reports [316].

The limitations encountered in terms of compatibility testing procedures (e.g. drug combination contact time and exposure temperature) were similar as described in Chapter 3.

As caffeine was compatible with all the filter types tested (NY, RC and PES), with a drug recovery of more than 99% at all filtrate volume levels, it gives health professionals reassurance that these filter membrane types can be used in the syringe filter process without a risk of drug loss. Furthermore, as the drug recovery was optimum regardless of the filtrate volume, filter priming prior to filtration by discarding the first millilitre is not necessary. This avoids drug solution wastage and is particularly advantageous for expensive drugs. In contrast, as discussed in Chapter 3,

filter priming was required for filtration process for sildenafil lower clinically relevant concentration in all types of filter membranes except for NY.

4.5 Conclusion

All secondary test drugs and PN solutions, except aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine, were physicochemically compatible with caffeine citrate injection (20 mg/mL equivalent to caffeine base 10 mg/mL), whereas caffeine base injection (10 mg/mL) was physicochemically compatible with all test drugs and PN solutions tested. Caffeine citrate (20 mg/mL & 10 mg/mL) and caffeine base injection (10 mg/mL) solutions were physically compatible with the lipid emulsion. A caffeine recovery was more than 99% by all tested filters, at all filtrate volume levels at both caffeine (base) concentrations tested (10 and 5 mg/mL).

Chapter 5

General conclusions and recommendations for future work

Well-conducted systematic reviews and meta-analyses provide the highest level of evidence for informed decisions in policy and practice, hence methodological rigour of systematic reviews is an important aspect.

The methodology utilized for the current systematic review composed of the SPIDER systematic review model, a broad search strategy to capture over 27,000 deduplicated articles and screening via the machine learning tool, Research Screener, to expedite the extraction of eligible articles.

The search strategy involved several generic terms, and it was concluded that this requirement to include common terms may be a broader issue for systematic reviews in pharmaceutical sciences and other scientific disciplines. Hence, the iterative process in refining the search strategy of the pilot study was an important evaluation step in developing the systematic review, to maximize the capture of relevant references, and this course of action is highly recommended in future systematic reviews. The literature search and screening process were tested using a pilot study and assessment of inter-reviewer reliability. Many situations in the healthcare industry rely on multiple researchers/ reviewers, hence, the question of consistency, or agreement among the individuals collecting data immediately arises due to the variability among human observers. Well-designed research studies must therefore include procedures that measure agreement among the various individuals involved.

Semi-automated machine learning tools such as Research Screener may then be utilized to efficiently screen the large sets of results (selected articles) of the search strategy, providing a manageable workload and confidence in the outcomes and

scientific rigor of the systematic review. In the present systematic review, all studies were extracted from <10% of the references available for screening, proving the value of machine learning screening tools in screening large databases from search strategies enabling efficient management of extracting articles for full-text review.

Using English language as a limiter during data base searching resulted in a likelihood of overlooking key articles pertaining to the subject of interest, which are published in languages other than English. Hence, it is recommended to prudently use language limiters in database searching if English abstracts and translation resources are available.

The systematic review concluded that there's a clear trend in the past decade of a higher proportion of physicochemical studies, however, combined physicochemical compatibility data has been reported in <30% of published literature. Hence, there should be a higher focus on combined physicochemical compatibility studies in future research, to support clinical decisions.

According to the systematic review results, it's evident that well established NICU medications such as inotropes have very limited chemical compatibility data, hence future physicochemical compatibility studies are recommended for these medicines.

Sildenafil, caffeine, alprostadil, morphine are of higher importance in the clinical setting due to their clinical indications and long duration infusions.

Furthermore, future studies can be directed towards testing compatibility of a wide range of other drugs which were not investigated in the present study. These may include anti-cancer and anti-arrhythmic medications according to their use as IV infusions in the neonatal settings. Furthermore, diluents such as plasma-lyte 148, which is a calcium-free, balanced, crystalloid and isotonic IV fluid can be used, in addition to the commonly used NS and D5W. As plasma-lyte 148 has additional

benefits compared to NS and D5W such as reduced rates of hyponatraemia and seizures and has become a more viable alternative in paediatric medicine.

Furthermore, future studies can include methodological improvements to contemporary Y-site compatibility studies, however, this would incur additional drug/consumable costs and time commitment.

One such suggestion is simulation of higher humidicrib/incubator temperatures in addition to room temperature testing. Although compatibility testing at higher contact temperatures has been reported in previous studies, not all infants in NICU are in humidicribs and infants in other settings are usually in wards/units at room temperature. Furthermore, a proportion of the total length of the Y-site tubing will not be exposed to the higher humidicrib temperature. However, compatibility testing at multiple temperatures will give a comparative conclusion on the impact of temperature to drug compatibility.

Another aspect that could be considered is the exact simulation of Y-site infusion, using Y-site tubing, in addition to static mixing of drug combinations. It's noteworthy, this would add a large cost to the experimental procedure since each set of tubing is expensive and could only be used once, however, it could be interesting for specific investigations to confirm if the static method is a valid alternative. Further, this will reveal any influence of fluid dynamics on the compatibility outcome, particularly if the two drugs are infused at different infusion rates via the Y-site.

Although an order of mixing does not exist in Y-site drug administration, it could be investigated by alternating the order of mixing in a portion of the replicates.

Testing of pH in drug combinations can be added to the compatibility testing experimental protocol to complement the analytical determination of drug concentrations.

Although HPLC analysis was carried out only for the primary drug of interest in the present study, based on the availability of analytical equipment and other resources, secondary drug measurement too could be considered in future compatibility studies.

The present study considered a drug combination contact time of 2 hours based on a previous report than 60 minutes was a plausible maximum contact time during Y-site administration, however, a majority of reported compatibility studies have used a contact time of 4 hours. It is recommended that future researchers consider a range of 'worst-case' scenarios (e.g. longer contact times, high-end concentrations) depending on their practical feasibility.

A recommended compatibility study protocol and compatibility interpretation criteria would be as follows.

Step 1: Sample preparation

Set 1 – Test drug solution + secondary drug solution - n=3; 1:1 volume ratio (test samples)

Set 2 – Test drug + diluent of the secondary drug solution - n=3; 1:1 volume ratio (positive control samples)

Set 3 – Secondary drug + diluent of the test drug solution - n=3; 1:1 volume ratio (negative control samples)

Step 2: Physical compatibility evaluation

Observation for evidence of physical incompatibility (precipitation, colour change, haze, evolution of gas and particles under polarized light) for 4 hours at room temperature and humidicrib temperature

Step 3: Chemical compatibility evaluation

In the absence of physical incompatibility, HPLC assay of the test and control samples to determine chemical compatibility (a drug concentration change of >10% is regarded as a chemical incompatibility)

Sildenafil 600 μg/mL was physicochemically compatible with approximately 70% of the 45 clinically relevant IV drugs used in NICU settings that were tested in the present study. A further seven drugs were compatible with sildenafil 60 μg/mL. Six drugs (amphotericin, flucloxacillin, furosemide, ibuprofen, meropenem and sodium bicarbonate) were incompatible with sildenafil and should not be co-administered via Y-site infusions. Combined physicochemical compatibility should be an important consideration in future studies as physical compatibility does not always guarantee chemical compatibility. For example, in the sildenafil compatibility testing with ibuprofen, the drug concentration ratio was only 74% although no evidence of physical incompatibility was obtained in any of the test samples.

Six 2-in-1 PN solutions were incompatible with sildenafil 600 μg/mL; however, three appeared to be compatible with sildenafil 60 μg/mL and three were deemed incompatible. Sildenafil solution was compatible with nylon syringe filters; however, absorption/adsorption loss from the first millilitre of filtrate occurred with polyethersulfone and cellulose ester filters, which should be avoided for small volumes and/or low concentrations of sildenafil solution. If a requirement arises to use filters with considerable drug loss in the first millilitre portion of the filtrate (polyethersulfone and cellulose ester filters) the first millilitre portion can be discarded in the process of priming the filter, prior to it being injected. This issue can be particularly important in the neonatal setting, as one millilitre can comprise the whole dose for the patient, due to volume restriction.

Regarding physicochemical compatibility of caffeine injections with other NICU drugs, all secondary test drugs and PN solutions, except aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine, were physicochemically compatible with caffeine citrate injection (20 mg/mL equivalent to caffeine base 10 mg/mL), whereas caffeine base injection (10 mg/mL) was physicochemically compatible with all test drugs and PN solutions tested. Experimental investigations of compatibility of the citrate buffer and the drugs which were incompatible with caffeine citrate revealed the fact that it's the citrate component in the excipients in the caffeine citrate formulation, that leads to incompatibility. It's an important finding that there can be instances where the excipient components of the drug formulation could also give rise to incompatibility, not only the main drug molecule. Hence, it's important to consider the components of the formulation during compatibility studies. This also highlights the importance of identifying the batches and manufacturers of different drugs in compatibility studies, due to the diversity of excipients used in different formulations. Furthermore, different formulations of the same drug may exist giving rise to differences in compatibility outcomes e.g. aciclovir is formulated as both a concentrated solution for injection and a powder for reconstitution.

In terms of compatibility, problematic drugs include furosemide, aciclovir, sodium bicarbonate and some antimicrobials, hence these might require more detailed investigation in future research.

There is considerable variability in reported compatibility studies in regard to multiple factors such as drug concentrations, drug combination mixing techniques, clinical setting (NICU/ older children and adults). Consequently, the present study addresses gaps in the IV compatibility literature, due to its clinical relevance to NICU.

Furthermore, as sildenafil and caffeine citrate were very limitedly studied for compatibility in NICU setting, and caffeine base injection had no compatibility information previously reported, the present study findings will contribute to the current compatibility information database with novel data. According to anecdotal information from the local clinicians, the findings of the present study could be directly applied in the clinical treatment protocols in the NICU which is frequently accessed by healthcare professionals on a daily basis.

References

1. Born too soon: decade of action on preterm birth (2023) World Health Organization. https://www.who.int/publications/i/item/9789240073890

2. Ohuma EO, Moller A-B, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, Okwaraji YB, Mahanani WR, Johansson EW, Lavin T (2023) National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. The Lancet. 402:1261-1271. https://doi.org/10.1016/S0140-6736(23)00878-4

3. Australian Preterm Birth Prevention Alliance (2018) https://www.pretermalliance.com.au/ Accessed 25/06/2024

4. Australia's mothers and babies (2023) Australian Institute of Health and Welfare. https://www.aihw.gov.au/reports/mothers-babies/australias-mothers babies/contents/about

5. Perin J, Mulick A, Yeung D, Villavicencio F, Lopez G, Strong KL, Prieto-Merino D, Cousens S, Black RE, Liu L (2022) Global, regional, and national causes of under-5 mortality in 2000-19: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet Child Adolesc Health. 6:106-115. http://dx.doi.org/10.1016/s2352-4642(21)00311-4

6. Howson CP, Kinney MV, McDougall L, Lawn JE (2013) Born too soon: preterm birth matters. Reprod Health. 10:1-9. https://doi.org/10.1186/1742-4755-10-S1-S1

7. Newborn health - World Health Organization. https://www.who.int/westernpacific/health-topics/newborn-health Accessed

8. Drews MB, Ludwig AC, Leititis JU, Daschner FD (1995) Low birth weight and nosocomial infection of neonates in a neonatal intensive care unit. J Hosp Infect. 30:65-72. https://doi.org/10.1016/0195-6701(95)90250-3

9. Carter MF, Xenakis E, Holden A, Dudley D (2012) Neonatal intensive care unit admissions and their associations with late preterm birth and maternal risk factors in a population-based study. J Matern-Fetal Neonatal Med. 25:343-345. https://doi.org/10.3109/14767058.2011.576723

10. Garite TJ, Clark R, Thorp JA (2004) Intrauterine growth restriction increases morbidity and mortality among premature neonates. Am J Obstet Gynecol. 191:481-487. https://doi.org/10.1016/j.ajog.2004.01.036

11. Foglia EE, Langeveld R, Heimall L, Deveney A, Ades A, Jensen EA, Nadkarni VM (2017) Incidence, characteristics, and survival following cardiopulmonary resuscitation in the quaternary neonatal intensive care unit. Resuscitation. 110:32-36. https://doi.org/10.1016/j.resuscitation.2016.10.012

12. Dees E, Lin H, Cotton RB, Graham TP, Dodd DA (2000) Outcome of preterm infants with congenital heart disease. J Pediatr. 137:653-659. https://doi.org/10.1067/mpd.2000.108568

13. Kommawar A, Borkar R, Vagha J, Lakhkar B, Meshram R, Taksandae A (2017) Study of respiratory distress in newborn. Int J Contemp Pediatr. 4:490-494. http://dx.doi.org/10.18203/2349-3291.ijcp20170695

14. Haidari ES, Lee HC, Illuzzi JL, Phibbs CS, Lin H, Xu X (2021) Hospital variation in admissions to neonatal intensive care units by diagnosis severity and category. J Perinatol. 41:468-477. https://doi.org/10.1038/s41372-020-00775-z

15. Linakis MW, Roberts JK, Lala AC, Spigarelli MG, Medlicott NJ, Reith DM, Ward RM, Sherwin CM (2016) Challenges associated with route of administration in neonatal drug delivery. Clin Pharmacokinet. 55:185-196. http://dx.doi.org/10.1007/s40262-015-0313-z

16. Skouroliakou M, Kountouri AM, Hatziantoniou S, Koutri K, Chiou A (2012) Physicochemical stability assessment of all-in-one parenteral emulsion for neonates containing SMOFlipid. Eur J Hosp Pharm. 19:514-518. http://dx.doi.org/10.1136/ejhpharm-2012-000121

17. Matsushita FY, Krebs VLJ, de Carvalho WB (2022) Association between fluid overload and mortality in newborns: a systematic review and meta-analysis. Pediatr Nephrol.1-10. https://doi.org/10.1007/s00467-021-05281-8

18. Stiers JL, Ward RM (2014) Newborns, one of the last therapeutic orphans to be adopted. JAMA Pediatr. 168:106-108. http://dx.doi.org/10.1001/jamapediatrics.2013.4604

19. Usher R, Shephard M, Lind J (1963) The blood volume of the newborn infant and placental transfusion. Acta Paediatr. 52:497-512. https://doi.org/10.1111/j.1651-2227.1963.tb03809.x

20. Sherwin CMT, Medlicott NJ, Reith DM, Broadbent RS (2014) Intravenous drug delivery in neonates: lessons learnt. Arch Dis Child. 99:590-594. http://dx.doi.org/10.1136/archdischild-2013-304887

21. Koren G (1997) Therapeutic drug monitoring principles in the neonate. Clin Chem. 43:222-227. https://doi.org/10.1093/clinchem/43.1.222

22. O'Brien F, Clapham D, Krysiak K, Batchelor H, Field P, Caivano G, Pertile M, Nunn A, Tuleu C (2019) Making medicines baby size: The challenges in bridging the formulation gap in neonatal medicine. Int J Mol Sci. 20:2688. https://dx.doi.org/10.3390/ijms20112688

23. Krzyżaniak N, Pawłowska I, Bajorek B (2016) Review of drug utilization patterns in NICU s worldwide. J Clin Pharm Ther. 41:612-620. https://doi.org/10.1111/jcpt.12440

24. Gaetani M, Frndova H, Seto W, Parshuram C (2017) Concurrent intravenous drug administration to critically ill children: Evaluation of frequency and compatibility. J Crit Care. 41:198-203. http://dx.doi.org/10.1016/j.jcrc.2017.05.027

25. Kalikstad B, Skjerdal Å, Hansen TWR (2010) Compatibility of drug infusions in the NICU. Arch Dis Child. 95:745-748. http://dx.doi.org/10.1136/adc.2009.174268

26. Beauman SS, Swanson A (2006) Neonatal infusion therapy: preventing complications and improving outcomes. Newborn Infant Nurs Rev. 6:193-201. https://doi.org/10.1053/j.nainr.2006.09.001

27. ASHP Injectable Drug Information : A Comprehensive Guide to Compatibility and Stability. (2022).American Society of Health-System Pharmacists: Bethesda (MD).

28. Driscoll DF, Bistrian BR, Demmelmair H, Koletzko B (2008) Pharmaceutical and clinical aspects of parenteral lipid emulsions in neonatology. Clin Nutr. 27:497-503. http://dx.doi.org/10.1016/j.clnu.2008.05.003

29. Hanifah S, Putri NS, Hadi NS, Sari CP, Nafiah Z (2022) Intravenous drugs use and potency of incompatibility in a neonatal intensive care unit (NICU). Eur J Clin Pharm. 24:79-83.

30. Tissot E, Cornette C, Demoly P, Jacquet M, Barale F, Capellier G (1999) Medication errors at the administration stage in an intensive care unit. Intensive Care Med. 25:353-359. https://doi.org/10.1007/s001340050857

31. Leopoldino RW, Costa HT, Costa TX, Martins RR, Oliveira AG (2018) Potential drug incompatibilities in the neonatal intensive care unit: A network analysis approach. BMC Pharmacol Toxicol. 19:1-6. http://dx.doi.org/10.1186/s40360-018-0265-7

32. Kanji S, Lam J, Johanson C, Singh A, Goddard R, Fairbairn J, Lloyd T, Monsour D, Kakal J (2010) Systematic review of physical and chemical compatibility of commonly used medications administered by continuous infusion in intensive care units. Crit Care Med. 38:1890-1898. https://doi.org/10.1097/CCM.0b013e3181e8adcc

33. Staven V, Wang S, Grønlie I, Tho I (2016) Development and evaluation of a test program for Y-site compatibility testing of total parenteral nutrition and intravenous drugs. Nutr J. 15:1-18. http://dx.doi.org/10.1186/s12937-016-0149-x

34. Gikic M, Di Paolo ER, Pannatier A, Cotting J (2000) Evaluation of physicochemical incompatibilities during parenteral drug administration in a paediatric intensive care unit. Pharm World Sci. 22:88-91. https://doi.org/10.1023/A:1008780126781

35. Häni C, Vonbach P, Fonzo-Christe C, Russmann S, Cannizzaro V, Niedrig DF (2019) Evaluation of incompatible coadministration of continuous intravenous infusions in a pediatric/neonatal intensive care unit. J Pediatr Pharmacol Ther. 24:479-488. http://dx.doi.org/10.5863/1551-6776-24.6.479

36. Hecq J-D, Krämer I, Vigneron J (2019) European Databases on Stability and Compatibility of Injectable Medicinal Products in Europe. Pharm Technol Hosp Pharm. 4:113-117. https://doi.org/10.1515/pthp-2019-0012

37. Belgado BS, Hatton RC, Doering PL (1997) Evaluation of electronic drug information resources for answering questions received by decentralized pharmacists. Am J Health Syst Pharm. 54:2592-2596. https://doi.org/10.1093/ajhp/54.22.2592

38. De Giorgi I, Guignard B, Fonzo-Christe C, Bonnabry P (2010) Evaluation of tools to prevent drug incompatibilities in paediatric and neonatal intensive care units. Pharm World Sci. 32:520-529. https://doi.org/10.1007/s11096-010-9403-z

39. King Edward Memorial Hospital - Clinical Neonatal Medication Protocols. https://www.kemh.health.wa.gov.au/For-Health-Professionals/Clinical-Guidelines/Neonatal Accessed 16/07/2023

40. South Australian Neonatal Medication Guidelines - Intravenous medication compatibility chart.: https://www.sahealth.sa.gov.au Accessed 25/06/2024

41. Maison O, Tardy C, Cabelguenne D, Parat S, Ducastelle S, Piriou V, Lepape A, Lalande L (2019) Drug incompatibilities in intravenous therapy: evaluation and proposition of preventive tools in intensive care and hematology units. Eur J Clin Pharmacol. 75:179-187. https://doi.org/10.1007/s00228-018-2602-6

42. Abdelkader A, Fathi HA, Hamad MA, Elsabahy M (2020) Nanomedicine: a new paradigm to overcome drug incompatibilities. J Pharm Pharmacol. 72:1289-1305. https://doi.org/10.1111/jphp.13292

43. Newton DW (2009) Drug incompatibility chemistry. Am J Health Syst Pharm. 66:348-357. https://doi.org/10.2146/ajhp080059

44. Walker SE, Fan-Lun C, Wyllie A, Iazzetta J, Law S (2004) Physical compatibility of pantoprazole with selected medications during simulated Y-site administration. Can J Hosp Pharm. 57:90-97. https://doi.org/10.4212/cjhp.v57i2.358

45. Ambados F (2002) Incompatibility between calcium and sulfate ions in solutions for injection. JPPR. 32:307-309. http://dx.doi.org/10.1002/jppr2002324307

46. Bentley J, Heard K, Collins G, Chung C (2015) Mixing medicines: how to ensure patient safety. Pharm J. 294. https://doi.org/10.1211/pj.2015.20068289

47. Morris ME (1978) Compatibility and Stability of Diazepam Injection Following Dilution with Intravenous Fluids. Am J Hosp Pharm. 35:669-672. https://doi.org/10.1093/ajhp/35.6.669

48. Onuki Y, Hasegawa N, Kida C, Ikegami-Kawai M, Tsubuki M, Shirozu S, Obata Y, Takayama K (2015) Supersaturated state of diazepam injection following dilution with infusion fluid. JPHCS. 1:9. https://doi.org/10.1186/s40780-014-0009-9

49. Mantong ML, Marquardt ED (1995) Visual compatibility of midazolam hydrochloride with selected drugs during simulated Y-site injection. Am J Health Syst Pharm. 52:2567-2568. http://dx.doi.org/10.1093/ajhp/52.22.2567

50. Dasta JF, Hale KN, Stauffer GL, Tschampel MM (1988) Comparison of visual and turbidimetric methods for determining short-term compatibility of intravenous critical-care drugs. Am J Hosp Pharm. 45:2361-2366. http://dx.doi.org/10.1093/ajhp/45.11.2361

51. Ribas Nicolau B, Pérez Juan E, Amorós Cerdá SM, Arévalo Rubert MJ, Maqueda Palau M (2011) Physical compatibility of sodium bicarbonate with other drugs often administered in the intensive care unit. Enferm Intensiva. 22:78-82. https://doi.org/10.1016/j.enfi.2010.09.004

52. Pesko L, Arend K, Hagman D (1988) Physical compatibility and stability of metoclopramide injection. Parenterals. 5:1-3.

53. Staven V, Iqbal H, Wang S, Grønlie I, Tho I (2017) Physical compatibility of total parenteral nutrition and drugs in Y-site administration to children from neonates to adolescents. J Pharm Pharmacol. 69:448-462. http://dx.doi.org/10.1111/jphp.12647

54. Newton DW (1978) Physicochemical determinants of incompatibility and instability in injectable drug solutions and admixtures. Am J Health Syst Pharm. 35:1213-1222. https://doi.org/10.1093/ajhp/35.10.1213

55. Matoi J, Jeffrey LP (1978) Formulation of a trace element solution for long-term parenteral nutrition. Am J Hosp Pharm. 35:165-168.

56. Uccello-Barretta G, Balzano F, Aiello F, Falugiani N, Desideri I (2015) Stability of hydrophilic vitamins mixtures in the presence of electrolytes and trace elements for parenteral nutrition: A nuclear magnetic resonance spectroscopy investigation. J Pharm Biomed Anal. 107:7-10. https://doi.org/10.1016/j.jpba.2014.12.008

57. Bouchoud L, Sadeghipour F, Klingmuller M, Fonzo-Christe C, Bonnabry P (2010) Long-term physico-chemical stability of standard parenteral nutritions for neonates. Clin Nutr. 29:808-812. http://dx.doi.org/10.1016/j.clnu.2010.04.004

58. Watrobska-Swietlikowska D, MacLoughlin R (2019) The effect of UV-protected ethylene vinyl acetate (EVA) bags on the physicochemical stability of pediatric parenteral nutrition admixtures. DARU. 27:255-264. http://dx.doi.org/10.1007/s40199-019-00270-7

59. Ferguson TI, Emery S, Price-Davies R, Cosslett AG (2014) A review of stability issues associated with vitamins in parenteral nutrition. ESPEN J. 9:e49-e53. https://doi.org/10.1016/j.clnme.2014.01.001

60. Dupertuis YM, Morch A, Fathi M, Sierro C, Genton L, Kyle UG, Pichard C (2002) Physical characteristics of total parenteral nutrition bags significantly affect the stability of vitamins C and B1: a controlled prospective study. J Parenter Enteral Nutr. 26:310-316. https://doi.org/10.1177/0148607102026005310

61. Edward SM (1967) pH—An Important Factor in the Compatibility of Additives in Intravenous Therapy. Am J Hosp Pharm. 24:440-449. https://doi.org/10.1093/ajhp/24.8.440

62. Webb JW (1969) A pH Pattern for I.V. Additives. Am J Hosp Pharm. 26:31-35. https://doi.org/10.1093/ajhp/26.1.31

63. Newton DW, Kluza RB (1978) pKa values of medicinal compounds in pharmacy practice. Drug Intell Clin Pharm. 12:546-554. https://doi.org/10.1177/106002807801200906

64. Carlin HS, Perkins AJ (1968) Predicting Pharmaceutical Incompatibilities of Parenteral Medications. Am J Hosp Pharm. 25:271-279. https://doi.org/10.1093/ajhp/25.6.271

65. Parker WA (1976) Physical compatibilities of preanesthetic medications. Can J Hosp Pharm. 29:91-92.

66. Allen Jr LV, Levinson RS, Phisutsinthop D (1977) Compatibility of various admixtures with secondary additives at Y-injection sites of intravenous administration sets. Am J Hosp Pharm. 34:939-943. https://dx.doi.org/10.1093/ajhp/34.9.939

67. Laegeler WL, Tio JM, Blake MI (1974) Stability of certain amino acids in a parenteral nutrition solution. Am J Health Syst Pharm. 31:776-779. https://doi.org/10.1093/ajhp/31.8.776

68. Shoup LK (1967) Reconstitution of Parenterals. Am J Hosp Pharm. 24:692-695. https://doi.org/10.1093/ajhp/24.12.692

69. Benlabed M, Perez M, Gaudy R, Genay S, Lannoy D, Barthélémy C, Odou P, Lebuffe G, Décaudin B (2019) Clinical implications of intravenous drug incompatibilities in critically ill patients. Anaesth Crit Care Pain Med. 38:173-180. https://doi.org/10.1016/j.accpm.2018.04.003

70. Bertsche T, Mayer Y, Stahl R, Hoppe-Tichy T, Encke J, Haefeli WE (2008) Prevention of intravenous drug incompatibilities in an intensive care unit. Am J Health Syst Pharm. 65:1834-1840. http://dx.doi.org/10.2146/ajhp070633

71. Leal KDB, Leopoldino RWD, Martins RR, Veríssimo LM (2016) Potential intravenous drug incompatibilities in a pediatric unit. Einstein (Sao Paulo). 14:185-189. http://dx.doi.org/10.1590/S1679-45082016AO3723

72. Boehne M, Jack T, Köditz H, Seidemann K, Schmidt F, Abura M, Bertram H, Sasse M (2013) In-line filtration minimizes organ dysfunction: new aspects from a prospective, randomized, controlled trial. BMC Pediatr. 13:1-8. https://doi.org/10.1186/1471-2431-13-21

73. Jack T, Brent BE, Boehne M, Müller M, Sewald K, Braun A, Wessel A, Sasse M (2010) Analysis of particulate contaminations of infusion solutions in a pediatric intensive care unit. Intensive Care Med. 36:707-711. http://dx.doi.org/10.1007/s00134-010-1775-y

74. Flamein F, Storme L, Maiguy-Foinard A, Perez M, Décaudin B, Masse M, Genay S, Odou P (2017) Avoid drug incompatibilities: clinical context in neonatal intensive care unit (NICU). Pharm Technol Hosp Pharm. 2:71-78. https://doi.org/10.1515/pthp-2017-0009

75. Bradley JS, Wassel RT, Lee L, Nambiar S (2009) Intravenous ceftriaxone and calcium in the neonate: assessing the risk for cardiopulmonary adverse events. Pediatrics. 123:e609-e613. https://doi.org/10.1542/peds.2008-3080

76. McNearney T, Bajaj C, Boyars M, Cottingham J, Haque A (2003) CASE REPORT: total parenteral nutrition associated crystalline precipitates resulting in pulmonary artery occlusions and alveolar granulomas. Dig Dis Sci. 48:1352-1354.

77. Reedy JS, Kuhlman JE, Voytovich M (1999) Microvascular Pulmonary Emboli Secondary to Precipitated Crystals in a Patient Receiving Total Parenteral Nutrition: A Case Report and Description of the High-Resolution CT Findings. Chest. 115:892-895. https://doi.org/10.1378/chest.115.3.892

78. Sasse M, Dziuba F, Jack T, Köditz H, Kaussen T, Bertram H, Beerbaum P, Boehne M (2015) In-line Filtration Decreases Systemic Inflammatory Response Syndrome, Renal and Hematologic Dysfunction in Pediatric Cardiac Intensive Care Patients. Pediatr Cardiol. 36:1270-1278. https://doi.org/10.1007/s00246-015-1157-x

79. Boehne M, Jack T, Dzuiba F, Köditz H, Kaussen T, Bertram H, Beerbaum P, Sasse M (2014) In-line filtration decreases systemic inflammatory response syndrome, renal and hematologic dysfunction in pediatric cardiac intensive care patients. Thorac Cardiovasc Surg. 62:v37. http://dx.doi.org/10.1055/s-0034-1394013

80. Jack T, Boehne M, Brent BE, Hoy L, Köditz H, Wessel A, Sasse M (2012) In-line filtration reduces severe complications and length of stay on pediatric intensive care unit: a prospective, randomized, controlled trial. Intensive Care Med. 38:1008-1016. http://dx.doi.org/10.1007/s00134-012-2539-7

81. Martins AM, McDougal A, Hamilton D, Igwemezie L, McErlane K (1991) In vitro assessment of vancomycin HCl compatibility after coinfusion with a specialized amino acid formulation. J Parenter Enteral Nutr. 15:536-539. http://dx.doi.org/10.1177/0148607191015005536

82. Ziegler TR, Leader LM, Jonas CR, Griffith DP (1997) Adjunctive therapies in nutritional support. Nutrition. 13:64-72. http://dx.doi.org/10.1016/s0899-9007%2897%2983046-8

83. Klein-Gitelman MS, Pachman LM (1998) Intravenous corticosteroids: Adverse reactions are more variable than expected in children. J Rheumatol. 25:1995-2002.

84. De Basagoiti A, Katsumiti A, Abascal S, Bustinza A, Lopez-Gimenez LR, Pascual P, De Miguel M, Campino A (2021) Physical compatibility of alprostadil with selected drugs commonly used in the neonatal intensive care units. Eur J Pediatr. 180:1169-1176. http://dx.doi.org/10.1007/s00431-020-03854-7

85. Veltri MA, Conner KG (2002) Physical compatibility of milrinone lactate injection with intravenous drugs commonly used in the pediatric intensive care unit. Am J Health Syst Pharm. 59:452-454. https://dx.doi.org/10.1093/ajhp/59.5.452

86. Newton DW, Driscoll DF (2008) Calcium and phosphate compatibility: Revisited again. Am J Health Syst Pharm. 65:73-80. http://dx.doi.org/10.2146/ajhp070138

87. Driscoll DF, Bhargava HN, Li L, Zaim RH, Babayan VK, Bistrian BR (1995) Physicochemical stability of total nutrient admixtures. Am J Health Syst Pharm. 52:623-634. https://dx.doi.org/10.1093/ajhp/52.6.623

88. Nezvalova-Henriksen K, Nilsson N, Osterberg CT, Berge VS, Tho I (2020) Y-site physical compatibility of numeta g13e with drugs frequently used at neonatal intensive care. Pharmaceutics. 12:1-13. http://dx.doi.org/10.3390/pharmaceutics12070677

89. Aeberhard C, Steuer C, Saxer C, Huber A, Stanga Z, Mühlebach S (2017) Physicochemical stability and compatibility testing of levetiracetam in all-in-one parenteral nutrition admixtures in daily practice. Eur J Pharm Sci. 96:449-455. http://dx.doi.org/10.1016/j.ejps.2016.10.015

90. Garcia J, Garg A, Song Y, Fotios A, Andersen C, Garg S (2018) Compatibility of intravenous ibuprofen with lipids and parenteral nutrition, for use as a continuous infusion. PLoS One. 13:1-14. http://dx.doi.org/10.1371/journal.pone.0190577

91. Gellis C, Sautou-Miranda V, Arvouet A, Vasson MP, Chopineau J (2001) Stability of methylprednisolone sodium succinate in pediatric parenteral nutrition mixtures. Am J Health Syst Pharm. 58:1139-1142. http://dx.doi.org/10.1093/ajhp/58.12.1139

92. Ross EL, Salinas A, Petty K, Her C, Carpenter JF (2020) Compatibility of medications with intravenous lipid emulsions: Effects of simulated Y-site mixing. Am J Health Syst Pharm. 77:1980-1985. https://doi.org/10.1093/ajhp/zxaa299

93. Staven V, Wang S, Grønlie I, Tho I (2018) Physical stability of an all-in-one parenteral nutrition admixture for preterm infants upon mixing with micronutrients and drugs. Eur J Hosp Pharm. 27:36-42. http://dx.doi.org/10.1136/ejhpharm-2018-001562

94. AlSalman F, Howlett M, Breatnach C, Kelly H, O'Brien F (2020) Supporting the use of sildenafil infusions in paediatric and neonatal intensive care – A compatibility study. Eur J Pharm Biopharm. 151:153-161. http://dx.doi.org/10.1016/j.ejpb.2020.04.008

95. Bhatt-Mehta V, Nahata MC (1990) Stability of dopamine hydrochloride injection in the presence of dobutamine hydrochloride, tolazoline hydrochloride, and theophylline injections. J Perinatol. 10:129-133.

96. Campbell AL, Petrovski M, Senarathna S, Mukadam N, Strunk T, Batty KT (2020) Compatibility of pentoxifylline and parenteral medications. Arch Dis Child. 105:395-397. http://dx.doi.org/10.1136/archdischild-2019-317912

97. Colding H, Andersen GE (1978) Stability of antibiotics and amino acids in two synthetic L-amino acid solutions commonly used for total parenteral nutrition in children. Antimicrob Agents Chemother. 13:555-558. http://dx.doi.org/10.1128/AAC.13.4.555

98. Dawson R, Wignell A, Cooling P, Barrett D, Vyas H, Davies P (2019) Physico-chemical stability of Plasma-Lyte 148® and Plasma-Lyte 148® + 5% Glucose with eight common intravenous medications. Paediatr Anaesth. 29:186-192. http://dx.doi.org/10.1111/pan.13554

99. Dice JE (2006) Physical Compatibility of Alprostadil with Commonly Used IV Solutions and Medications in the Neonatal Intensive Care Unit. J Pediatr Pharmacol Ther. 11:233-236. http://dx.doi.org/10.5863/1551-6776-11.4.233

100. Feigin RD, Moss KS, Shackelford PG (1973) Antibiotic stability in solutions used for intravenous nutrition and fluid therapy. Pediatrics. 51:1016-1026.

101. Fox LM, Wilder AG, Foushee JA (2013) Physical compatibility of various drugs with neonatal total parenteral nutrient solution during simulated Y-site administration. Am J Health Syst Pharm. 70:520-524. http://dx.doi.org/10.2146/ajhp110715

102. Greenhill K, Hornsby E, Gorman G (2019) Investigations of physical compatibilities of commonly used intravenous medications with and without parenteral nutrition in pediatric cardiovascular intensive care unit patients. Pharmaceuticals. 12:1-9. http://dx.doi.org/10.3390/ph12020067

103. Hammond S, Wignell A, Cooling P, Barrett DA, Davies P (2020) Plasma-Lyte 148 and Plasma-Lyte 148 + 5% glucose compatibility with commonly used critical care drugs. Intensive Care Med Exp. 8:25-25. http://dx.doi.org/10.1186/s40635-020-00311-5

104. Holt RJ, Siegert SWK, Krishna A (2008) Physical Compatibility of Ibuprofen Lysine Injection with Selected Drugs During Simulated Y-site Injection. J Pediatr Pharmacol Ther. 13:156-161. https://dx.doi.org/10.5863/1551-6776-13.3.156

105. Izgi M, Basaran B, Muderrisoglu A, Ankay Yilbas A, Uluer MS, Celebioglu B (2018) Evaluation of the stability and stratification of propofol and ketamine mixtures for pediatric anesthesia. Paediatr Anaesth. 28:275-280. http://dx.doi.org/10.1111/pan.13318

106. Luu Y, Thigpen J, Brown SD (2017) Stability of sildenafil in combination with heparin and dopamine. Am J Health Syst Pharm. 74:e64-e71. http://dx.doi.org/10.2146/ajhp150853

107. Mayhew SL, Quick MW (1997) Compatibility of iron dextran with neonatal parenteral nutrient solutions. Am J Health Syst Pharm. 54:570-571. http://dx.doi.org/10.1093/ajhp/54.5.570

108. Palmero D, Chavan E, Berger-Gryllaki M, Tolsa JF, Di Paolo ER, Pannatier A, Henry H, Sadeghipour F (2018) Stability of prostaglandin E1 solutions stored in polypropylene syringes for continuous intravenous administration to newborns. Eur J Hosp Pharm. 25:e109-e114. http://dx.doi.org/10.1136/ejhpharm-2017-001205

109. Senarathna SMDKG, Strunk T, Petrovski M, Batty KT (2019) Physical compatibility of pentoxifylline and intravenous medications. Arch Dis Child. 104:292-295. http://dx.doi.org/10.1136/archdischild-2018-315376

110. Seto W, Trope A, Carfrae L, Walker S (2001) Visual compatibility of sodium nitroprusside with other injectable medications given to pediatric patients. Am J Health Syst Pharm. 58:1422-1426. http://dx.doi.org/10.1093/ajhp/58.15.1422

111. Sykes R, McPherson C, Foulks K, Wade J, Gal P (2008) Aminophylline compatibility with neonatal total parenteral nutrition. J Pediatr Pharmacol Ther. 13:76-769. http://dx.doi.org/10.5863/1551-6776-13.2.76

112. Tounian P, Jehl F, Pauliat S, Morgant G, Ghirardi L, Selva MA, Fontaine JL, Aymard P, Girardet JP (1999) Stability and compatibility of Teicoplanin in parenteral nutrition solutions used in pediatrics. Clin Nutr. 18:159-165. http://dx.doi.org/10.1016/S0261-5614(99)80006-5

113. Veltri M, Lee CKK (1996) Compatibility of neonatal parenteral nutrient solutions with selected intravenous drugs. Am J Health Syst Pharm. 53:2611-2613. http://dx.doi.org/10.1093/ajhp/53.21.2611

114. Volonté MG, Valora PD, Cingolani A, Ferrara M (2005) Stability of ibuprofen in injection solutions. Am J Health Syst Pharm. 62:630-633. http://dx.doi.org/10.1093/ajhp/62.6.630

115. Watson D (1985) Piggyback Compatibility of Antibiotics with Pediatric Parenteral Nutrition Solutions. J Parenter Enteral Nutr. 9:220-224. http://dx.doi.org/10.1177/0148607185009002220

116. Kirupakaran K, Mahoney L, Rabe H, Patel BA (2017) Understanding the Stability of Dopamine and Dobutamine Over 24 h in Simulated Neonatal Ward Conditions. Pediatric Drugs. 19:487-495. http://dx.doi.org/10.1007/s40272-017-0234-4

117. Bouchoud L, Fonzo-Christe C, Sadeghipour F, Bonnabry P (2010) Maximizing calcium and phosphate content in neonatal parenteral nutrition solutions using organic calcium and phosphate salts. J Parenter Enteral Nutr. 34:542-545. http://dx.doi.org/10.1177/0148607110374615

118. Watrobska-Swietlikowska D, Szlagatys-Sidorkiewicz A, MacLoughlin R (2018) The presence of inorganic calcium in pediatric parenteral admixtures. Nutr Hosp. 35:11-18. http://dx.doi.org/10.20960/nh.1340

119. Chaieb SD, Chaumeil J-C, Jebnoun S, Khrouf N, Hedhili A, Sfar S (2009) Effect of high calcium and phosphate concentrations on the physicochemical properties of two lipid emulsions used as total parenteral nutrition for neonates. PDA J Pharm Sci Technol. 63:27-41.

120. Ribeiro DDO, Lobo BW, Volpato NM, Da Veiga VF, Cabral LM, De Sousa VP (2009) Influence of the calcium concentration in the presence of organic phosphorus on the physicochemical compatibility and stability of all-in-one admixtures for neonatal use. Nutr J. 8. http://dx.doi.org/10.1186/1475-2891-8-51

121. Blackmer AB, Partipilo ML (2015) Three-in-one parenteral nutrition in neonates and pediatric patients: Risks and benefits. Nutr Clin Pract. 30:337-343. http://dx.doi.org/10.1177/0884533615580596

122. United States Pharmacopeia and National Formulary. General chapters:<729>Globule size distribution in lipid Injectable emulsions. (2020) United States Pharmacopeial Convention. USP 43-NF 38 ed.

123. Watrobska-Swietlikowska D (2019) Stability of commercial parenteral lipid emulsions repacking to polypropylene syringes. PLoS One. 14:e0214451. http://dx.doi.org/10.1371/journal.pone.0214451

124. Driscoll DF, Nehne J, Peterss H, Klutsch K, Bistrian BR, Niemann W (2003) Physicochemical stability of intravenous lipid emulsions as all-in-one admixtures intended for the very young. Clin Nutr. 22:489-495. http://dx.doi.org/10.1016/S0261-5614%2803%2900046-3

125. MacKay M, Anderson C (2015) Physical Compatibility of Sodium Glycerophosphate and Calcium Gluconate in Pediatric Parenteral Nutrition Solutions. J Parenter Enteral Nutr. 39:725-728. http://dx.doi.org/10.1177/0148607114528982

126. Pereira-da-Silva L, Nurmamodo A, Videira Amaral JM, Rosa ML, Almeida MC, Ribeiro ML (2003) Compatibility of calcium and phosphate in four parenteral nutrition solutions for preterm neonates. Am J Health Syst Pharm. 60:1041-1044. http://dx.doi.org/10.1093/ajhp/60.10.1041

127. Venkataraman PS, Brissie Jr EO, Tsang RC (1983) Stability of calcium and phosphorus in neonatal parenteral nutrition solutions. J Pediatr Gastroenterol Nutr. 2:640-643. https://doi.org/10.1002/j.1536-4801.1983.tb08563.x

128. Riera P, Garrido-Alejos G, Cardenete J, Moliner E, Zapico-Muñiz E, Cardona D, Garin N (2018) Physicochemical Stability and Sterility of Standard Parenteral Nutrition Solutions and Simulated Y-Site Admixtures for Neonates. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 33:694-700. http://dx.doi.org/10.1002/ncp.10013

129. Anderson C, MacKay M (2016) Physical Compatibility of Calcium Chloride and Sodium Glycerophosphate in Pediatric Parenteral Nutrition Solutions. J Parenter Enteral Nutr. 40:1166-1169. http://dx.doi.org/10.1177/0148607115592673

130. Huston RK, Christensen JM, Alshahrani SM, Mohamed SM, Clark SM, Nason JA, Wu YX (2015) Calcium chloride in neonatal parenteral nutrition solutions with and without added cysteine: Compatibility studies using laser and micro-flow imaging methodology. PLoS One. 10:1-13. http://dx.doi.org/10.1371/journal.pone.0136894

131. Huston RK, Christensen JM, Alshahrani SM, Mohamed SM, Heisel CF, Stout KN (2019) Calcium Chloride and Calcium Gluconate in Neonatal Parenteral Nutrition Solutions with Added Cysteine: Compatibility Studies Using Laser Light Obscuration Methodology. J Parenter Enteral Nutr. 43:426-433. http://dx.doi.org/10.1002/jpen.1434

132. Watrobska-Swietlikowska D (2019) Compatibility of Maximum Inorganic and Organic Calcium and Phosphate Content in Neonatal Parenteral Solutions. Sci Rep. 9:1-11. http://dx.doi.org/10.1038/s41598-019-46987-y

133. MacKay M, Rusho W, Jackson D, McMillin G, Winther B (2009) Physical and chemical stability of iron sucrose in parenteral nutrition. Nutr Clin Pract. 24:733-737. http://dx.doi.org/10.1177/0884533609351528

134. Parikh MJ, Dumas G, Silvestri A, Bistrian BR, Driscoll DF (2005) Physical compatibility of neonatal total parenteral nutrient admixtures containing organic calcium and inorganic phosphate salts. Am J Health Syst Pharm. 62:1177-1183. http://dx.doi.org/10.1093/ajhp/62.11.1177

135. Raupp P, Kries RV, Pfahl HG, Manz F (1991) Glycero- vs glucose-phosphate in parenteral nutrition of premature infants: A comparative in vitro evaluation of calcium/phosphorus compatibility. J Parenter Enteral Nutr. 15:469-473. http://dx.doi.org/10.1177/0148607191015004469

136. Watrobska-Swietlikowska D, Kwidzynska A, Szlagatys-Sidorkiewicz A, Sznitowska M, Klek S (2014) Finding new solutions in pediatric parenteral admixtures: how to improve quality and to deal with shortages. Nutr Hosp. 30:84-93. http://dx.doi.org/10.3305/nh.2014.30.1.7500

137. Dorota WS, Agnieszka SS, Łuszkiewicz K (2015) Evaluation of physical stability of all in one parenteral admixtures for pediatric home care with high electrolytes concentrations. Nutr Hosp. 31:236-243. http://dx.doi.org/10.3305/nh.2015.31.1.7965

138. Lobo BW, Da Veiga VF, Cabral LM, Michel RC, Volpato NM, De Sousa VP (2012) Influence of the relative composition of trace elements and vitamins in physicochemical stability of total parenteral nutrition formulations for neonatal use. Nutr J. 11. http://dx.doi.org/10.1186/1475-2891-11-26

139. Sayed OAEA, Hassan SB, Abdelkader A, Elsabahy M, Abdelaziz NHR, El-Sayed AM (2021) Stability Study and Clinical Evaluation of Lipid Injectable Emulsion in Parenteral Nutrition Admixtures Used for Preterm Neonates. Nutr Clin Pract. 36:696-703. http://dx.doi.org/10.1002/ncp.10556

140. Télessy IG, Balogh J, Turmezei J, Dredán J, Zelkó R (2011) Stability assessment of o/w parenteral nutrition emulsions in the presence of high glucose and calcium concentrations. J Pharm Biomed Anal. 56:159-164. http://dx.doi.org/10.1016/j.jpba.2011.05.002

141. Murphy S, Craig DQM, Murphy A (1996) An investigation into the physical stability of a neonatal parenteral nutrition formulation. Acta Paediatr. 85:1483-1486. http://dx.doi.org/10.1111/j.1651-2227.1996.tb13956.x

142. Athanasiou C, Hatziantoniou S, Skouroliakou M, Markantonis-Kyroudis S (2014) Assessment of the Physicochemical Stability of All-in-One Parenteral Emulsions for Neonates According to USP Specifications. J Parenter Enteral Nutr. 38:867-872. http://dx.doi.org/10.1177/0148607113499589

143. Bourcier E, Poullain-Termeau S (2015) Parenteral nutrition in a neonatal intensive care unit: Galenic stability of four all-in-one admixtures. Eur J Hosp Pharm. 22:285-290. http://dx.doi.org/10.1136/ejhpharm-2014-000625

144. Forchielli ML, Bonoli A, Preite I, Stancari A, Maselli S, Guarguaglini AM, Mignini I, Masi M, Puggioli C, Bersani G (2014) Parenteral nutrition admixtures for pediatric patients compounded with highly refined fish oil-based emulsion: Assessment of physicochemical stability. Clin Nutr. 33:1127-1131. http://dx.doi.org/10.1016/j.clnu.2013.12.011

145. Forchielli ML, Bonoli A, Stancari A, Bruno LL, Piro F, Piazza G, Albertini C, Pession A, Puggioli C, Bersani G (2019) Do carnitine and extra trace elements

change stability of paediatric parenteral nutrition admixtures? Clin Nutr. 38:2369-2374. http://dx.doi.org/10.1016/j.clnu.2018.10.016

146. Yailian AL, Serre C, Fayard J, Faucon M, Thomaré P, Filali S, Pivot C, Vételé F, Pirot F, Olivier E (2019) Production and stability study of a hospital parenteral nutrition solution for neonates. J Pharm Anal. 9:83-90. http://dx.doi.org/10.1016/j.jpha.2018.01.002

147. Bullock L, Fitzgerald JF, Walter WV (1992) Emulsion stability in total nutrient admixtures containing a pediatric amino acid formulation. J Parenter Enteral Nutr. 16:64-68. http://dx.doi.org/10.1177/014860719201600164

148. Dahl GB, Svensson L, Kinnander NJG, Zander M, Bergström UK (1994) Stability of Vitamins in Soybean Oil Fat Emulsion Under Conditions Simulating Intravenous Feeding of Neonates and Children. J Parenter Enteral Nutr. 18:234-239. http://dx.doi.org/10.1177/0148607194018003234

149. De Cloet J, Van Biervliet S, Van Winckel M (2018) Physicochemical stable standard all-in-one parenteral nutrition admixtures for infants and children in accordance with the ESPGHAN/ESPEN guidelines. Nutrition. 49:41-47. http://dx.doi.org/10.1016/j.nut.2017.11.019

150. Huston RK, Christensen JM, Karnpracha C, Rosa JE, Clark SM, Migaki EA, Wu Y (2014) Calcium Chloride in Neonatal Parenteral Nutrition: Compatibility Studies Using Laser Methodology. PLoS One. 9:e106825-e106825. http://dx.doi.org/10.1371/journal.pone.0106825

151. Skouroliakou M, Matthaiou C, Chiou A, Panagiotakos D, Gounaris A, Nunn T, Andrikopoulos N (2008) Physicochemical stability of parenteral nutrition supplied as all-in-one for neonates. J Parenter Enteral Nutr. 32:201-209. http://dx.doi.org/10.1177/0148607108314768

152. Singh H, Dumas GJ, Silvestri AP, Young S, Martin CR, Bistrian BR, Driscoll DF (2009) Physical compatibility of neonatal total parenteral nutrition admixtures containing organic calcium and inorganic phosphate salts in a simulated infusion at 37°. Pediatr Crit Care Med. 10:213-216. http://dx.doi.org/10.1097/PCC.0b013e31819a3bf4

153. Wang HJ, Hsieh YT, Liu LY, Huang CF, Lin SC, Tsao PN, Chou HC, Yen TA, Chen CY (2020) Use of sodium glycerophosphate in neonatal parenteral nutrition solutions to increase calcium and phosphate compatibility for preterm infants. Pediatr Neonatol. 61:331-337. http://dx.doi.org/10.1016/j.pedneo.2020.02.004

154. Silvers KM, Darlow BA, Winterbourn CC (1998) Pharmacologic levels of heparin do not destabilize neonatal parenteral nutrition. J Parenter Enteral Nutr. 22:311-314. https://doi.org/10.1177/014860719802200531l

155. Ribeiro DO, Pinto DC, Lima LMTR, Volpato NM, Cabral LM, De Sousa VP (2011) Chemical stability study of vitamins thiamine, riboflavin, pyridoxine and ascorbic acid in parenteral nutrition for neonatal use. Nutr J. 10:1-9. http://dx.doi.org/10.1186/1475-2891-10-47

156. Schilling CG, Watson DM, McCoy HG, Uden DL (1989) Stability and delivery of vancomycin hydrochloride when admixed in a total parenteral nutrition solution. J Parenter Enteral Nutr. 13:63-64. http://dx.doi.org/10.1177/014860718901300163

157. Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs - A review. J Pharm Anal. 4:159-165. http://dx.doi.org/10.1016/j.jpha.2013.09.003

158. Sengupta P, Chatterjee B, Tekade RK (2018) Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: A comprehensive review. Int J Pharm. 543:328-344. http://dx.doi.org/10.1016/j.ijpharm.2018.04.007

159. Bansal R, Saini B, Bansal Y, Bansal G (2013) MSn, LC-MS-TOF and LC-PDA studies for identification of new degradation impurities of bupropion. Biomed Chromatogr. 27:1387-1397. https://dx.doi.org/10.1002/bmc.2933

160. Qiu F, Norwood DL (2007) Identification of pharmaceutical impurities. J Liq Chromatogr Relat Technol. 30:877-935. https://doi.org/10.1080/10826070701191151

161. ICH Topic Q 1 A (R2) Stability Testing of new Drug Substances and Products (2003) European Medicines Agency. London.

162. Ngwa G (2010) Forced degradation as an integral part of HPLC stability-indicating method development. Drug Delivery Technology. 10:56-59.

163. Szepesi G, Gazdag M, Mihalyfi K (1991) Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. III. Method validation. J Chromatogr A. 464:265-278. http://dx.doi.org/10.1016/S0021-9673%2800%2994245-6

164. Jenke DR (1996) Chromatographic Method Validation: A Review of Current Practices and Procedures. I. General Concepts and Guidelines. J Liq Chromatogr Relat Technol. 19:719-736. http://dx.doi.org/10.1080/10826079608005533

165. ICH Topic Q 5 C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (1996) European Medicines Agency. London.

166. ICH Topic Q 1 B Photostability Testing of New Active Substances and Medicinal Products (1998) European Medicines Agency. London.

167. Maheswaran R (2012) Scientific considerations of forced degradation studies in ANDA submissions. J Valid Technol. 18:92-96.

168. Klick S, Muijselaar PG, Waterval J, Eichinger T, Korn C, Gerding TK, Debets AJ, Sanger-Van De Griend C, Van Den Beld C, Somsen GW, De Jong GJ (2005) Toward a generic approach for: Stress testing of drug substances and drug products. Pharm Technol. 29:48-66.

169. Bakshi M, Singh S (2002) Development of validated stability-indicating assay methods - Critical review. J Pharm Biomed Anal. 28:1011-1040. http://dx.doi.org/10.1016/S0731-7085(02)00047-X

170. Singh S, Bakshi M (2000) Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Technol. 4:1-14.

171. Alsante KM, Ando A, Brown R, Ensing J, Hatajik TD, Kong W, Tsuda Y (2007) The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Deliv Rev. 59:29-37. http://dx.doi.org/10.1016/j.addr.2006.10.006

172. Gupta A, Yadav JS, Rawat S, Gandhi M (2011) Method development and hydrolytic degradation study of doxofylline by RP-HPLC and LC-MS/MS. Asian J Pharm Ana. 1:14-18.

173. Allwood M, Plane J (1986) The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation. Int J Pharm. 31:1-7. https://doi.org/10.1016/0378-5173(86)90206-1

174. Baertschi SW, Thatcher SR. Sample presentation for photostability studies: problems and solutions (2006) Pharmaceutical Photostability and Stabilization Technology.CRC Press: p. 201-226.

175. Ahuja S, Alsante KM. Handbook of isolation and characterization of impurities in pharmaceuticals(2003).Academic press: San Diego, London.

176. Snyder LR, Kirkland JJ, Glajch JL. Ionic Samples: Reversed-Phase, Ion-Pair, and Ion-Exchange HPLC (1997) Practical HPLC Method Development. p. 292-349.

177. Lim C-K, Lord G (2002) Current developments in LC-MS for pharmaceutical analysis. Biol Pharm Bull. 25:547-557. https://doi.org/10.1248/bpb.25.547

178. Patel RM, Patel PM, Patel NM (2011) Stability indicating HPLC method development–a review. IRJP. 2:79-87.

179. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology (1995) European Medicines Agency. London.

180. Bioanalytical Method Validation Guidance for Industry (2018) U.S. Department of Health and Human Services Food and Drug Administration.

181. Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry (2015) U.S. Department of Health and Human Services Food and Drug Administration.

182. United States Pharmacopoeia. (2003) 26 ed.United States Pharmacopeial Convention: Rockville, MD.

183. AOAC Peer-verified Methods Program: Manual on Policies and Procedures (1993) Association of Official Analytical Chemists. Arlington, VA.

184. Guideline on bioanalytical method validation (2011) European Medicines Agency. London, United Kingdom.

185. Araujo P (2009) Key aspects of analytical method validation and linearity evaluation. J Chromatogr B. 877:2224-2234. https://doi.org/10.1016/j.jchromb.2008.09.030

186. Chandran S, Singh R (2007) Comparison of various international guidelines for analytical method validation. Pharmazie. 62:4-14. https://doi.org/10.1691/ph2007.1.5064

187. Taverniers I, De Loose M, Van Bockstaele E (2004) Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends Analyt Chem. 23:535-552. https://doi.org/10.1016/j.trac.2004.04.001

188. Lao GC, Reyes MR, Turet JR, Dot MP, Muner DS, Cabezas CL (2020) Compatibility of drugs administered as Y-site infusion in intensive care units: A systematic review. Med Intensiva. 44:80-87. https://doi.org/10.1016/j.medin.2018.08.004

189. Tawfik GM, Dila KAS, Mohamed MYF, Tam DNH, Kien ND, Ahmed AM, Huy NT (2019) A step by step guide for conducting a systematic review and meta-analysis with simulation data. Trop Med Health. 47:1-9. https://doi.org/10.1186/s41182-019-0165-6

190. Eriksen MB, Frandsen TF (2018) The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: a systematic review. J Med Libr Assoc. 106:420. https://doi.org/10.5195/jmla.2018.345

191. Cooke A, Smith D, Booth A (2012) Beyond PICO: The SPIDER Tool for Qualitative Evidence Synthesis. Qual Health Res. 22:1435-1443. https://doi.org/10.1177/1049732312452938

192. Booth A (2006) Clear and present questions: formulating questions for evidence based practice. Library hi tech. 24:355-368. http://dx.doi.org/10.1108/07378830610692127

193. Wildridge V, Bell L (2002) How CLIP became ECLIPSE: a mnemonic to assist in searching for health policy/management information. Health Inf Libr J. 19:113-115. https://doi.org/10.1046/j.1471-1842.2002.00378.x

194. Chai KEK, Lines RLJ, Gucciardi DF, Ng L (2021) Research Screener: a machine learning tool to semi-automate abstract screening for systematic reviews. Syst Rev. 10:93. http://dx.doi.org/10.1186/s13643-021-01635-3

195. Michelson M, Reuter K (2019) The significant cost of systematic reviews and meta-analyses: A call for greater involvement of machine learning to assess the promise of clinical trials. Contemp Clin Trials Commun. 16:100443-100443. https://doi.org/10.1016/j.conctc.2019.100443

196. Olofsson H, Brolund A, Hellberg C, Silverstein R, Stenstrom K, Osterberg M, Dagerhamn J (2017) Can abstract screening workload be reduced using text mining? User experiences of the tool Rayyan. Res Synth Methods. 8:275-280. https://dx.doi.org/10.1002/jrsm.1237

197. Gates A, Johnson C, Hartling L (2018) Technology-assisted title and abstract screening for systematic reviews: A retrospective evaluation of the Abstrackr machine learning tool. Syst Rev. 7:45. https://dx.doi.org/10.1186/s13643-018-0707-8

198. Przybyla P, Brockmeier AJ, Kontonatsios G, Le Pogam MA, McNaught J, von Elm E, Nolan K, Ananiadou S (2018) Prioritising references for systematic reviews with RobotAnalyst: A user study. Res Synth Methods. 9:470-488. https://dx.doi.org/10.1002/jrsm.1311

199. van de Schoot R, de Bruin J, Schram R, Zahedi P, de Boer J, Weijdema F, Kramer B, Huijts M, Hoogerwerf M, Ferdinands G (2021) An open source machine learning framework for efficient and transparent systematic reviews. Nat Mach Intell. 3:125-133. https://doi.org/10.1038/s42256-020-00287-7

200. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A (2016) Rayyan-a web and mobile app for systematic reviews. Syst Rev. 5. https://doi.org/10.1186/s13643-016-0384-4

201. Przybyła P, Brockmeier AJ, Kontonatsios G, Le Pogam MA, McNaught J, von Elm E, Nolan K, Ananiadou S (2018) Prioritising references for systematic reviews with RobotAnalyst: a user study. Res Synth Methods. 9:470-488. https://doi.org/10.1002/jrsm.1311

202. Kellermeyer L, Harnke B, Knight S (2018) Covidence and rayyan. J Med Libr Assoc. 106:580–583. https://doi.org/10.5195/jmla.2018.513

203. Bramer WM, Rethlefsen ML, Kleijnen J, Franco OH (2017) Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. Syst Rev. 6:1-12. https://doi.org/10.1186/s13643-017-0644-y

204. McHugh ML (2012) Interrater reliability: the kappa statistic. Biochem Med. 22:276-282.

205. Guimarães NS, Ferreira AJF, Ribeiro Silva RdC, de Paula AA, Lisboa CS, Magno L, Ichiara MY, Barreto ML (2022) Deduplicating records in systematic reviews: there are free, accurate automated ways to do so. J Clin Epidemiol. 152:110-115. https://doi.org/10.1016/j.jclinepi.2022.10.009

206. LaPlante KL, Woodmansee S, Mermel LA (2012) Compatibility and stability of telavancin and vancomycin in heparin or sodium citrate lock solutions. Am J Health Syst Pharm. 69:1405-1409. http://dx.doi.org/10.2146/ajhp110256

207. Raverdy V, Ampe E, Hecq JD, Tulkens PM (2013) Stability and compatibility of vancomycin for administration by continuous infusion. J Antimicrob Chemother. 68:1179-1182. http://dx.doi.org/10.1093/jac/dks510

208. Souney PF, Colucci RD, Mariani G, Campbell D (1984) Compatibility of magnesium sulfate solutions with various antibiotics during simulated Y-site injection. Am J Hosp Pharm. 41:323-324. http://dx.doi.org/10.1093/ajhp/41.2.323

209. Taylor A (1997) Review clarithromycin mixtures. Pharmacy in Practice. 7:473-474.

210. Cohen MH, Johnston-Early A, Hood MA (1985) Drug precipitation within iv tubing: A potential hazard of chemotherapy administration. Cancer Treat Rep. 69:1325-1326.

211. Bhatt-Mehta V, Johnson CE, Leininger N, Agarwal M (1995) Stability of fentanyl citrate and midazolam hydrochloride during simulated intravenous coadministration. Am J Health Syst Pharm. 52:511-513. http://dx.doi.org/10.1093/ajhp/52.5.511

212. Foushee JA, Meredith P, Fox LM, Wilder AG (2020) Y-site physical compatibility of hydrocortisone continuous infusions with admixtures used in critically ill patients. Am J Health Syst Pharm. 77:1144-1148. http://dx.doi.org/10.1093/ajhp/zxaa118

213. Ghazi IM, El Nekidy WS, Asay R, Fimognari P, Knarr A, Awad M (2020) Simultaneous administration of imipenem/cilastatin/relebactam with selected intravenous antimicrobials, a stewardship approach. PLoS One. 15:e0233335. https://dx.doi.org/10.1371/journal.pone.0233335

214. Ghazi IM, El Nekidy WS, Sood A, Dulku A, Patel R, Patel K, Li P (2020) Y-site Administration of Imipenem/Cilastatin/Relebactam With Common Intravenous Medications. Clin Ther. 42:475-485. http://dx.doi.org/10.1016/j.clinthera.2020.01.017

215. Kidd JM, Avery LM, Asempa TE, Nicolau DP, Kuti JL (2018) Physical Compatibility of Meropenem and Vaborbactam With Select Intravenous Drugs During Simulated Y-site Administration. Clin Ther. 40:261-269. http://dx.doi.org/10.1016/j.clinthera.2017.12.007

216. Parker WA (1985) Physical compatibility of ranitidine HCl with preoperative injectable medications. Can J Hosp Pharm. 38:160-161.

217. Semark AJ, Venkatesh K, McWhinney BC, Pretorius C, Roberts JA, Cohen J, Venkatesh B (2013) The compatibility of a low concentration of hydrocortisone sodium succinate with selected drugs during a simulated Y-site administration. Crit Care Resusc. 15:63-66. https://doi.org/10.1016/S1441-2772(23)02191-9

218. Smythe M, Malouf E (1991) Visual compatibility of insulin with secondary intravenous drugs in admixtures. Am J Hosp Pharm. 48:125-126. http://dx.doi.org/10.1093/ajhp/48.1.125

219. Smythe MA, Patel MA, Gasloli RA (1990) Visual compatibility of narcotic analgesics with selected intravenous admixtures. Am J Hosp Pharm. 47:819-820. http://dx.doi.org/10.1093/ajhp/47.4.819

220. Chandler SW, Trissel LA, Weinstein SM (1996) Combined administration of opioids with selected drugs to manage pain and other cancer symptoms: Initial safety screening for compatibility. J Pain Symptom Manage. 12:168-171. http://dx.doi.org/10.1016/0885-3924%2896%2900127-3

221. Ibezim EC, Attama AA (1998) Compatibility studies on some commercially available gentamicin sulphate injections with commonly co-administered parenterals. Afr J Health Sci. 5:103-107.

222. Inagaki K, Takagi J, Lor E, Lee KJ, Nii L, Gill MA (1993) Stability of fluconazole in commonly used intravenous antibiotic solutions. Am J Hosp Pharm. 50:1206-1208. https://dx.doi.org/10.1093/ajhp/50.6.1206

223. Knudsen L, Eisend S, Haake N, Kunze T (2014) Physicochemical compatibility of commonly used analgesics and sedatives in the intensive care medicine. Eur J Hosp Pharm. 21:161-166. http://dx.doi.org/10.1136/ejhpharm-2014-000444

224. Szof C, Walker PC (1993) Incompatibility of cefotaxime sodium and vancomycin sulfate during Y-site administration. Am J Hosp Pharm. 50:2054-2057. https://doi.org/10.1093/ajhp/50.10.2054

225. Ayuso EF, Hernandez PV, Cabezas CL, Molina LG, Muner DS (2013) Physical compatibility of highly concentrated midazolam infusions with other drugs commonly used in intensive care units. Atencion Farmaceutica. 15:335-338.

226. Koller AK, Krebs S, Dorje F (2020) Medication Safety in Intravenous Therapy: A Compatibility Study of Clonidine with Drugs Frequently Used in Intensive Care. Pharmaceutics. 13:21. https://dx.doi.org/10.3390/pharmaceutics13010021

227. Kufel WD, Miller CD, Johnson PR, Reid K, Zahra JJ, Seabury RW (2017) Y-site incompatibility between premix concentrations of vancomycin and piperacillin-tazobactam: Do current compatibility testing methodologies tell the whole story? Hosp Pharm. 52:132-137. http://dx.doi.org/10.1310/hpj5202-132

228. Pauwels J, Spriet I, Fu X, Von Winckelmann S, Willems L, Hoogmartens J, Van Schepdael A (2011) Chemical stability and compatibility study of vancomycin for administration by continuous infusion in intensive care units. J Liq Chromatogr Relat Technol. 34:1965-1975. http://dx.doi.org/10.1080/10826076.2011.582909

229. López-Cabezas C, Guerrero L, Molas G, Anglada H, Soy D (2015) Physicochemical compatibility of high concentration drugs usually Y-site

administered in intensive care units. Eur J Hosp Pharm. 22:107-112. http://dx.doi.org/10.1136/ejhpharm-2014-000539

230. Yamashita SK, Walker SE, Choudhury T, Iazzetta J (1996) Compatibility of selected critical care drugs during simulated Y-site administration. Am J Health Syst Pharm. 53:1048-1051. https://dx.doi.org/10.1093/ajhp/53.9.1048

231. Jasti B, Saraf P (2011) Compatibility of parenteral furosemide with seventeen secondary drugs used in standard concentrations. IJPC. 15:259-261.

232. Humbert-delaloye V, Berger-gryllaki M, Voirol P, Gattlen L, Pannatier A (2013) In vitro compatibility of various cardioactive drugs during simulated Y-site administration. Eur J Hosp Pharm. 20:110-116. http://dx.doi.org/10.1136/ejhpharm-2012-000239

233. Cruz KCE, Churchwell MD, Mauro VF, Boddu Sai HS (2018) Physical compatibility of levetiracetam injection with heparin, dobutamine, and dopamine. Am J Health Syst Pharm. 75:510-512. https://doi.org/10.2146/ajhp180069

234. Nemec K, Kopelent-Frank H, Greif R (2008) Standardization of infusion solutions to reduce the risk of incompatibility. Am J Health Syst Pharm. 65:1648-1654. http://dx.doi.org/10.2146/ajhp070471

235. Lee TM, Villareal CL, Meyer LM (2021) Y-Site Compatibility of Intravenous Levetiracetam With Commonly Used Critical Care Medications. Hosp Pharm. 56:282-286. http://dx.doi.org/10.1177/0018578719893376

236. Baririan N, Chanteux H, Viaene E, Servais H, Tulkens PM (2003) Stability and compatibility study of cefepime in comparison with ceftazidime for potential administration by continuous infusion under conditions pertinent to ambulatory treatment of cystic fibrosis patients and to administration in intensive care units. J Antimicrob Chemother. 51:651-658. http://dx.doi.org/10.1093/jac/dkg134

237. Humbert-Delaloye V, Berger-Gryllaki M, Voirol P, Testa B, Pannatier A (2015) Screening for physicochemical incompatibilities of intravenous drugs in intensive care units: The case of monobasic potassium phosphate and furosemide. Eur J Hosp Pharm. 22:56-58. http://dx.doi.org/10.1136/ejhpharm-2013-000431

238. Servais H, Tulkens PM (2001) Stability and compatibility of ceftazidime administered by continuous infusion to intensive care patients. Antimicrob Agents Chemother. 45:2643-2647. http://dx.doi.org/10.1128/AAC.45.9.2643-2647.2001

239. Swart EL, Mooren RA, van Loenen AC (1995) Compatibility of midazolam hydrochloride and lorazepam with selected drugs during simulated Y-site administration. Am J Health Syst Pharm. 52:2020-2022. https://doi.org/10.1093/ajhp/52.18.2020

240. Gilbert DL, Jr., Trissel LA, Martinez JF (1997) Compatibility of ciprofloxacin lactate with sodium bicarbonate during simulated Y-site administration. Am J Health Syst Pharm. 54:1193-1195. https://doi.org/10.1093/ajhp/54.10.1193

241. Meyer K, Santarossa M, Danziger LH, Wenzler E (2017) Compatibility of ceftazidime-avibactam, ceftolozane- tazobactam, and piperacillin-tazobactam with vancomycin in dextrose 5% in water. Hosp Pharm. 52:221-228. http://dx.doi.org/10.1310/hpj5203-221

242. Trissel LA, Martinez JF (1994) Compatibility of piperacillin sodium plus tazobactam with selected drugs during simulated Y-site injection. Am J Hosp Pharm. 51:672-678. http://dx.doi.org/10.1093/ajhp/51.5.672

243. Trissel LA (1996) Concentration-dependent precipitation of sodium bicarbonate with ciprofloxacin lactate Am J Health Syst Pharm. 53:84-85. https://dx.doi.org/10.1093/ajhp/53.1.84

244. Wade J, Cooper M, Ragan R (2015) Simulated Y-site compatibility of vancomycin and piperacillin-tazobactam. Hosp Pharm. 50:376-379. http://dx.doi.org/10.1310/hpj5005-376

245. Hanifah S, Nugroho BH, Chabib L (2020) Compatibility of acetaminophen with central nervous system medications during simulated Y-site injection. Anaesthesiol Intensive Ther. 52:23-27. https://doi.org/10.5114/ait.2020.92684

246. Anderson C, Boehme S, Ouellette J, Stidham C, Mackay M (2014) Physical and chemical compatibility of injectable acetaminophen during simulated Y-site administration. Hosp Pharm. 49:42-47. http://dx.doi.org/10.1310/hpj4901-42

247. Canann D, Tyler LS, Barker B, Condie C (2009) Visual compatibility of i.v. medications routinely used in bone marrow transplant recipients. Am J Health Syst Pharm. 66:727-729. http://dx.doi.org/10.2146/ajhp070572

248. Najari Z, Rusho WJ (1997) Compatibility of commonly used bone marrow transplant drugs during Y- site delivery. Am J Health Syst Pharm. 54:181-184. http://dx.doi.org/10.1093/ajhp/54.2.181

249. Voytilla KL, Tyler LS, Rusho WJ (2002) Visual compatibility of azithromycin with 24 commonly used drugs during simulated Y-site delivery. Am J Health Syst Pharm. 59:853-855. http://dx.doi.org/10.1093/ajhp/59.9.853

250. United States Pharmacopeia and National Formulary. General chapters<788> Particulate Matter in Injections. (2012) United States Pharmacopeial Convention. Rockville, MD.

251. Lessard J-J, Caron E, Scherer H, Forest J-M, Leclair G (2020) Compatibility of Y-Site Injection of Meropenem Trihydrate With 101 Other Injectable Drugs. Hosp Pharm. 55:332-337. https://dx.doi.org/10.1177/0018578719844168

252. Sullivan T, Forest J-M, Leclair G (2015) Compatibility of Cloxacillin Sodium with Selected Intravenous Drugs During Simulated Y-Site Administration. Hosp Pharm. 50:214-220. https://dx.doi.org/10.1310/hpj5003-214

253. Monograph 2.10.19. (2020) European Pharmacopoeia. 10 ed.EDQM: Strasbourg, France.

254. Akkerman SR, Zhang H, Mullins RE, Yaughn K (1999) Stability of milrinone lactate in the presence 29 critical care drugs and 4 i.v. solutions. Am J Health Syst Pharm. 56:63-68. http://dx.doi.org/10.1093/ajhp/56.1.63

255. Nahata MC, Durrell DE (1985) Stability of tobramycin sulfate in admixtures with calcium gluconate. Am J Hosp Pharm. 42:1987-1988. https://dx.doi.org/10.1093/ajhp/42.9.1987

256. Hamdi M, Lentschener C, Bazin C, Ozier Y, Havard L (2009) Compatibility and stability of binary mixtures of acetaminophen, nefopam, ketoprofen and ketamine in

infusion solutions. Eur J Anaesthesiol. 26:23-27. http://dx.doi.org/10.1097/EJA.0b013e328319c04b

257. Forman JK, Lachs JR, Souney PF (1987) Visual compatibility of acyclovir sodium with commonly used intravenous drugs during simulated Y-site injection. Am J Hosp Pharm. 44:1408-1409. https://doi.org/10.1093/ajhp/44.6.1408

258. Patel PR (1996) Compatibility of meropenem with commonly used injectable drugs. Am J Health Syst Pharm. 53:2853-2855. https://doi.org/10.1093/ajhp/53.23.2853

259. Chalmers JR, Bobek MB, Militello MA (2001) Visual compatibility of amiodarone hydrochloride injection with various intravenous drugs. Am J Health Syst Pharm. 60:1095-1096. https://doi.org/10.1093/ajhp/58.6.504

260. Lor E, Sheybani T, Takagi J (1991) Visual compatibility of fluconazole with commonly used injectable drugs during simulated Y-site administration. Am J Hosp Pharm. 48:744-746. http://dx.doi.org/10.1093/ajhp/48.4.744

261. Trissel LA, Gilbert DL, Jr., Martinez JE (1998) Concentration dependency of vancomycin hydrochloride compatibility with beta-lactam antibiotics during simulated Y-site administration. Hosp Pharm. 33:1515-1522.

262. Hasegawa GR, Eder JF (1984) Visual compatibility of dobutamine hydrochloride with other injectable drugs. Am J Hosp Pharm. 41:949-951. http://dx.doi.org/10.1093/ajhp/41.5.949

263. Cervenka P, DeJong DJ, Butler BL, Monzingo MD (1992) Visual compatibility of injectable ciprofloxacin lactate with selected injectable drugs during simulated y-site administration. Hosp Pharm. 27:957-962.

264. Chiu MF, Schwartz ML (1997) Visual compatibility of injectable drugs used in the intensive care unit. Am J Health Syst Pharm. 54:64-65. https://dx.doi.org/10.1093/ajhp/54.1.64

265. Hasegawa GR, Eder JF (1984) Dobutamine-heparin mixture inadvisable. Am J Hosp Pharm. 41:2588-2590.

266. Pugh CB, Pabis DJ, Rodriguez C (1991) Visual compatibility of morphine sulfate and meperidine hydrochloride with other injectable drugs during simulated Y-site injection. Am J Hosp Pharm. 48:123-125. http://dx.doi.org/10.1093/ajhp/48.1.123

267. Hanci V, Boztas N, Omur D, Ozbilgin S, Hanci SY (2014) Evaluation of the Precipitation Characteristics of Ertapenem, Tigecycline, Colistin, Daptomycin, Vancomycin and Teicoplanin. Lat Am J Pharm. 33:1678-1683.

268. Ozbilgin S, Boztas N, Tosun M, Omur D, Kucukguclu S, Hanci V (2017) Evaluation of ibuprofens precipitation characteristics. Anestezi Dergisi. 25:38-46.

269. Voirol P, Berger-Gryllaki M, Pannatier A, Eggimann P, Sadeghipour F (2015) Visual compatibility of insulin aspart with intravenous drugs frequently used in ICU. Eur J Hosp Pharm. 22:123-124. http://dx.doi.org/10.1136/ejhpharm-2014-000478

270. D'Huart E, Vigneron J, Demore B (2019) Physical Compatibility of Intravenous Drugs Commonly Used in Intensive Care Units: An Observational Study and Physical Compatibility Laboratory Tests on Anti-Infective Drugs. Pharm Technol Hosp Pharm. 4:29-40. http://dx.doi.org/10.1515/pthp-2019-0005

271. Forman JK, Souney PF (1987) Visual compatibility of midazolam hydrochloride with common preoperative injectable medications. Am J Hosp Pharm. 44:2298-2299. http://dx.doi.org/10.1093/ajhp/44.10.2298

272. Haddaway NR, Westgate MJ (2019) Predicting the time needed for environmental systematic reviews and systematic maps. Conserv Biol. 33:434-443. https://dx.doi.org/10.1111/cobi.13231

273. Shemilt I, Khan N, Park S, Thomas J (2016) Use of cost-effectiveness analysis to compare the efficiency of study identification methods in systematic reviews. Syst Rev. 5:1-13. https://doi.org/10.1186/s13643-016-0315-4

274. Audet M-A, Forest E, Friciu M, Forest J-M, Leclair G (2017) Compatibilité du citrate de caféine injectable avec plusieurs autres médicaments. Pharmactuel. 50:27-33.

275. Mitchell A, Gailey R (1999) Compatibility of caffeine citrate with other medications commonly used in a neonatal intensive care unit. J Pediatr Pharm Pract. 4:239–242.

276. DeMonaco HJ (1990) Intravenous drug compatibility. Crit Care Med. 18:896. https://dx.doi.org/10.1097/00003246-199008000-00025

277. Kanji S, Lam J, Goddard RD, Johanson C, Singh A, Petrin L, Coons P, McIntyre LA, Turgeon AF (2013) Inappropriate medication administration practices in Canadian adult ICUs: a multicenter, cross-sectional observational study. Ann Pharmacother. 47:637-643. https://doi.org/10.1345/aph.1R414

278. O'Donnell JN, Venkatesan N, Manek M, Rhodes NJ, Scheetz MH (2016) Visual and absorbance analyses of admixtures containing vancomycin and piperacillin-tazobactam at commonly used concentrations. Am J Health Syst Pharm. 73:241-246. http://dx.doi.org/10.2146/ajhp150170

279. Michelakis ED. The role of the NO axis and its therapeutic implications in pulmonary arterial hypertension (2004) The Role of Nitric Oxide in Heart Failure.Springer: Boston, MA.p. 213-229.

280. Ghofrani HA, Osterloh IH, Grimminger F (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nat Rev Drug Discov. 5:689-702. http://dx.doi.org/10.1038/nrd2030

281. Al Omari MM, Zughul MB, Davies JED, Badwan AA (2006) Sildenafil/cyclodextrin complexation: Stability constants, thermodynamics, and guest–host interactions probed by 1H NMR and molecular modeling studies. J Pharm Biomed Anal. 41:857-865. https://doi.org/10.1016/j.jpba.2006.01.055

282. Terrett NK, Bell AS, Brown D, Ellis P (1996) Sildenafil (VIAGRATM), a potent and selective inhibitor of type 5 cGMP phosphodiesterase with utility for the treatment of male erectile dysfunction. Bioorg Med Chem Lett. 6:1819-1824. https://doi.org/10.1016/0960-894X(96)00323-X

283. Turko IV, Ballard SA, Francis SH, Corbin JD (1999) Inhibition of cyclic GMP-binding cyclic GMP-specific phosphodiesterase (Type 5) by sildenafil and related compounds. Mol Pharmacol. 56:124-130. https://doi.org/10.1124/mol.56.1.124

284. Sanchez LS, de la Monte SM, Filippov G, Jones RC, Zapol WM, Bloch KD (1998) Cyclic-GMP-binding, cyclic-GMP-specific phosphodiesterase (PDE5) gene

expression is regulated during rat pulmonary development. Pediatr Res. 43:163-168. http://dx.doi.org/10.1203/00006450-199802000-00002

285. Ziegler JW, Ivy DD, Wiggins JW, Kinsella JP, Clarke WR, Abman SH (1998) Effects of dipyridamole and inhaled nitric oxide in pediatric patients with pulmonary hypertension. Am J Respir Crit Care Med. 158:1388-1395. https://doi.org/10.1164/ajrccm.158.5.9710117

286. Ichinose F, Adrie C, Hurford WE, Bloch KD, Zapol WM (1998) Selective pulmonary vasodilation induced by aerosolized zaprinast. Anesthesiology. 88:410-416. http://dx.doi.org/10.1097/00000542-199802000-00020

287. Ichinose F, Adrie C, Hurford WE, Zapol WM (1995) Prolonged pulmonary vasodilator action of inhaled nitric oxide by Zaprinast in awake lambs. J Appl Physiol. 78:1288-1295. https://doi.org/10.1152/jappl.1995.78.4.1288

288. Nagamine J, Hill LL, Pearl RG (2000) Combined therapy with zaprinast and inhaled nitric oxide abolishes hypoxic pulmonary hypertension. Crit Care Med. 28:2420-2424. http://dx.doi.org/10.1097/00003246-200007000-00038

289. Thusu KG, Morin FC, 3rd, Russell JA, Steinhorn RH (1995) The cGMP phosphodiesterase inhibitor zaprinast enhances the effect of nitric oxide. Am J Respir Crit Care Med. 152:1605-1610. https://doi.org/10.1164/ajrccm.152.5.7582302

290. Barst RJ, McGoon M, Torbicki A, Sitbon O, Krowka MJ, Olschewski H, Gaine S (2004) Diagnosis and differential assessment of pulmonary arterial hypertension. J Am Coll Cardiol. 43:40s-47s. http://dx.doi.org/10.1016/j.jacc.2004.02.032

291. Simonneau G, Galiè N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, Gibbs S, Lebrec D, Speich R, Beghetti M (2004) Clinical classification of pulmonary hypertension. J Am Coll Cardiol. 43:S5-S12. http://dx.doi.org/10.1016/j.jacc.2004.02.037

292. Rubin LJ (1997) Primary pulmonary hypertension. N Engl J Med. 336:111-117. http://dx.doi.org/10.1056/NEJM199701093360207

293. Pietra G, Edwards W, Kay J, Rich S, Kernis J, Schloo B, Ayres S, Bergofsky E, Brundage B, Detre K (1989) Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. Circulation. 80:1198-1206. https://doi.org/10.1161/01.CIR.80.5.1198

294. Barnett CF, Machado RF (2006) Sildenafil in the treatment of pulmonary hypertension. Vasc Health Risk Manag. 2:411-422. https://doi.org/10.2147/vhrm.s24411

295. Leuchte HH, Schwaiblmair M, Baumgartner RA, Neurohr CF, Kolbe T, Behr J (2004) Hemodynamic response to sildenafil, nitric oxide, and iloprost in primary pulmonary hypertension. Chest. 125:580-586. https://doi.org/10.1378/chest.125.2.580

296. Greenough A, Khetriwal B (2005) Pulmonary hypertension in the newborn. Paediatr Respir Rev. 6:111-116. https://doi.org/10.1016/j.prrv.2005.03.005

297. Yaseen H, Darwich M, Hamdy H (2012) Is sildenafil an effective therapy in the management of persistent pulmonary hypertension? J Clin Neonatol. 1:171-175. https://doi.org/10.4103/2249-4847.105958

298. Wardle AJ, Tulloh RMR (2013) Paediatric pulmonary hypertension and sildenafil: Current practice and controversies. Arch Dis Child Educ Pract Ed. 98:141-147. http://dx.doi.org/10.1136/archdischild-2013-303981

299. Simonca L, Tulloh R (2017) Sildenafil in Infants and Children. Children 4:60. https://doi.org/10.3390/children4070060

300. Nichols DJ, Muirhead GJ, Harness JA (2002) Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. Br J Clin Pharmacol. 53 Suppl 1:5s-12s. http://dx.doi.org/10.1046/j.0306-5251.2001.00027.x

301. Hyland R, Roe EG, Jones BC, Smith DA (2001) Identification of the cytochrome P450 enzymes involved in the N-demethylation of sildenafil. Br J Clin Pharmacol. 51:239-248. http://dx.doi.org/10.1046/j.1365-2125.2001.00318.x

302. Fraisse A, Wessel DL (2010) Acute pulmonary hypertension in infants and children cGMP-related drugs. Pediatr Crit Care Med. 11:S37-S40. https://dx.doi.org/10.1097/PCC.0b013e3181c8e6e9

303. Steinhorn RH, Kinsella JP, Pierce C, Butrous G, Dilleen M, Oakes M, Wessel DL (2009) Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. J Pediatr. 155:841-847. http://dx.doi.org/10.1016/j.jpeds.2009.06.012

304. Steiner M, Salzer U, Baumgartner S, Waldhoer T, Klebermass-Schrehof K, Wald M, Langgartner M, Berger A (2014) Intravenous sildenafil i.v. as rescue treatment for refractory pulmonary hypertension in extremely preterm infants. Klin Padiatr. 226:211-215. https://dx.doi.org/10.1055/s-0034-1375697

305. Provenza N, Calpena AC, Mallandrich M, Halbaut L, Clares B (2014) Design and physicochemical stability studies of paediatric oral formulations of sildenafil. Int J Pharm. 460:234-239. http://dx.doi.org/10.1016/j.ijpharm.2013.11.006

306. Daraghmeh N, Al-Omari M, Badwan AA, Jaber AMY (2001) Determinaton of sildenafil citrate and related substances in the commercial products and tablet dosage form using HPLC. J Pharm Biomed Anal. 25:483-492. http://dx.doi.org/10.1016/S0731-7085(00)00512-4

307. Dinesh ND, Vishukumar BK, Nagaraja P, Made Gowda NM, Rangappa KS (2002) Stability indicating RP-LC determination of sildenafil citrate (Viagra) in pure form and in pharmaceutical samples. J Pharm Biomed Anal. 29:743-748. http://dx.doi.org/10.1016/S0731-7085(02)00123-1

308. Fejos I, Neumajer G, Béni S, Jankovics P (2014) Qualitative and quantitative analysis of PDE-5 inhibitors in counterfeit medicines and dietary supplements by HPLC-UV using sildenafil as a sole reference. J Pharm Biomed Anal. 98:327-333. http://dx.doi.org/10.1016/j.jpba.2014.06.010

309. Hashem H, Ibrahim AE, Elhenawee M (2014) Chromatographic analysis of some drugs employed in erectile dysfunction therapy: Qualitative and quantitative studies using calixarene stationary phase. J Sep Sci. 37:2814-2824. http://dx.doi.org/10.1002/jssc.201400276

310. Shi S, Wu Y, Zhou M, Cheng Q (2020) Simultaneous analysis of 31 anti-impotence compounds potentially illegally added to herbal-based dietary supplements by ultra-high-performance liquid chromatography coupled with

quadrupole time-of-flight mass spectrometry. J Chromatogr B. 1144:122077. http://dx.doi.org/10.1016/j.jchromb.2020.122077

311. Atipairin A, Woradechakul C, Chee KS, Sawatdee S, Yoon AS (2014) Method validation for determination of sildenafil citrate in extemporaneous oral suspension. Int J Pharm Pharm Sci. 6:131-136.

312. Al Hadithy AFY, De Goede AL, Eckhardt M, Hanff L, Koch BCP (2011) Stability of sildenafil (Revatio ®) dilutions in dextrose 5%. Intensive Care Med. 37:1899. http://dx.doi.org/10.1007/s00134-011-2356-4

313. Bell MS, Nolt DH (2003) Visual compatibility of doxapram hydrochloride with drugs commonly administered via a Y-site in the intensive care nursery Am J Health Syst Pharm. 60:193-194. http://dx.doi.org/10.1093/ajhp/60.2.193

314. Johnson CE, Bhatt-Mehta V, Mancari SC, McKown JA (1994) Stability of midazolam hydrochloride and morphine sulfate during simulated intravenous coadministration. Am J Hosp Pharm. 51:2812-2815. https://dx.doi.org/10.1093/ajhp/51.22.2812

315. Bouchoud L, Fonzo-Christe C, Klingmüller M, Bonnabry P (2013) Compatibility of intravenous medications with parenteral nutrition: in vitro evaluation. J Parenter Enteral Nutr. 37:416-424. https://doi.org/10.1177/0148607112464239

316. Senarathna SG, Strunk T, Petrovski M, Woodland S, Martinez J, Chuang VT, Batty KT (2023) Physical compatibility of lipid emulsions and intravenous medications used in neonatal intensive care settings. Eur J Hosp Pharm. http://dx.doi.org/10.1136/ejhpharm-2023-003870

317. United States Pharmacopeia and National Formulary. General chapters <1790> Visual Inspection of Injections. (2017) United States Pharmacopeial Convention. USP 40–NF 35 ed.

318. Allen Jr LV, Stiles ML (1981) Compatibility of various admixtures with secondary additives at Y-injection sites of intravenous administration sets. Part 2. Am J Hosp Pharm. 38:380-381. http://dx.doi.org/10.1093/ajhp/38.3.380

319. Bashaw ED, Amantea MA, Minor JR, Gallelli JF (1988) Visual compatibility of zidovudine with other injectable drugs during simulated Y-site administration. Am J Hosp Pharm. 45:2532-2533. https://dx.doi.org/10.1093/ajhp/45.12.2532

320. Benedict MK, Roche VF, Banakar UV, Hilleman DE (1988) Visual compatibility of amiodarone hydrochloride with various antimicrobial agents during simulated Y-site injection. Am J Hosp Pharm. 45:1117-1118. https://doi.org/10.1093/ajhp/45.5.1117

321. Faudone G, Arifi S, Merk D (2021) The medicinal chemistry of caffeine. J Med Chem. 64:7156-7178. https://doi.org/10.1021/acs.jmedchem.1c00261

322. Marchai F, Bairam A, Vert P (1987) Neonatal apnea and apneic syndromes. Clin Perinatol. 14:509-529. https://doi.org/10.1016/S0095-5108(18)30748-6

323. Mathew OP (2011) Apnea of prematurity: pathogenesis and management strategies. J Perinatol. 31:302-310. http://dx.doi.org/10.1038/jp.2010.126

324. Task Force on Prolonged Infantile Apnea, American Academy of Pediatrics. Prolonged infantile apnea: 1985. (1985) Pediatrics. 76:129-131.

325. Jenni OG, Wolf M, Hengartner M, von Siebenthal K, Keel M, Bucher H-U (1996) Impact of central, obstructive and mixed apnea on cerebral hemodynamics in preterm infants. Neonatology. 70:91-100. https://doi.org/10.1159/000244353

326. Perlman JM, Volpe JJ (1985) Episodes of apnea and bradycardia in the preterm newborn: impact on cerebral circulation. Pediatrics. 76:333-338. https://doi.org/10.1542/peds.76.3.333

327. Bhatt-Mehta V, Schumacher RE (2003) Treatment of apnea of prematurity. Paediatr Drugs. 5:195-210. http://dx.doi.org/10.2165/00128072-200305030-00006

328. Natarajan G, Lulic-Botica M, Aranda J (2007) Pharmacology review: clinical pharmacology of caffeine in the newborn. Neoreviews. 8:e214-e221. https://doi.org/10.1542/neo.8-5-e214

329. Bairam A, Boutroy MJ, Badonnel Y, Vert P (1987) Theophylline versus caffeine: comparative effects in treatment of idiopathic apnea in the preterm infant. J Pediatr. 110:636-639. http://dx.doi.org/10.1016/s0022-3476(87)80569-3

330. Brouard C, Moriette G, Murat I, Flouvat B, Pajot N, Walti H, de Gamarra E, Relier J-P (1985) Comparative efficacy of theophylline and caffeine in the treatment of idiopathic apnea in premature infants. Am J Dis Child. 139:698-700. http://dx.doi.org/10.1001/archpedi.1985.02140090060028

331. Erenberg A, Leff RD, Haack DG, Mosdell KW, Hicks GM, Wynne BA (2000) Caffeine citrate for the treatment of apnea of prematurity: a double-blind, placebo-controlled study. Pharmacotherapy. 20:644-652. http://dx.doi.org/10.1592/phco.20.7.644.35167

332. Moschino L, Zivanovic S, Hartley C, Trevisanuto D, Baraldi E, Roehr CC (2020) Caffeine in preterm infants: where are we in 2020? ERJ Open Res. 6:00330-02019. http://dx.doi.org/10.1183/23120541.00330-2019

333. Lelo A, Birkett D, Robson R, Miners J (1986) Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. Br J Clin Pharmacol. 22:177-182. https://doi.org/10.1111/j.1365-2125.1986.tb05246.x

334. Lelo A, Miners J, Robson R, Birkett D (1986) Quantitative assessment of caffeine partial clearances in man. Br J Clin Pharmacol. 22:183-186. https://doi.org/10.1111/j.1365-2125.1986.tb05247.x

335. Miners JO, Birkett DJ (1996) The use of caffeine as a metabolic probe for human drug metabolizing enzymes. Gen Pharmacol. 27:245-249. https://doi.org/10.1016/0306-3623(95)02014-4

336. Fredholm BB, Arnaud MJ. Pharmacokinetics and metabolism of natural methylxanthines in animal and man (2011) Methylxanthines. p. 33-91.

337. Aldridge A, Aranda J-V, Neims AH (1979) Caffeine metabolism in the newborn. Clin Pharmacol Ther. 25:447-453. https://doi.org/10.1002/cpt1979254447

338. Aranda JV, Sitar DS, Parsons WD, Loughnan PM, Neims AH (1976) Pharmacokinetic aspects of theophylline in premature newborns. N Engl J Med. 295:413-416. http://dx.doi.org/10.1056/NEJM197608192950803

339. Aranda JV, Cook CE, Gorman W, Collinge JM, Loughnan PM, Outerbridge EW, Aldridge A, Neims AH (1979) Pharmacokinetic profile of caffeine in the premature

newborn infant with apnea. J Pediatr. 94:663-668. http://dx.doi.org/10.1016/s0022-3476(79)80047-5

340. Aranda JV, Beharry KD (2020) Pharmacokinetics, pharmacodynamics and metabolism of caffeine in newborns. Semin Fetal Neonatal Med. 25:101183. https://doi.org/10.1016/j.siny.2020.101183

341. Gal P (2009) Optimum Use of Therapeutic Drug Monitoring and Pharmacokinetics-Pharmacodynamics in the NICU. J Pediatr Pharmacol Ther. 14:66-74. https://doi.org/10.5863/1551-6776-14.2.66

342. Lee TC, Charles B, Steer P, Flenady V, Shearman A (1997) Population pharmacokinetics of intravenous caffeine in neonates with apnea of prematurity. Clin Pharmacol Ther. 61:628-640. https://doi.org/10.1016/S0009-9236(97)90097-7

343. Charles BG, Townsend SR, Steer PA, Flenady VJ, Gray PH, Shearman A (2008) Caffeine citrate treatment for extremely premature infants with apnea: population pharmacokinetics, absolute bioavailability, and implications for therapeutic drug monitoring. Ther Drug Monit. 30:709-716. http://dx.doi.org/10.1097/FTD.0b013e3181898b6f

344. Steer P, Flenady V, Shearman A, Lee T, Tudehope D, Charles B (2003) Periextubation caffeine in preterm neonates: a randomized dose response trial. J Paediatr Child Health. 39:511-515. https://doi.org/10.1046/j.1440-1754.2003.00207.x

345. Lowry JA, Jarrett RV, Wasserman G, Pettett G, Kauffman RE (2001) Theophylline Toxicokinetics in Premature Newborns. Arch Pediatr Adolesc Med. 155:934-939. http://dx.doi.org/10.1001/archpedi.155.8.934

346. Leon AEC, Michienzi K, Ma C-X, Hutchison AA (2006) Serum caffeine concentrations in preterm neonates. Am J Perinatol. 24:039-047. http://dx.doi.org/10.1055/s-2006-958163

347. Dobson NR, Hunt CE (2013) Pharmacology Review: Caffeine Use in Neonates: Indications, Pharmacokinetics, Clinical Effects, Outcomes. Neoreviews. 14:e540-e550. https://doi.org/10.1542/neo.14-11-e540

348. Aranda JV, Turmen T (1979) Methylxanthines in apnea of prematurity. Clin Perinatol. 6:87-108. https://doi.org/10.1016/S0095-5108(18)31165-5

349. Schmidt B, Roberts RS, Davis P, Doyle LW, Barrington KJ, Ohlsson A, Solimano A, Tin W (2006) Caffeine therapy for apnea of prematurity. N Engl J Med. 354:2112-2121. http://dx.doi.org/10.1056/NEJMoa054065

350. Schmidt B, Roberts RS, Davis P, Doyle LW, Barrington KJ, Ohlsson A, Solimano A, Tin W (2007) Long-term effects of caffeine therapy for apnea of prematurity. N Engl J Med. 357:1893-1902. http://dx.doi.org/10.1056/NEJMoa073679

351. Schmidt B, Anderson PJ, Doyle LW, Dewey D, Grunau RE, Asztalos EV, Davis PG, Tin W, Moddemann D, Solimano A (2012) Survival without disability to age 5 years after neonatal caffeine therapy for apnea of prematurity. JAMA. 307:275-282. http://dx.doi.org/10.1001/jama.2011.2024

352. Schmidt B, Roberts RS, Anderson PJ, Asztalos EV, Costantini L, Davis PG, Dewey D, D'Ilario J, Doyle LW, Grunau RE (2017) Academic performance, motor function, and behavior 11 years after neonatal caffeine citrate therapy for apnea of

prematurity: an 11-year follow-up of the CAP randomized clinical trial. JAMA Pediatr. 171:564-572. http://dx.doi.org/10.1001/jamapediatrics.2017.0238

353. Doyle LW, Schmidt B, Anderson PJ, Davis PG, Moddemann D, Grunau RE, O'Brien K, Sankaran K, Herlenius E, Roberts R (2014) Reduction in developmental coordination disorder with neonatal caffeine therapy. J Pediatr. 165:356-359. https://doi.org/10.1016/j.jpeds.2014.04.016

354. Aranda JV, Beharry K, Valencia GB, Natarajan G, Davis J (2010) Caffeine impact on neonatal morbidities. J Matern-Fetal Neonatal Med. 23:20-23. https://doi.org/10.3109/14767058.2010.517704

355. Nahata MC, Zingarelli JR, Durrell DE (1989) Stability of caffeine injection in intravenous admixtures and parenteral nutrition solutions. DICP. 23:466-467. https://doi.org/10.1177/106002808902300606

356. Oliphant EA, Purohit TJ, Alsweiler JM, McKinlay CJ, Hanning SM (2022) Validation and application of a simple and rapid stability-indicating liquid chromatographic assay for the quantification of caffeine from human saliva. J Liq Chromatogr Relat Technol. 45:10-17. https://doi.org/10.1080/10826076.2022.2095402

357. Latheef F, Wahlgren H, Lilja HE, Diderholm B, Paulsson M (2021) The risk of necrotizing enterocolitis following the administration of hyperosmolar enteral medications to extremely preterm infants. Neonatology. 118:73-79. https://doi.org/10.1159/000513169

Appendix 1

Drugs included in the search strategies of the main systematic review and pilot review

List of NICU drugs and their clinically relevant concentration/ concentration ranges. This list was used for concept 3 of search strategy to capture the studies which investigated the physical and chemical compatibility of these drugs. Seven different guidelines from institutions representing different countries were used to construct the list. Extremely rare drugs, TPNs, most IV fluids and blood products were excluded.

Table 1: Drugs included in the main search strategy (Drugs listed in guidelines of two or more institutions selected, drugs with emerging role in NICUs and drugs of international relevance)

Drug	Clinically relevant concentration/ concentration range
Aciclovir/ acyclovir/ acycloguanosin	5 mg/mL
Adenosine	300 µg/mL
Adrenaline/Epinephrine	0.1 mg/mL or 1mg/mL
Alprostadil/ Prostaglandin E1/ PGE1	1/ 2 or 4 µg/mL
Amikacin	5 mg/mL
Amiodarone	300 µg/mL
Amoxicillin/ Amoxycillin	50/ 100 mg/mL
Amoxicillin + clavulanic acid/ Co-amoxiclav	20 mg/mL by amoxicillin content
Amphotericin B (Fungizone®)	0.1 mg/mL
Amphotericin (liposomal)	2 mg/mL
Ampicillin	50 mg/mL
Atropine (sulphate)/ Hyoscyamine	100 µg/mL
Azithromycin	2 mg/mL
Benzylpenicillin/ penicillin/ penicillin G	60 mg/mL
Caffeine/ Caffeine citrate	5 mg/mL
Calcium gluconate	0.11 mmol/mL
Cefazolin/ cephazolin	100 mg/mL & 20 mg/mL
Cefepime	100 mg/mL & 40mg/mL
Cefotaxime	100 mg/mL & 40 mg/mL
Ceftazidime	100 mg/mL & 40 mg/mL
Ceftriaxone	40 mg/mL
Cefuroxime	250 mg/ 2.5mL
Ciprofloxacin	2 mg/mL
Clarithromycin	2.5 mg/mL
Clindamycin	5 mg/mL
Clonazepam	100 µg/mL
Clonidine	150 µg/mL
Cloxacillin	50 mg/mL
Dexamethasone	100 µg/mL
Dexmedetomidine	1 µg/mL
Diazepam	10 mg/ 2 mL
Digoxin	50 µg/ 2mL
Dobutamine	1/ 2/ 4 mg/mL
Dopamine	0.8/ 1.6/ 3.2 mg/mL
Doxapram	2 mg/mL
Erythromycin	1mg/mL
Erythropoietin/EPO	1000 units/0.5mL
Fentanyl	10 µg/mL
Flecainide	10 mg/mL
Flucloxacillin/ Floxacillin	50 mg/mL
Fluconazole	2 mg/mL
Flumazenil	10 µg/mL
Folic Acid	100 µg/mL

Drug	Clinically relevant concentration/ concentration range
Furosemide (frusemide)	1 mg/mL
Ganciclovir	10 mg/mL
Gentamicin	10 mg/mL
Glucagon	40/ 80/ 160 µg/mL
Heparin/ Unfractionated Heparin	100 units/mL
Hydralazine	1 mg/mL
Hydrocortisone/ Cortisol	10 mg/mL & 1 mg/mL
Ibuprofen (& ibuprofen lysine)	5 mg/mL
Imipenem	5 mg/mL
Indometacin/ Indomethacin	0.5 mg/mL
Insulin neutral (soluble)	0.05/ 0.1/ 0.2 units/mL
Isoprenaline/ Isoproterenol	16/ 32/ 64 µg/mL
Ketamine	2 mg/mL
Lidocaine (Lignocaine)	1.75 mg/kg/hour
Levetiracetam	5/ 15 mg/mL
Linezolid	2 mg/mL
Magnesium sulfate	0.4 mmol/mL & 0.8mmol/mL
Meropenem	25/50 mg/mL
Metronidazole	5 mg/mL
Midazolam	50/ 100/ 200 µg/mL
Milrinone	50/ 100/ 200 µg/mL
Morphine	40/ 80/ 160 µg/mL
Naloxone	400 µg/mL
Neostigmine	150/ 500 µg/mL
Noradrenaline (norepinephrine)	40/ 80/ 120 µg/mL
Octreotide	5 µg/mL & 25 µg/mL
Omeprazole	0.4 mg/mL
Pancuronium	1 mg/mL
Pantoprazole	4 mg/mL & 0.4 mg/mL
Paracetamol/ Acetaminophen	10 mg/mL
Pentoxifylline	5 mg/mL
Phenobarbital/ Phenobarbitone	20 mg/mL
Phenytoin	5 mg/mL
Piperacillin-tazobactam	50 mg/mL
Potassium chloride	0.08 mmol/kg/mL
Propranolol	100 µg/mL
Pyridoxine	50 mg/mL
Ranitidine	2.5 mg/mL
Rifampicin/ Rifampin	6 mg/mL
Rocuronium	1 mg/mL
Salbutamol	5 µg/mL
Sildenafil	0.1/ 0.2/ 0.4 mg/mL
Sodium Benzoate	50 mg/mL
Sodium bicarbonate/ $NaHCO_3$	0.5 mmol/mL
Sodium Nitroprusside	100/ 200 µg/mL
Suxamethonium/ Succinylcholine	10 mg/mL
Tobramycin	10 mg/mL
Trimethoprim sulfamethoxazole/ Co-trimoxazole	0.64 mg/mL
Vancomycin	5 mg/mL
Vecuronium	1 mg/mL
Vitamin K/ Phytomenadione	2 mg/0.2mL
Zidovudine	1 – 2 mg/mL

Table 2: Drugs excluded in the main search strategy (Drugs listed in only one institution guideline, TPNs and IV fluids, blood products and drugs very rarely used in NICUs as per expert opinion)

Acetazolamide	Glycopyrrolate
Albumin	Glucose/ Dextrose
Alteplase	Gonadorelin - GnRH
Arginine	Iloprost
Atracurium	Immunoglobulin
Biotin	Labetalol
Calcitriol/ 1,25-dihydroxycolecalciferol	Levofloxacin
Carnitine	Lorazepam
Caspofungin	Methylene Blue
Colistin	Potassium Canrenoate
Dantrolene	Protamine sulfate
Diazoxide	Protirelin (TRH)
Dinoprostone	SMOFlipid with vitamins
Edrophonium	Sodium chloride
Enfuvirtide	Sodium Dihydrogen Phosphate
Epoprostenol	Sodium Phenylbutyrate (Ambutyrate)
Esmolol	Sotalol
Famotidine	Teicoplanin
Flucytosine	Thiamine
Fosfomycin	Thiopental/ Thiopentone
Fresh Frozen Plasma	Tranexamic Acid
Glyceryl Trinitrate (GTN)	

Table 3: Drugs used in the pilot test of the search strategy

Drug	**Clinically relevant concentration/ concentration range**
Aminophylline	2.5 mg/mL
Caffeine/ Caffeine citrate	5 mg/mL
Indometacin/ Indomethacin	0.5 mg/mL
Ketamine	2 mg/mL
Pentoxifylline	5 mg/mL
Sotalol	2 mg/mL

References:

1. Neonatal Medication Protocols, Government of Western Australia, North Metropolitan Health Services, King Edward Memorial Hospital https://www.wnhs.health.wa.gov.au/For-health-professionals/Clinical-guidelines/Neonatal-Medication-Protocols;
2. Neonatal Medication Guidelines, Government of South Australia https://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/clinical+resources/clinical+programs+and+practice+guidelines/womens+and+babies+health/neonatal+medication+guidelines/neonatal+medication+guidelines;
3. Neonatal Medication Guidelines, The Royal Children's hospital, Melbourne https://www.rch.org.au/piper/neonatal_medication_guidelines/Neonatal_Medication_Guidelines/;
4. Neonatal Drug Information Sheets, Canterbury District Health Board, New Zealand https://edu.cdhb.health.nz/Hospitals-Services/Health-Professionals/Neonatal-Clinical-Resources/Neonatal-Drug-Information-Sheets/Pages/default.aspx;
5. Neonatal Drug Formulary, West of Scotland, The Knowledge Network http://www.knowledge.scot.nhs.uk/child-services/communities-of-practice/neonatal-managed-clinical-networks/west-of-scotland/neonatal-drug-formulary-(wos).aspx;
6. Children's Hospital London Health Sciences Center, London, Ontario, Canada – NICU Medication Manual https://www.lhsc.on.ca/nicu/nicu-medication-manual;
7. Leeds Children's Hospital Formulary, Leeds Teaching Hospitals NHS Trust http://www.leedsformulary.nhs.uk/chaptersSubDetails.asp?FormularySectionID=24&SubSectionRef=24.16&SubSectionID=A100

Appendix 2

Search terms, subject headings and limiters used in data base searching

Table 1. Key search terms, subject headings, limiters applied and number of hits retrieved from each of the selected databases

Database	Concept 1	Concept 2	Concept 3	limiters	No of hits
KEYWORDS	compatib* or incompatib* or stability or instability	intravenous* or "intra-venous*" or iv or "y-site" or "y-site" or ysite or injection* or infusion* or parenteral or injectable* or mixture*	(Aciclovir or acyclovir or acycloguanosin or Adenosine or Adrenaline or Epinephrine or Alprostadil or "Prostaglandin E1" or PGE1 or Amikacin or Amiodarone or Amoxicillin or Amoxycillin or Amoxicillin or "clavulanic acid" or "Co-amoxiclav" or Coamoxiclav or "Amphotericin B" or Fungizone or "Amphotericin liposomal" or Ampicillin or Atropine or Hyoscyamine or Azithromycin or Benzylpenicillin or penicillin or Caffeine or "Calcium gluconate" or Cefazolin or cephazolin or Cefepime or Cefotaxime or Ceftazidime or Ceftriaxone or Cefuroxime or Ciprofloxacin or Clarithromycin or Clindamycin or Clonazepam or Clonidine or Cloxacillin or Dexamethasone or Dexmedetomidine or Diazepam or Digoxin or Dobutamine or Dopamine or Doxapram or Erythromycin or Erythropoietin or Fentanyl or Flecainide or Flucloxacillin or Floxacillin or Fluconazole or Flumazenil or "Folic Acid" or Furosemide or frusemide or Ganciclovir or Gentamicin or Glucagon or Heparin or Hydralazine or Hydrocortisone or Cortisol or Ibuprofen or Imipenem or Indometacin or Indomethacin or Insulin or Isoprenaline or Isoproterenol or Ketamine or Lidocaine or Lignocaine or Levetiracetam or Linezolid or "Magnesium sulfate" or Meropenem or Metronidazole or Midazolam or Milrinone or Morphine or Naloxone or Neostigmine or Noradrenaline or norepinephrine or Octreotide or Omeprazole or Pancuronium or Pantoprazole or Paracetamol or Acetaminophen or Pentoxifylline or Phenobarbital or Phenobarbitone or Phenytoin or "Piperacillin-tazobactam" or "Potassium chloride" or Propranolol or Pyridoxine or Ranitidine or Rifampicin or Rifampin or Rocuronium or Salbutamol or Sildenafil or "Sodium Benzoate" or "Sodium bicarbonate" or "Sodium Nitroprusside" or Suxamethonium or Succinylcholine or Tobramycin or Trimethoprim sulfamethoxazole or "Co-trimoxazole" or Cotrimoxazole or Vancomycin or Vecuronium or "Vitamin K" or Phytomenadione or Zidovudine) [Ovid - .mp. search] [CINAHL – default search]	English language	

Database	Concept 1	Concept 2	Concept 3	limiters	No of hits
			[Web of Science – Topic search, English] [Proquest – NOFT, Peer reviewed, English		
EMBASE SHs	"drug incompatibility"/ or "drug stability"/	"intravenous drug administration"/ or "continuous infusion"/ or "parenteral drug administration"/ or "drug infusion"/ or "drug mixture"/	Aciclovir/ or Adenosine/ or Epinephrine/ or Prostaglandin E1/ or Amikacin/ or Amiodarone/ or Amoxicillin/ or Amoxicillin plus clavulanic acid/ or Amphotericin B/ or Amphotericin B lipid complex/ or amphotericin B deoxycholate/ or Ampicillin/ or Atropine/ or Hyoscyamine/ or Azithromycin/ or Benzylpenicillin/ or penicillin G/ or Caffeine/ or Caffeine citrate/ or gluconate calcium/ or Cefazolin/ or Cefepime/ or Cefotaxime/ or Ceftazidime/ or Ceftriaxone/ or Cefuroxime/ or Ciprofloxacin/ or Clarithromycin/ or Clindamycin/ or Clonazepam/ or Clonidine/ or Cloxacillin/ or Dexamethasone/ or Dexmedetomidine/ or Diazepam/ or Digoxin/ or Dobutamine/ or Dopamine/ or Doxapram/ or Erythromycin/ or Erythropoietin/ or Fentanyl/ or Flecainide/ or Flucloxacillin/ or Fluconazole/ or Flumazenil/ or Folic Acid/ or Furosemide/ or Ganciclovir/ or Gentamicin/ or Glucagon/ or recombinant glucagon/ or Heparin/ or Hydralazine/ or Hydrocortisone/ or Ibuprofen/ or Imipenem/ or Indometacin/ or Insulin/ or Isoprenaline/ or Ketamine/ or Lidocaine/ or Levetiracetam/ or Linezolid/ or Magnesium sulfate/ or Meropenem/ or Metronidazole/ or Midazolam/ or Milrinone/ or Morphine/ or Naloxone/ or Neostigmine/ or Noradrenalin/ or Octreotide/ or Omeprazole/ or Pancuronium/ or Pantoprazole/ or Paracetamol/ or Pentoxifylline/ or Phenobarbital/ or Phenytoin/ or piperacillin plus tazobactam/ or Potassium chloride/ or Propranolol/ or Pyridoxine/ or Ranitidine/ or Rifampicin/ or Rocuronium/ or Salbutamol/ or Sildenafil/ or benzoic acid/ or bicarbonate/ or Nitroprusside Sodium/ or Suxamethonium/ or Tobramycin/ or Cotrimoxazole/ or Vancomycin/ or Vecuronium/ or vitamin K group/ or Zidovudine/	English	21880
MEDLINE SHs	"Drug Incompatibility"/ or "Drug Stability"/	"Infusions, Intravenous"/ or "injections, Intravenous"/ or "Infusions, Parenteral"/ or "Administration, Intravenous"/	Acyclovir/ or Adenosine/ or Epinephrine/ or Alprostadil/ or Amikacin/ or Amiodarone/ or amoxicillin/ or amoxicillin-potassium clavulanate combination/ or Amphotericin B/ or Ampicillin/ or Atropine/ or Hyoscyamine/ or Azithromycin/ or Penicillin G/ or Caffeine/ or Calcium gluconate/ or Cefazolin/ or Cefepime/ or Cefotaxime/ or Ceftazidime/ or Ceftriaxone/ or Cefuroxime/ or Ciprofloxacin/ or Clarithromycin/ or Clindamycin/ or Clonazepam/ or Clonidine/ or Cloxacillin/ or Dexamethasone/ or Dexmedetomidine/ or Diazepam/ or Digoxin/ or Dobutamine/	English	8526

Database	Concept 1	Concept 2	Concept 3	limiters	No of hits
			or Dopamine/ or Doxapram/ or Erythromycin/ or Erythropoietin/ or Fentanyl/ or Flecainide/ or Floxacillin/ or Fluconazole/ or Flumazenil/ or Folic Acid/ or Furosemide/ or Ganciclovir/ or Gentamicin/ or Glucagon/ or Heparin/ or Hydralazine/ or Hydrocortisone/ or Ibuprofen/ or Imipenem/ or Indomethacin/ or Insulin/ or Isoproterenol/ or Ketamine/ or Lidocaine/ or Levetiracetam/ or Linezolid/ or Magnesium sulfate/ or Meropenem/ or Metronidazole/ or Midazolam/ or Milrinone/ or Morphine/ or Naloxone/ or Neostigmine/ or norepinephrine/ or Octreotide/ or Omeprazole/ or Pancuronium/ or Pantoprazole/ or Acetaminophen/ or Pentoxifylline/ or Phenobarbital/ or Phenytoin/ or Piperacillin, Tazobactam Drug Combination/ or Potassium chloride/ or Propranolol/ or Pyridoxine/ or Ranitidine/ or Rifampin/ or Rocuronium/ or Albuterol/ or Sildenafil Citrate/ or Sodium Benzoate/ or Benzoic Acid/ or Sodium bicarbonate/ or Nitroprusside/ or Succinylcholine/ or Tobramycin/ or Trimethoprim, Sulfamethoxazole Drug Combination/ or Vancomycin/ or Vecuronium Bromide/ or Vitamin K 1/ or Zidovudine/		
CINAHL SHs	MH= "Drug incompatibility" or "Drug Stability"	MH= "Infusions, Intravenous" or "Injections, Intravenous" or "Infusions, Parenteral" or "Administration, Intravenous"	MH= "Acyclovir" or "Adenosine" or "Epinephrine" or "Prostaglandins E" or "Amikacin" or "Amiodarone" or "Amoxicillin" or "Amphotericin B" or "Ampicillin" or "Atropine" or "Azithromycin" or "Penicillin G" or "Caffeine" or "Cefazolin" or "Cefepime Hydrochloride" or "Cefotaxime" or "Ceftazidime" or "Ceftriaxone" or "Cefuroxime" or "Ciprofloxacin" or "Clarithromycin" or "Clindamycin" or "Clonazepam" or "Clonidine" or "Cloxacillin" or "Dexamethasone" or "Diazepam" or "Digoxin" or "Dobutamine" or "Dopamine" or "Doxapram" or "Erythromycin" or "Erythropoietin" or "Fentanyl" or "Flecainide Acetate" or "Fluconazole" or "Flumazenil" or "Folic Acid" or "Furosemide" or "Ganciclovir" or "Gentamicins" or "Glucagon" or "Heparin" or "Hydralazine" or "Hydrocortisone" or "Ibuprofen" or "Imipenem" or "Indomethacin" or "Insulin" or "Isoproterenol" or "Ketamine" or "Lidocaine" or "Linezolid" or "Magnesium sulfate" or "Meropenem" or "Metronidazole" or "Midazolam" or "Milrinone" or "Morphine" or "Naloxone" or "Neostigmine" or "norepinephrine" or "Octreotide Acetate" or "Omeprazole" or "Pancuronium" or "Pantoprazole Sodium" or "Acetaminophen" or "Pentoxifylline" or "Phenobarbital" or "Phenytoin" or "Potassium chloride" or "Propranolol" or	English	1262

Database	Concept 1	Concept 2	Concept 3	limiters	No of hits
			"Pyridoxine" or "Ranitidine" or "Rifampin" or "Rocuronium" or "Albuterol" or "Sildenafil" or "Sodium Benzoate" or "Sodium bicarbonate" or "Nitroprusside" or "Succinylcholine" or "Tobramycin" or "Trimethoprim-Sulfamethoxazole Combination" or "Vancomycin" or "Vecuronium Bromide" or "Vitamin K" or "Zidovudine"		
Proquest (Health & Medical Collection)				NOFT, Peer reviewed, English	1843
Web of Science				Topic search, English	9303

Appendix 3

Standardized data extraction sheet

Reviewer	**1**		**2**		**3**		
Title of the study							
First and corresponding authors							
Journal							
Year of publication							
Key words used							
Abbreviated study ID							
Main Objective/s related to physical/chemical compatibility testing (Please check)	**Physical**		**Chemical**		**Physical and Chemical**		
Context of application (if mentioned, please check)	**Neonatal**		**Paediatric**		**Adult**	**Not specified**	
Main drug tested, its concentration/s and diluent							
Sample preparation (Please check)	**Filtration of samples done**			**Order of mixing mentioned**			
Use of negative controls	**Yes**		**No**		**If yes, specify**		
Mixing methods (Please check)	**Vortex mixing**		**Gentle mixing**		**Not specified**		
Mixing ratio							
Replication of samples (Please check)	**Yes**			**No**			
Batches and/or manufacturers recorded	**Yes**			**No**			
Y-site simulation and test Vessels used (Please check)	**Y – Site simulation**				**Actual Y-site IV tube mixing**		
	Glass tubes		**Plastic tubes**				
Test temperatures (Please check)	**Humidicrib**		**RT**		**Refrigerator**		
Test duration (contact/exposure)							
Test time points							

Methods of physical compatibility testing (Please check)	**Visual observation for haze/ colour change/ precipitation/ gas evolution**		**Turbidimetry**		**Other (Specify)**	
Acceptance criteria for physical compatibility (Please check, if yes specify)	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**

Methods of chemical compatibility testing (Please check)	**HPLC**		**Other (specify)**	
Acceptance criteria for chemical compatibility (Please check, if yes specify)	**Yes**	**No**	**Yes**	**No**
Statistical methods used in analysis				
Any other information				

Drugs/ s tested with the main drug, concentrations, diluents, physical compatibility results and chemical compatibility results **Compatible = C** **Incompatible = I** **Inconclusive = Q** **Undiluted = U**	**Drug**	**Concentration**	**Diluent**	**Physical compatibility**	**Chemical compatibility**

Appendix 4

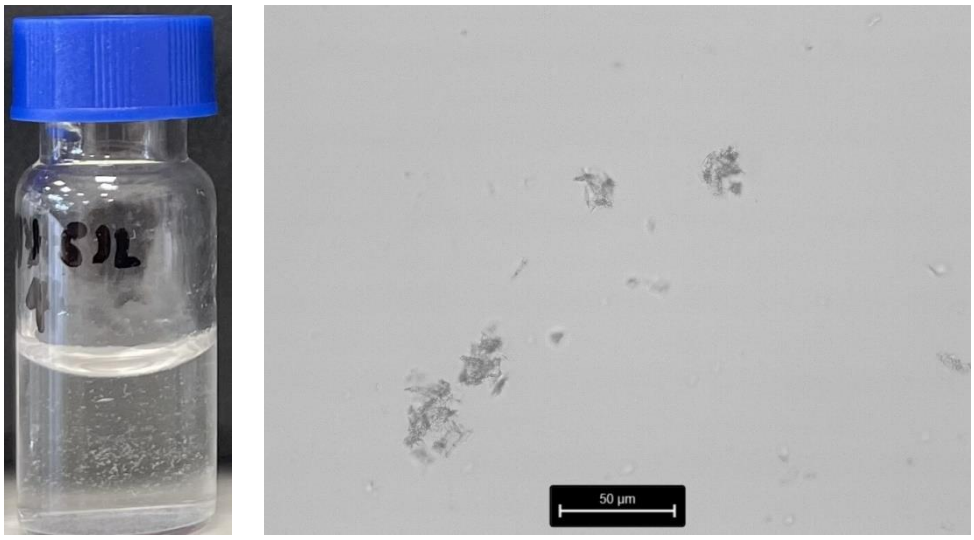
Quality assessment instrument

General quality Assessment Tool (applicable to all studies involved)	Yes / No
Drug manufacturers listed	Yes / No
Drug batches listed	Yes / No
Number and frequency of observations defined	Yes / No
Study duration defined	Yes / No
Study conditions: temperature	Yes / No
Study conditions: duration of exposure	Yes / No
Mixing vessels mentions (tubes/ containers/ Y-tubing)	Yes / No
Drug concentrations defined	Yes / No
Drug diluents reported for all drugs	Yes / No
Sampling times defined	Yes / No
Base line level testing (t = 0)	Yes / No
Parallel controls	
Testing performed in replicates	Yes / No
Mixing ratios defined	Yes / No
Study provides implications for future research and clinical practice	Yes / No
Physical compatibility component of the study	
Precipitate formation evaluated visually	Yes / No
Colour change evaluated	Yes / No
Gas production evaluated	Yes / No
Turbidity measured instrumentally	Yes / No
pH tested	Yes / No
Acceptance/compatibility criteria defined	Yes / No
Visual observation done by more than one assessor	Yes / No
Chemical compatibility component of the study	
Analytical method described or referenced	Yes / No
Method validation is reported/ referenced (selective or stability-indicating)	Yes / No
Analytical method QA data provided (LOQ, accuracy, precision)	Yes / No
Acceptance/compatibility criteria defined	Yes / No

Appendix 5

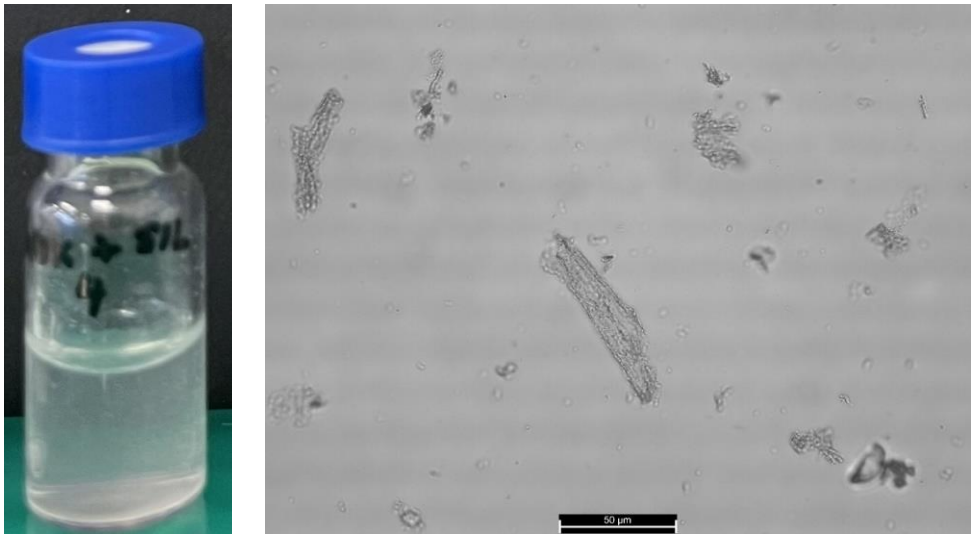
Physical incompatibilities of sildenafil with secondary test drugs

1. Sildenafil and aciclovir

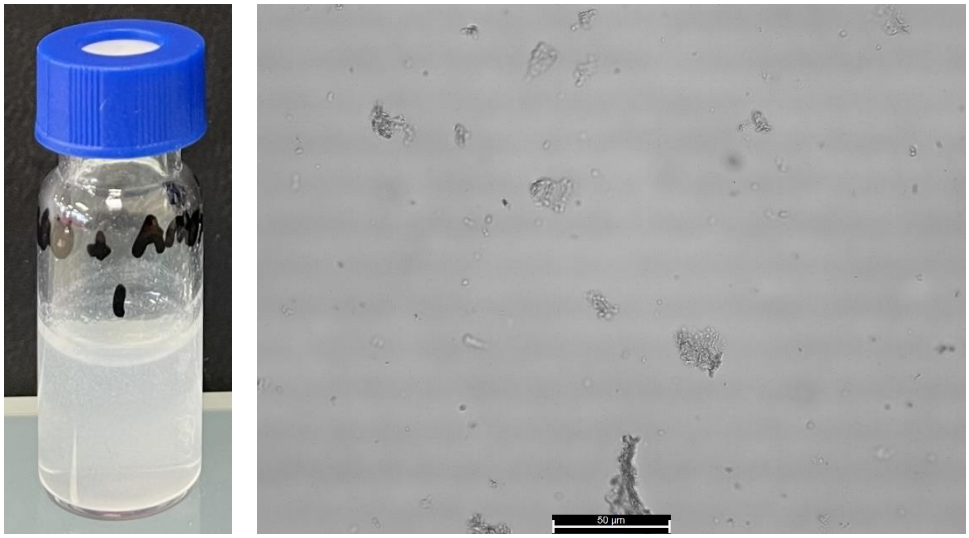


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and aciclovir (5 mg/mL)

2. Sildenafil and amoxicillin

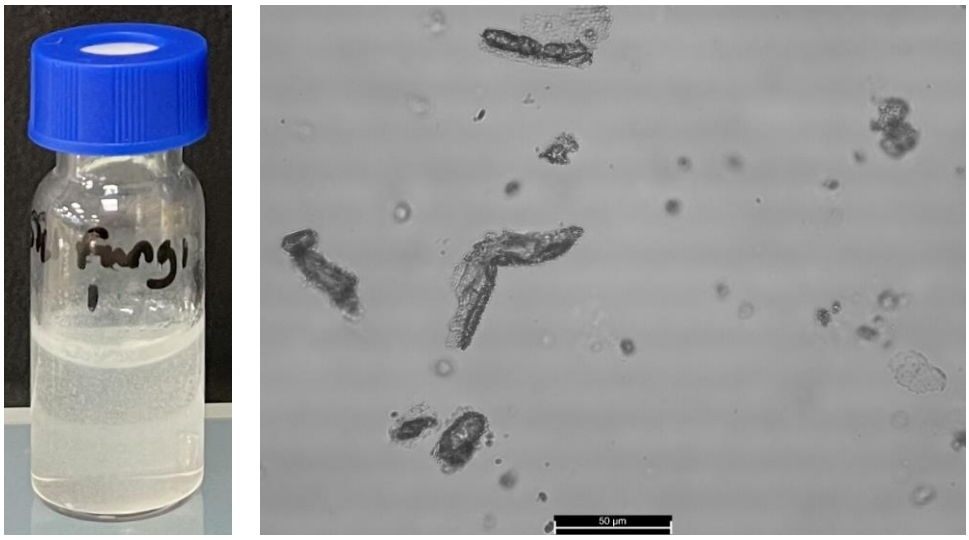


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and amoxicillin (100 mg/mL)



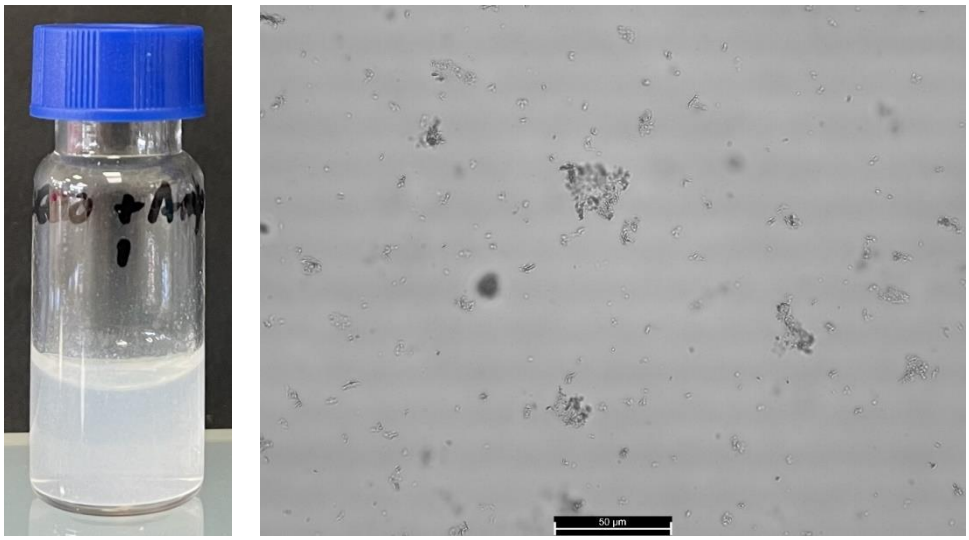
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and amoxicillin (50 mg/mL)

3. Sildenafil and amphotericin (fungizone)

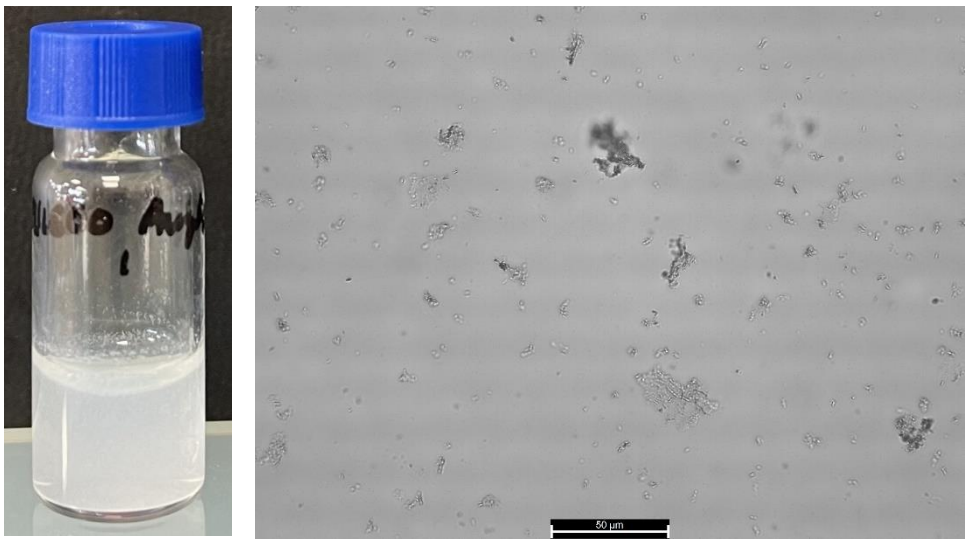


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and amphotericin (fungizone) (100 µg/mL)

4. Sildenafil and ampicillin

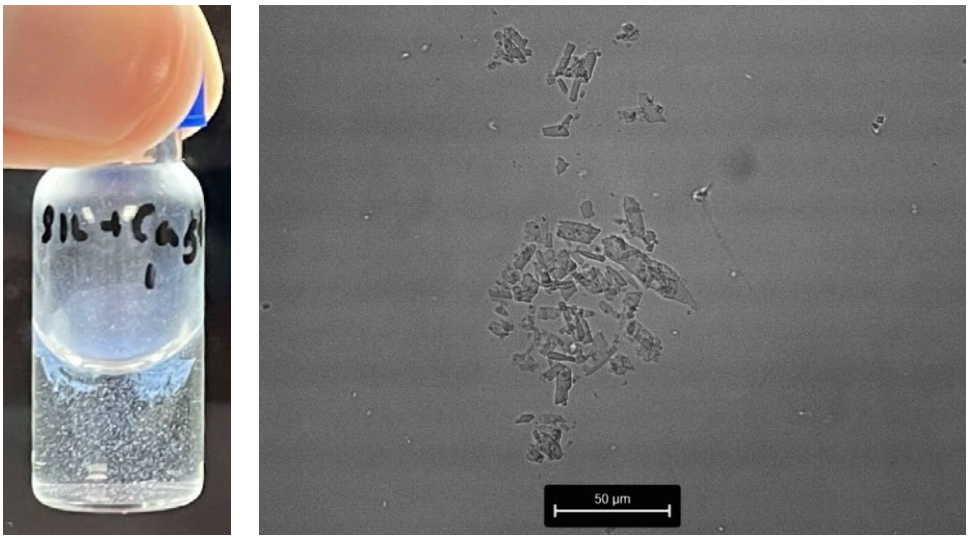


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and ampicillin (100 mg/mL)



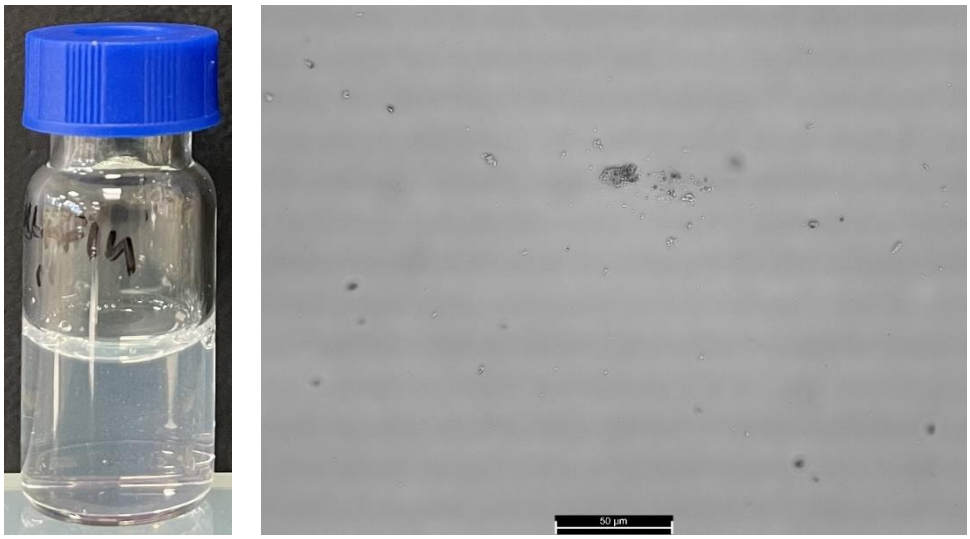
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and ampicillin (50 mg/mL)

5. Sildenafil and calcium gluconate



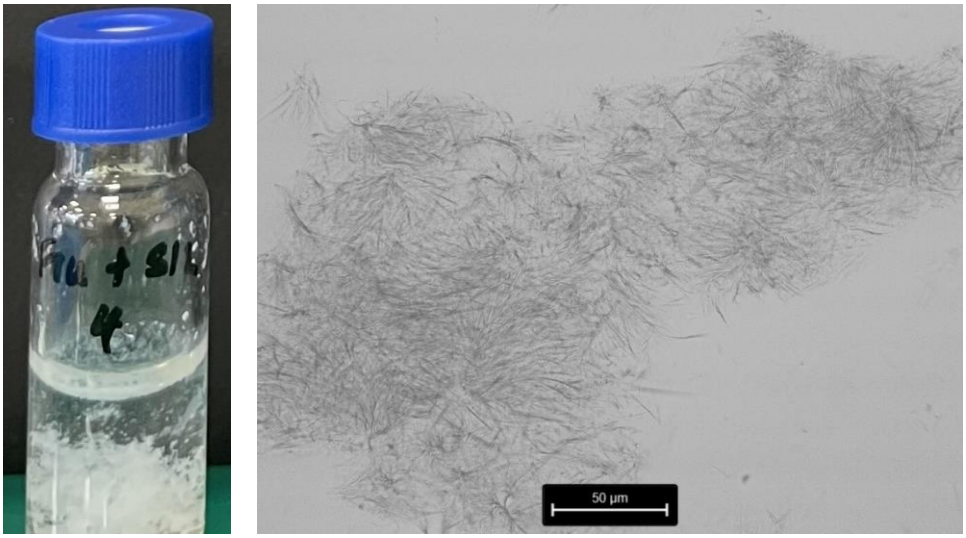
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles under polarized light) in the combination of sildenafil (600 µg/mL) and calcium gluconate (100 mg/mL)

6. Sildenafil and flucloxacillin

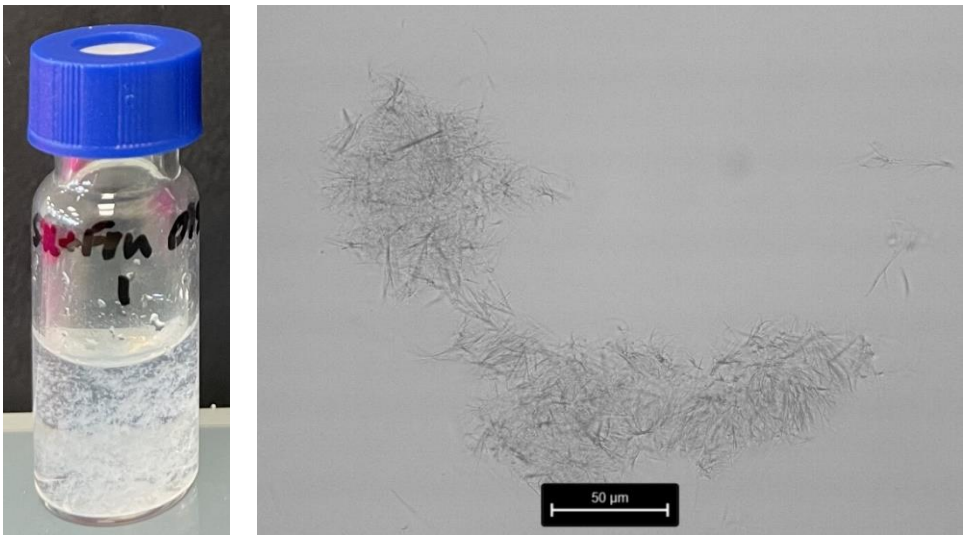


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (haze) in the combination of sildenafil (600 µg/mL) and flucloxacillin (50 mg/mL)

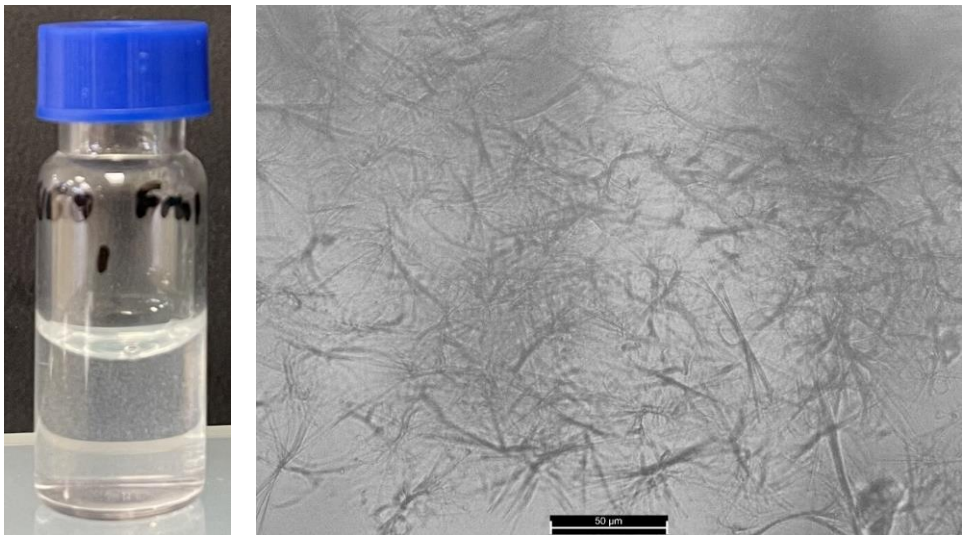
7. Sildenafil and furosemide



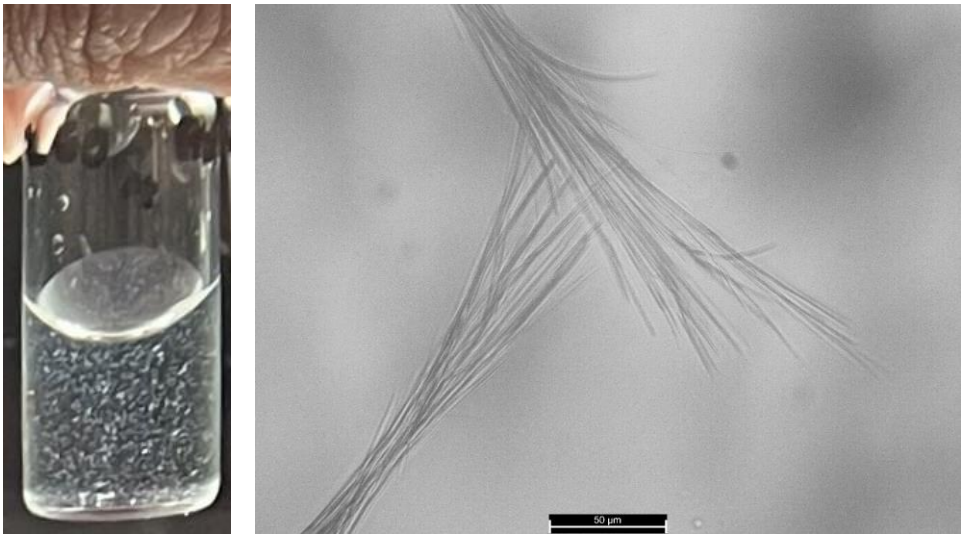
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and furosemide (1 mg/mL)



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and furosemide (0.2 mg/mL)

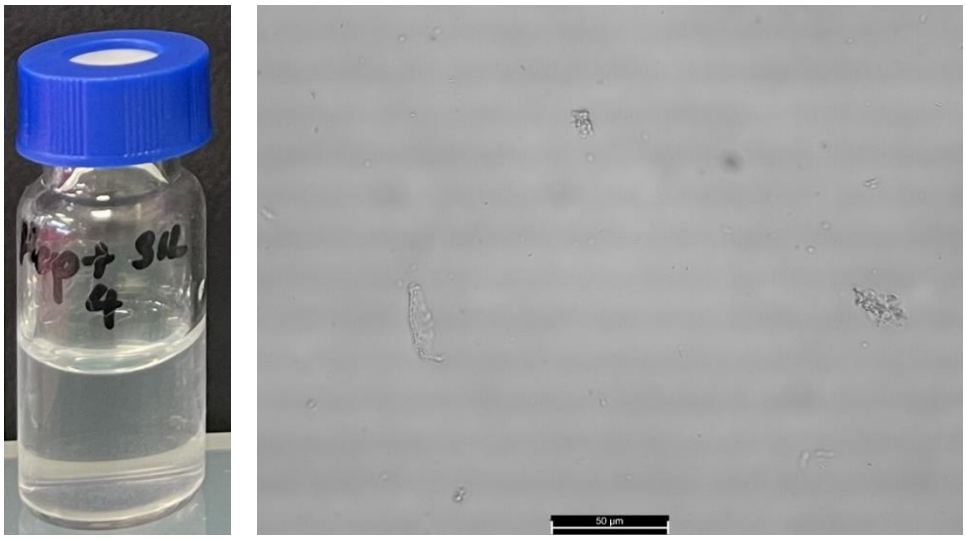


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (60 µg/mL) and furosemide (1 mg/mL)



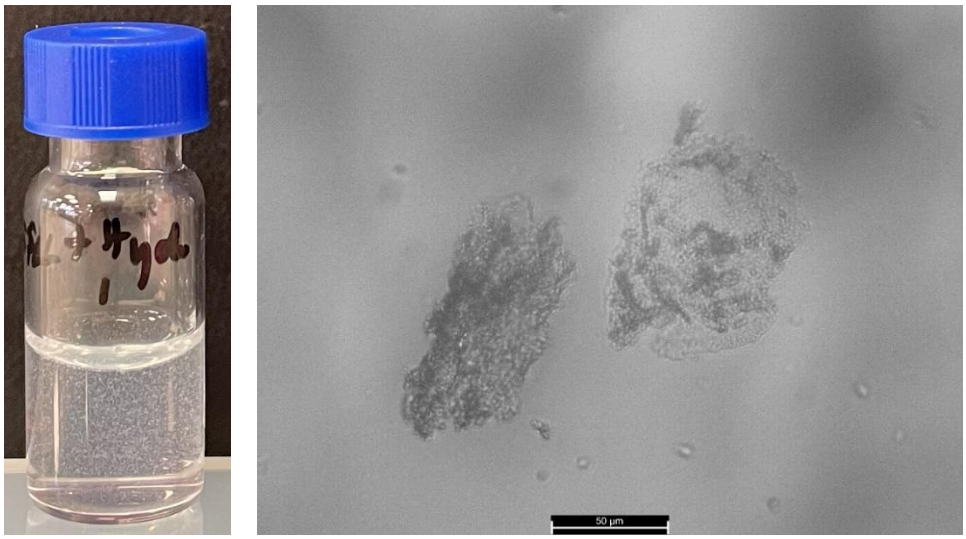
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles under polarized light) in the combination of sildenafil (60 µg/mL) and furosemide (0.2 mg/mL)

8. Sildenafil and heparin



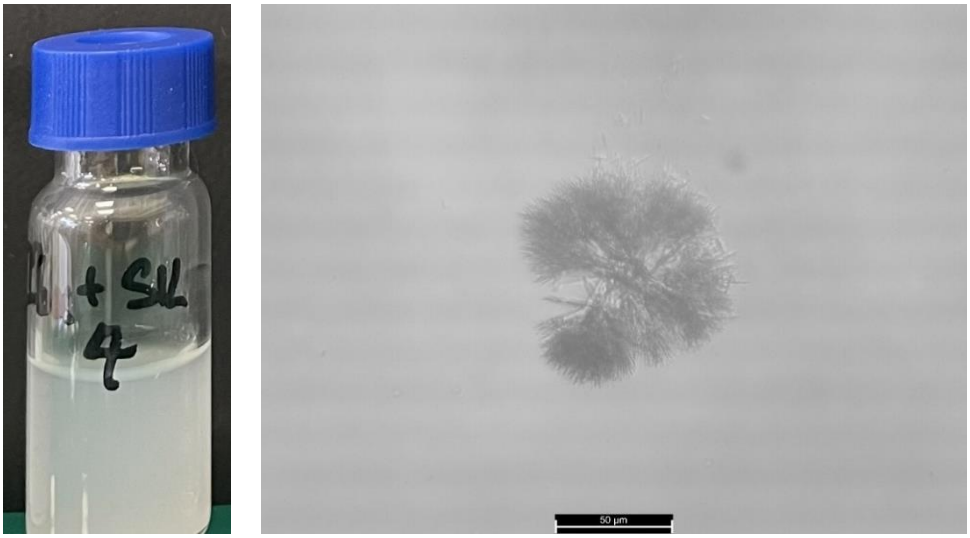
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (haze) in the combination of sildenafil (600 µg/mL) and heparin (100 units/mL)

9. Sildenafil and hydrocortisone



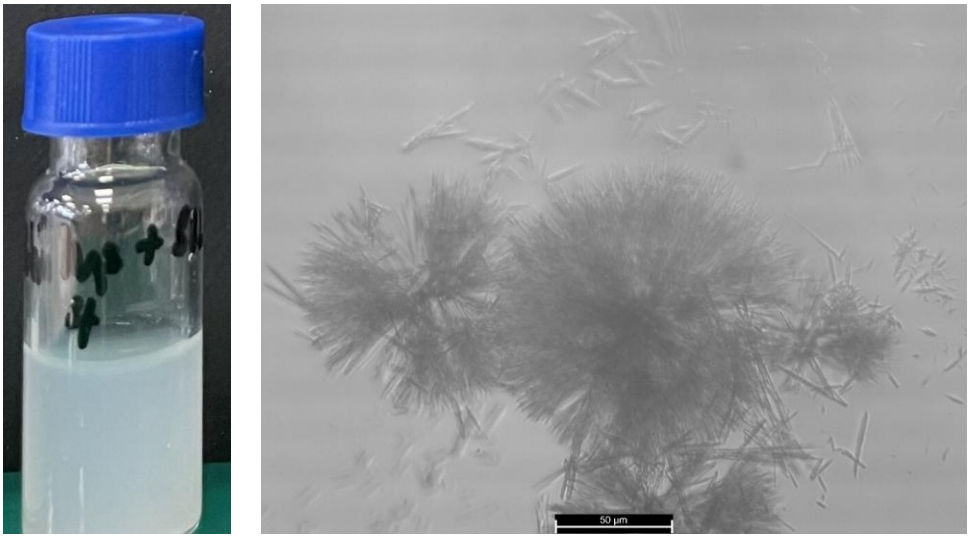
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and hydrocortisone (10 mg/mL)

10. Sildenafil and ibuprofen



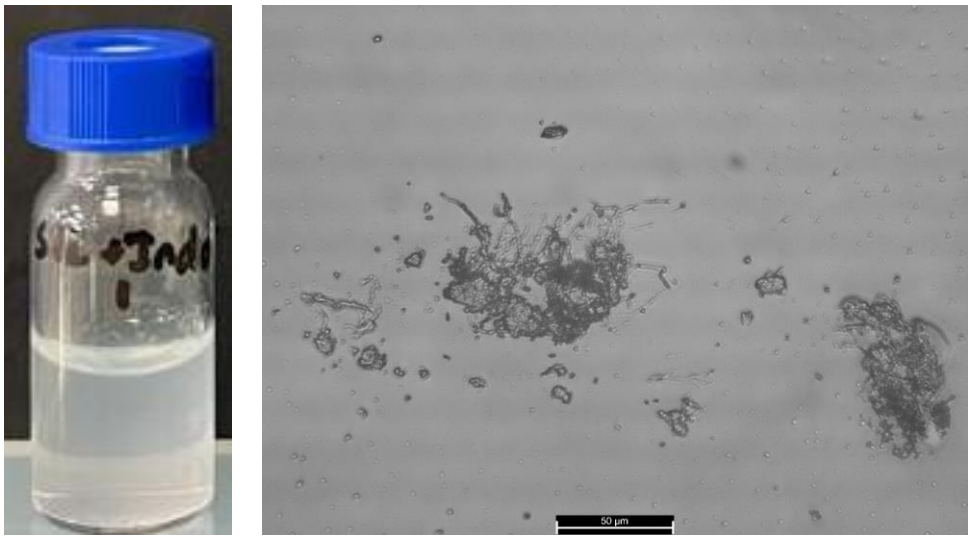
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of sildenafil (600 µg/mL) and ibuprofen (5 mg/mL)

11. Sildenafil and ibuprofen lysine



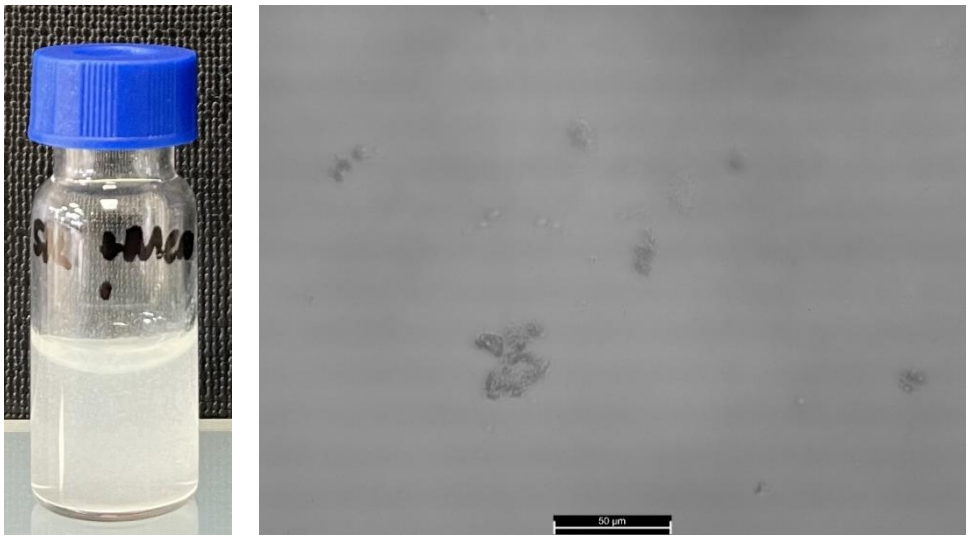
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of sildenafil (600 µg/mL) and ibuprofen lysine (4 mg/mL)

12. Sildenafil and indomethacin

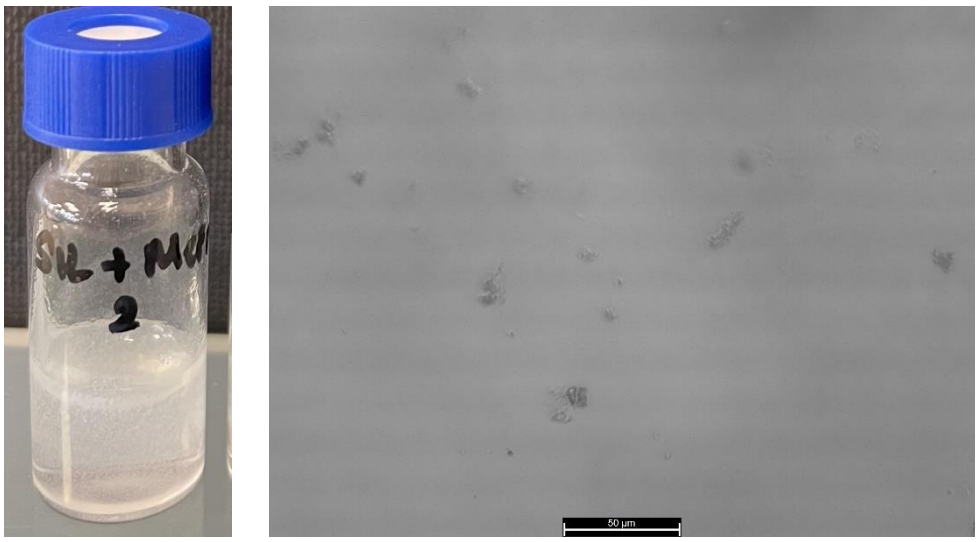


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of sildenafil (600 µg/mL) and indomethacin (200 µg/mL)

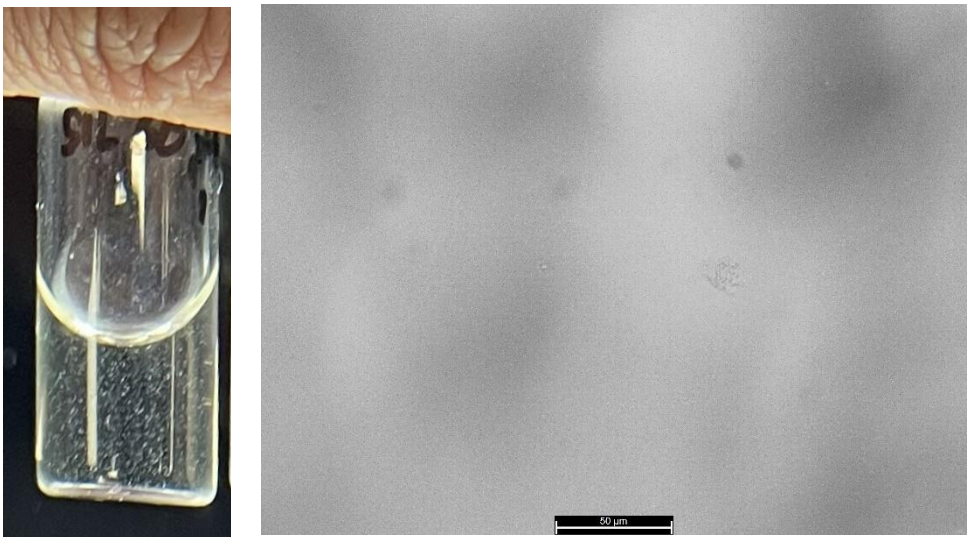
13. Sildenafil and meropenem



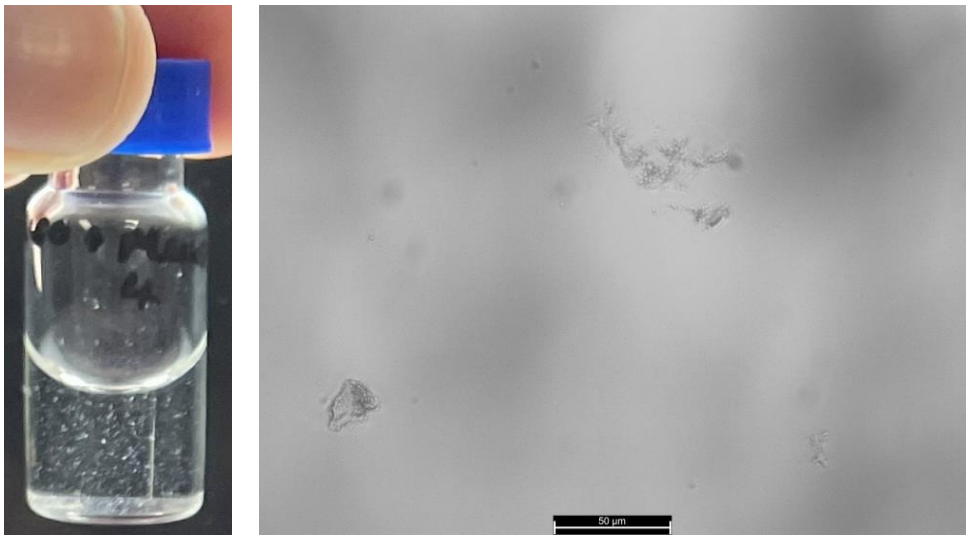
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and meropenem (50 mg/mL)



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and meropenem (25 mg/mL)

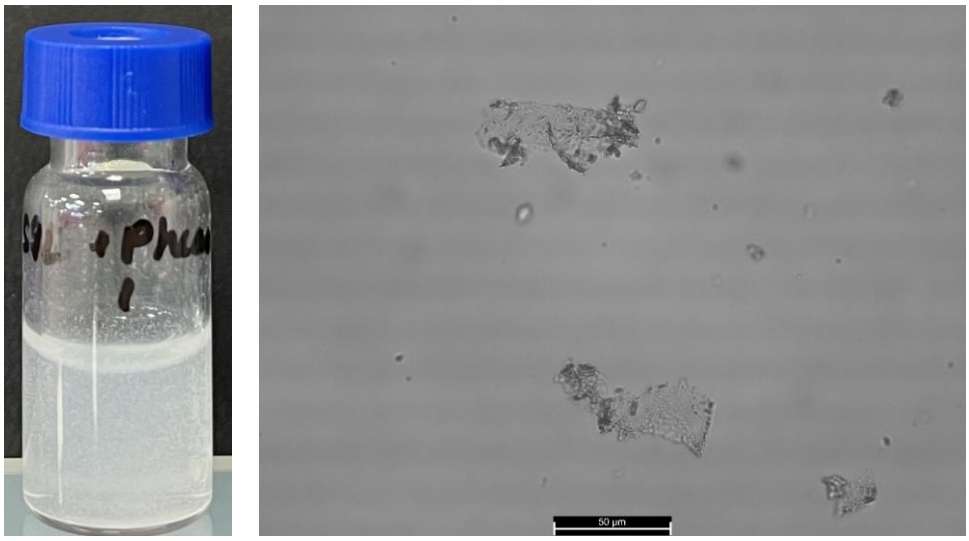


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles under polarized light) in the combination of sildenafil (60 µg/mL) and meropenem (50 mg/mL)



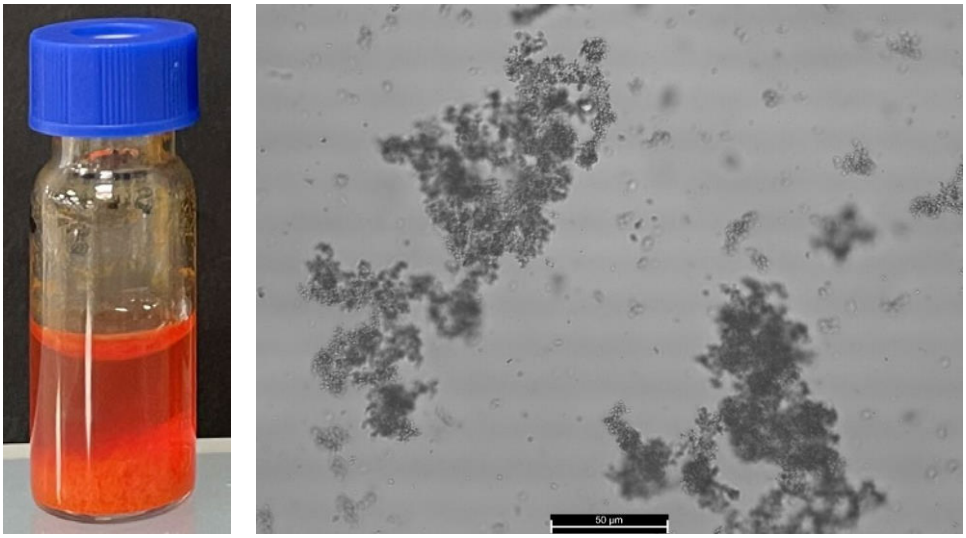
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles under polarized light) in the combination of sildenafil (60 µg/mL) and meropenem (25 mg/mL)

14. Sildenafil and phenobarbitone



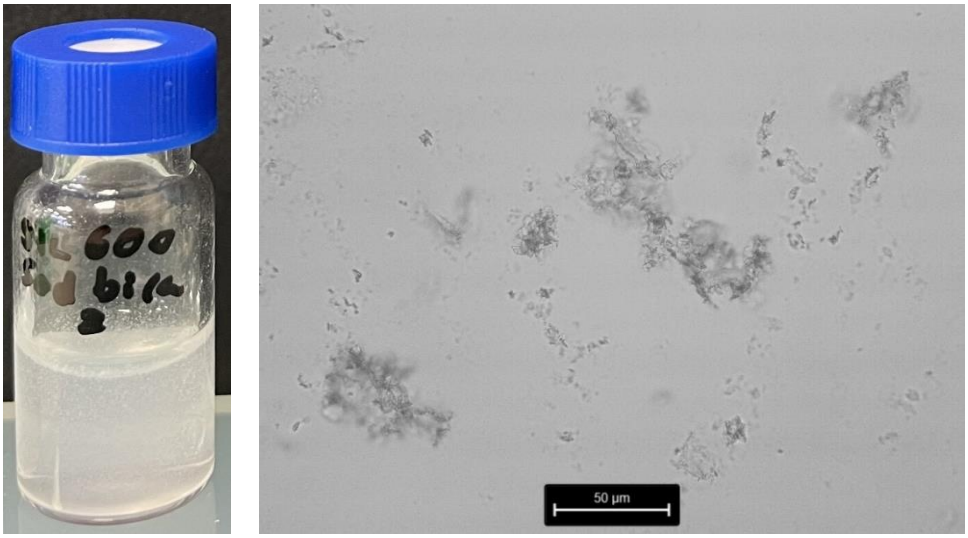
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and phenobarbitone (20 mg/mL)

15. Sildenafil and rifampicin

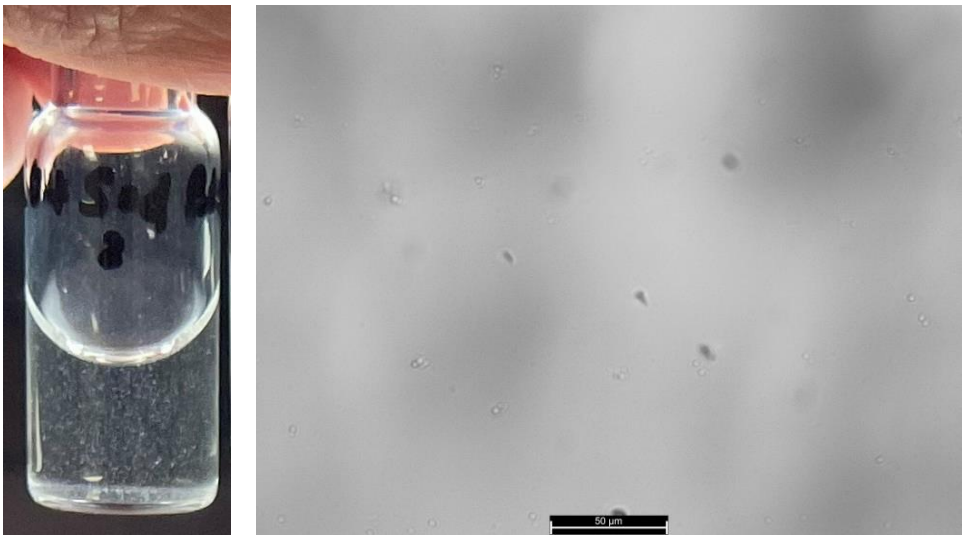


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (heavy precipitate) in the combination of sildenafil (60 µg/mL) and rifampicin (6 mg/mL)

16. Sildenafil and sodium bicarbonate

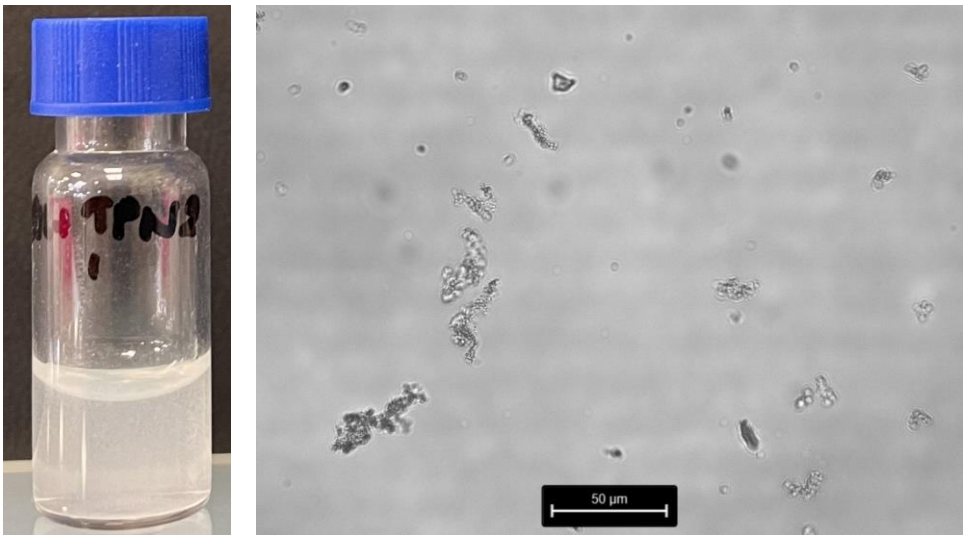


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and sodium bicarbonate (4.2 %) w/v)



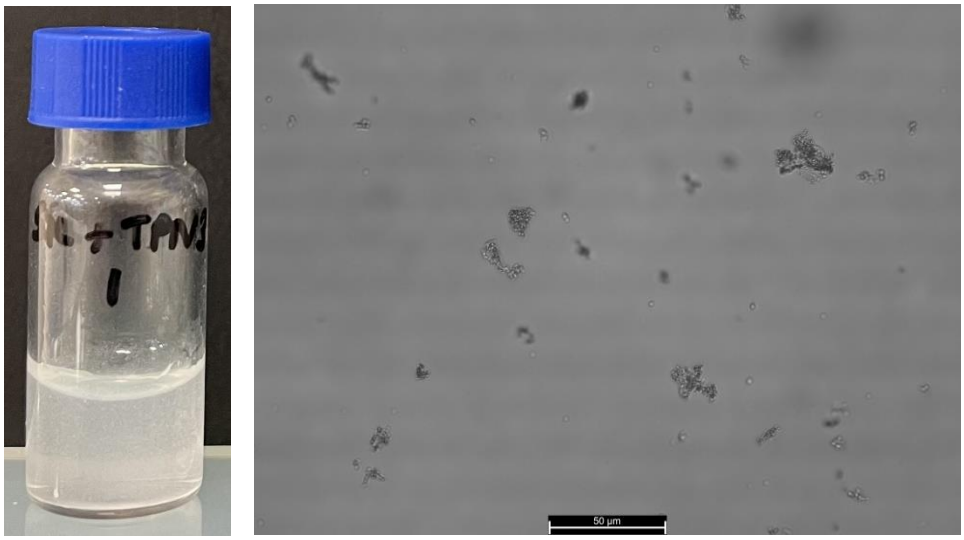
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles under polarized light) in the combination of sildenafil (60 µg/mL) and sodium bicarbonate (4.2 % w/v)

17. Sildenafil and 2-in-1 PN 1 (Preterm A)



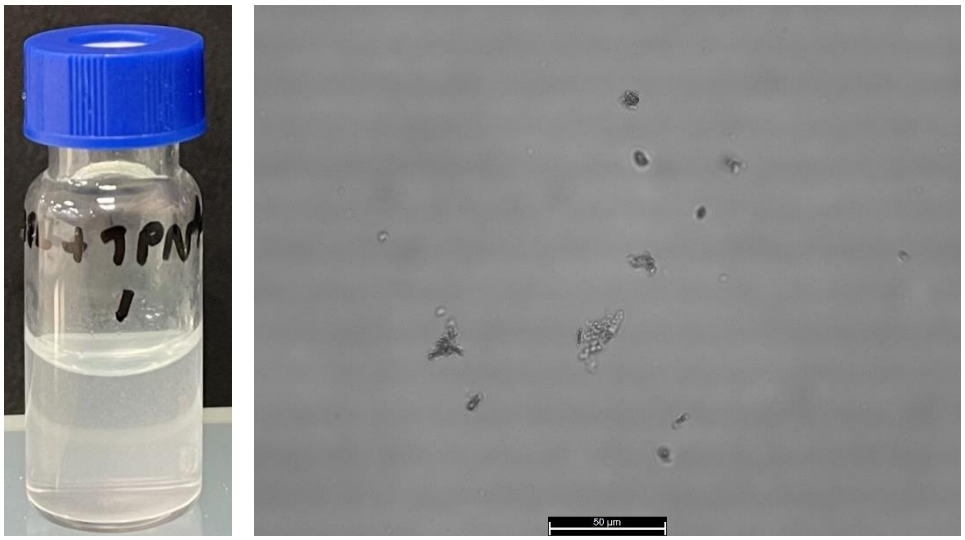
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and 2-in-1 PN 1 (Preterm A)

18. Sildenafil and 2-in-1 PN 2 (Preterm B)



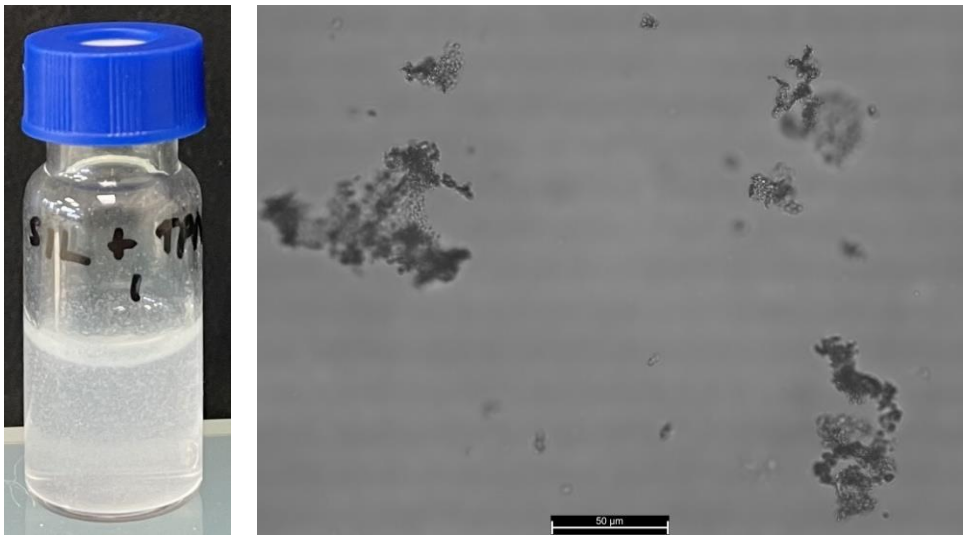
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and 2-in-1 PN 2 (Preterm B)

19. Sildenafil and 2-in-1 PN 3 (Term)



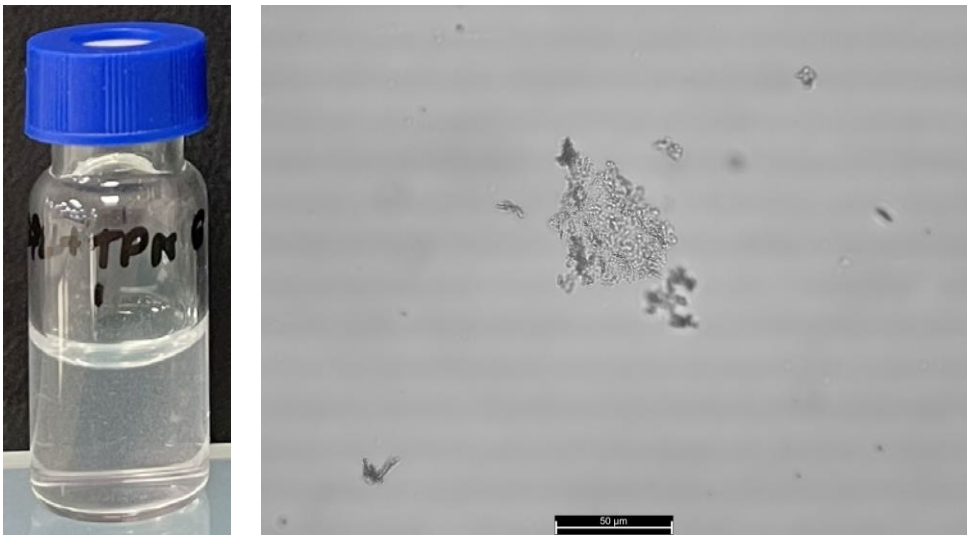
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and 2-in-1 PN 3 (Term)

20. Sildenafil and 2-in-1 PN 4 (Custom - 1)



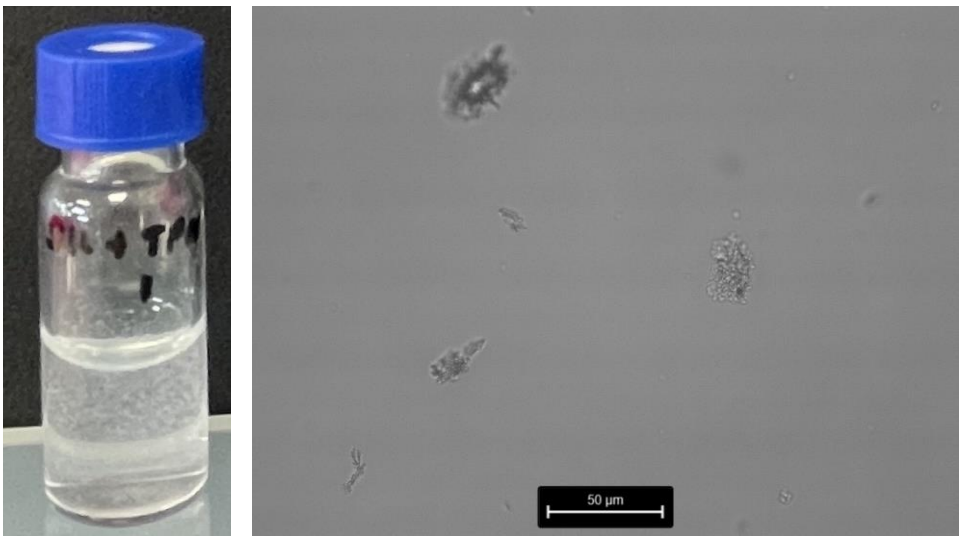
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and 2-in-1 PN 4 (Custom - 1)

21. Sildenafil and 2-in-1 PN 5 (Custom - 2)



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and 2-in-1 PN 5 (Custom - 2)

22. Sildenafil and 2-in-1 PN 6 (Custom - 3)



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of sildenafil (600 µg/mL) and 2-in-1 PN 6 (Custom - 3)

Appendix 6

Size distribution plots - Sildenafil compatibility with lipid emulsions

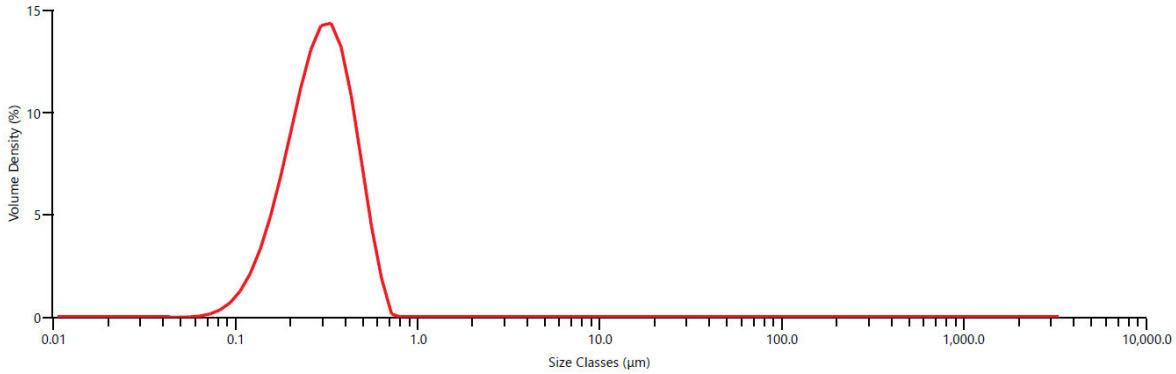


Figure 1. Sildenafil 600 µg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.308 µm; Dv50 = 0.294 µm; Dv10 = 0.162 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 92.5% and 7.5%, respectively (no droplets > 1 µm)

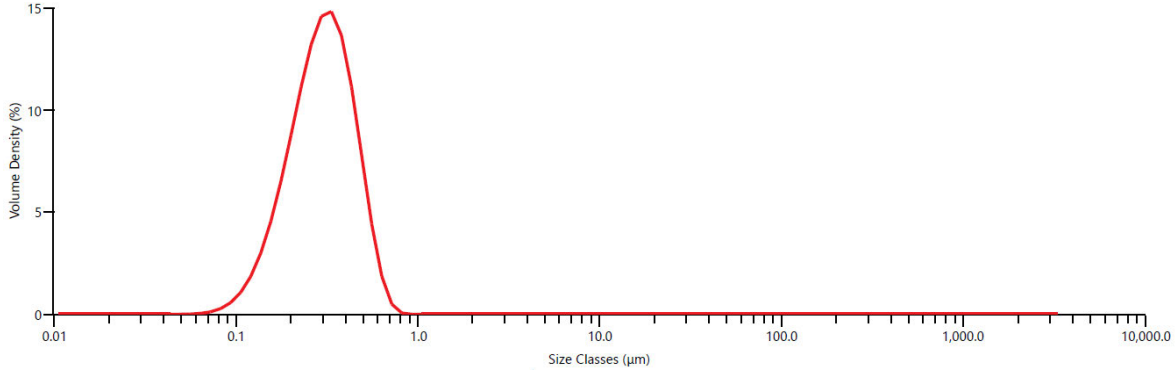


Figure 2. Sildenafil 600 µg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.313 µm; Dv50 = 0.299 µm; Dv10 = 0.168 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 92.2% and 7.8%, respectively (no droplets > 1 µm)

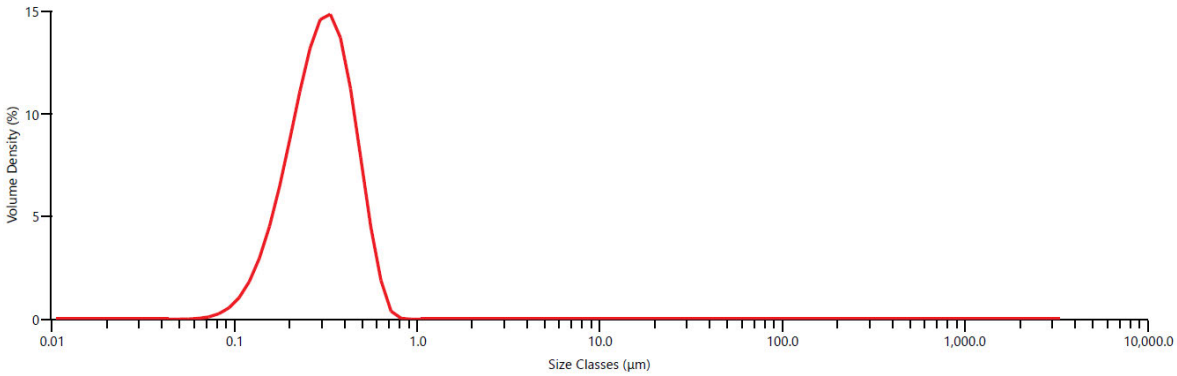


Figure 3. Sildenafil 60 µg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.313 µm; Dv50 = 0.299 µm; Dv10 = 0.168 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 92.2% and 7.8%, respectively (no droplets > 1 µm)

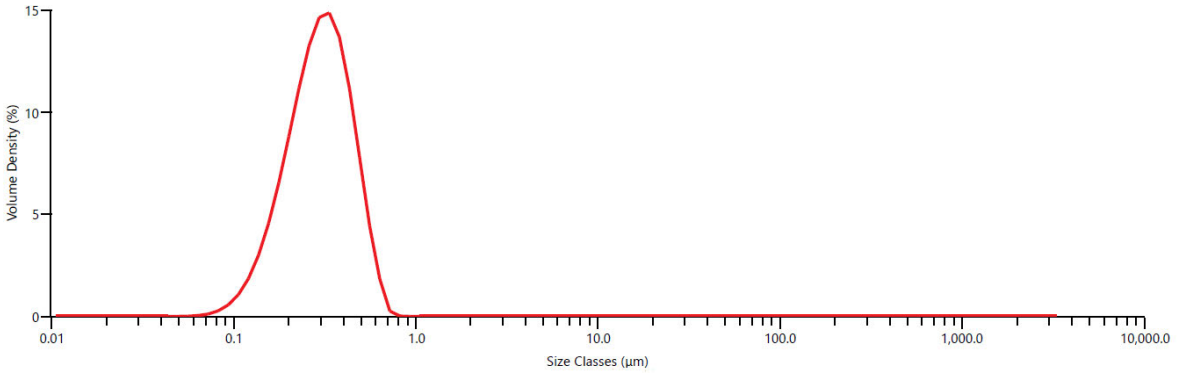


Figure 4. Sildenafil 60 µg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.312 µm; Dv50 = 0.298 µm; Dv10 = 0.168 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 92.4% and 7.6%, respectively (no droplets > 1 µm)

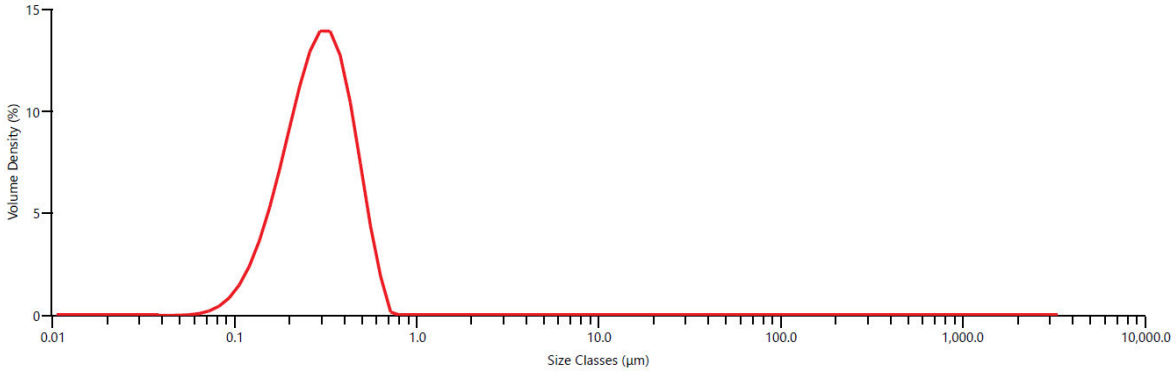


Figure 5. Glucose 5% and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.304 µm; Dv50 = 0.289 µm; Dv10 = 0.157 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 92.6% and 7.4%, respectively (no droplets > 1 µm)

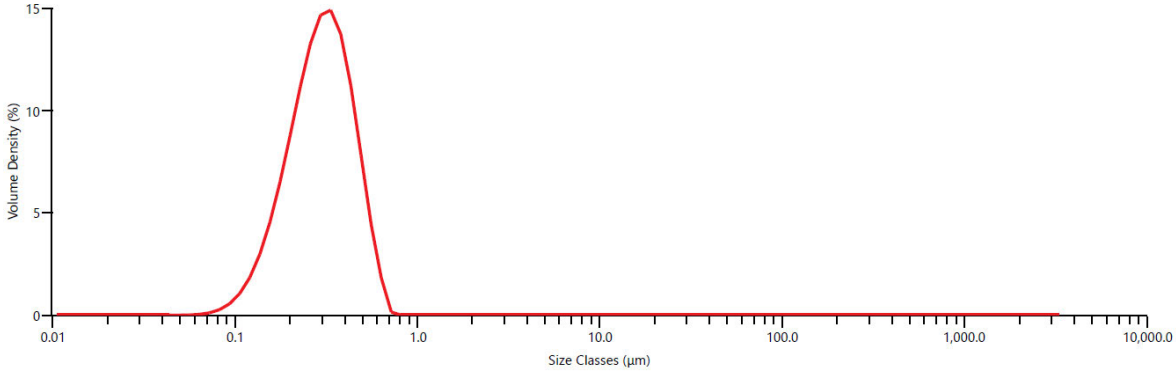
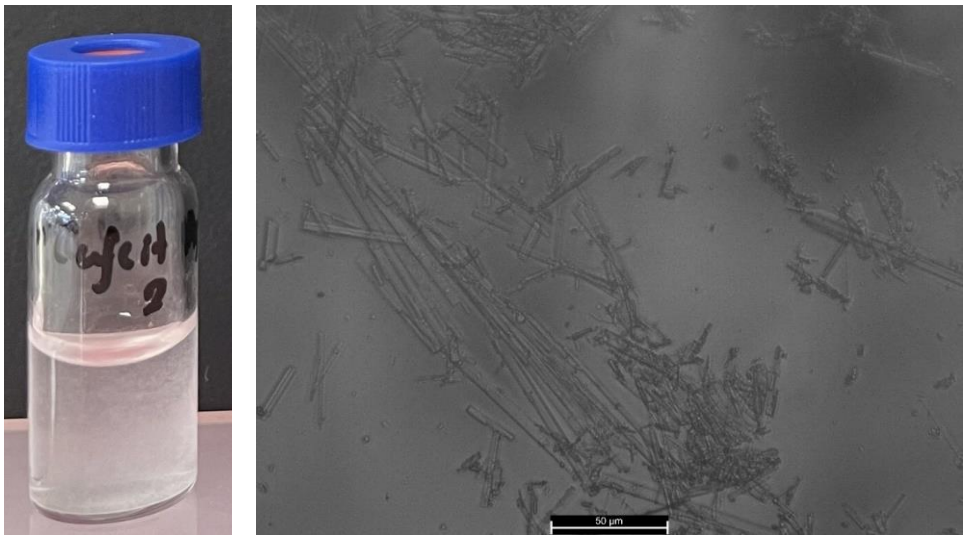


Figure 6. Glucose 5% and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.311 µm; Dv50 = 0.299 µm; Dv10 = 0.168 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 92.5% and 7.5%, respectively (no droplets > 1 µm)

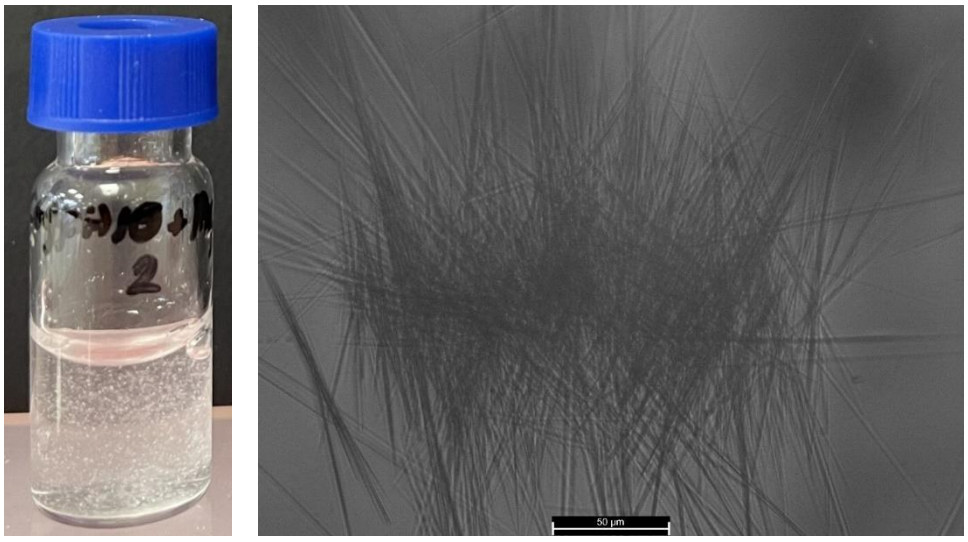
Appendix 7

Physical incompatibilities of caffeine citrate with secondary test drugs

1. Caffeine citrate and aciclovir

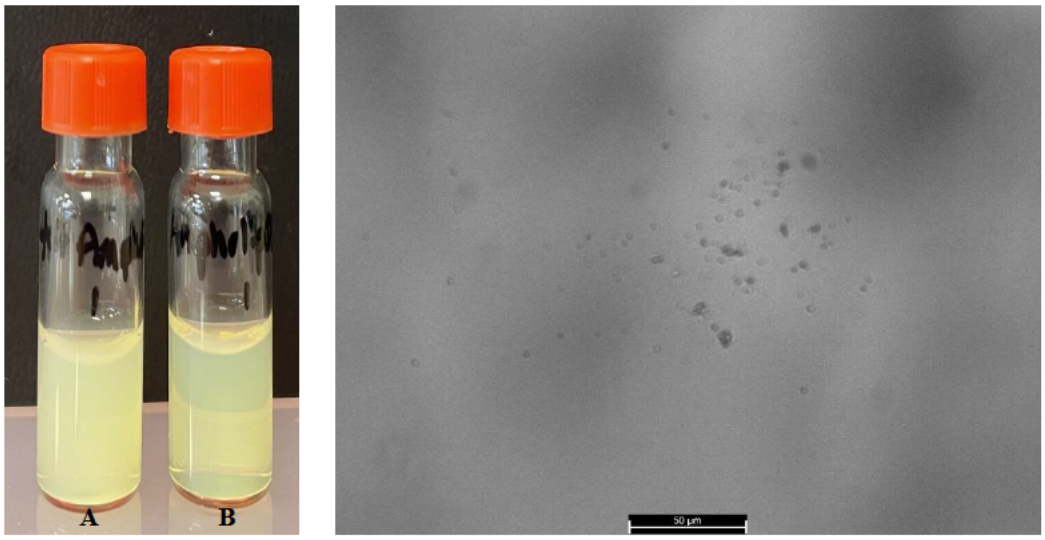


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of caffeine citrate (20 mg/mL) and aciclovir (5 mg/mL)

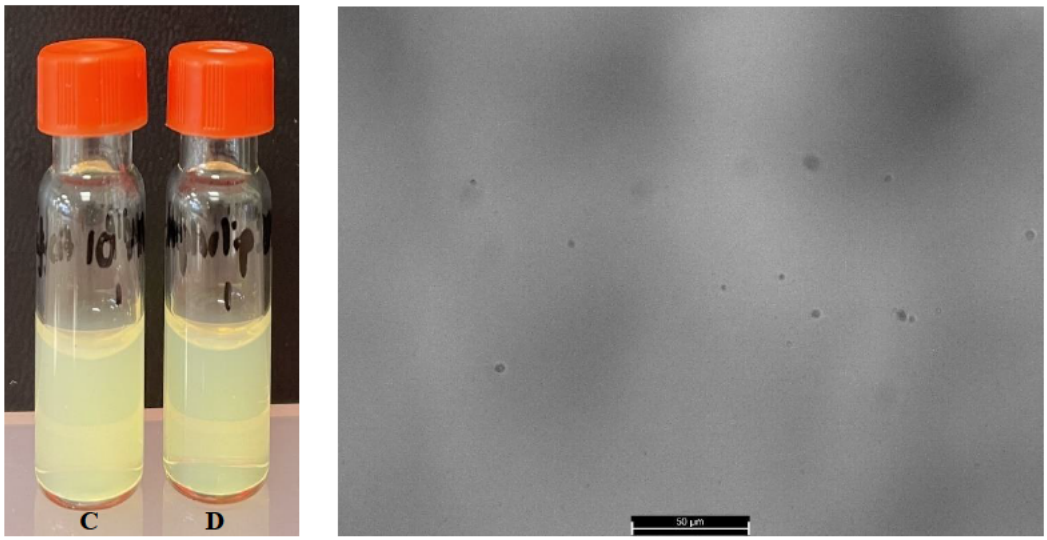


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of caffeine citrate (10 mg/mL) and aciclovir (5 mg/mL)

2. Caffeine citrate and amphotericin liposomal

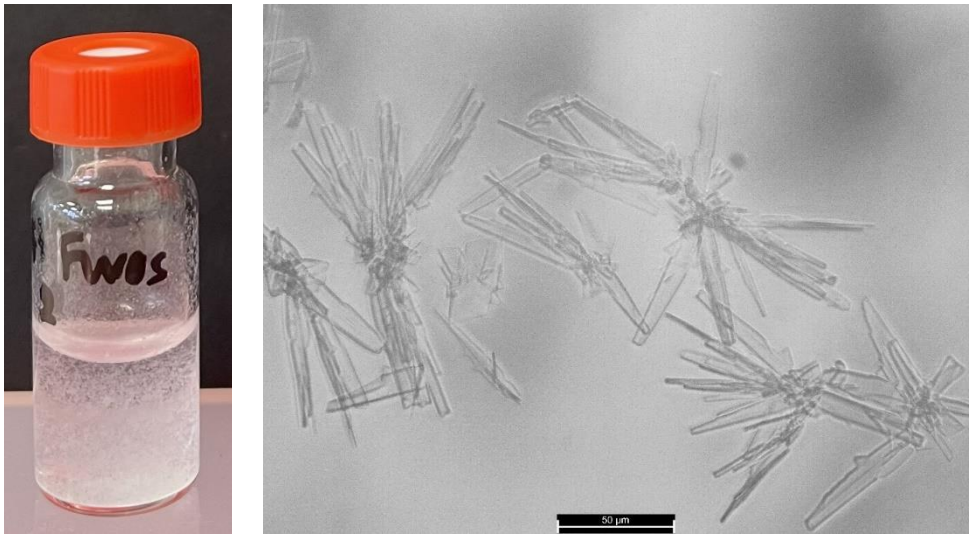


Photograph (left) of the increase in opacity in the combination of caffeine citrate (20 mg/mL) and amphotericin liposomal 2 mg/mL (A) compared to the control (B) (amphotericin + diluent); corresponding photomicrograph (right) (Leica MC190HD, objective x40) of test sample

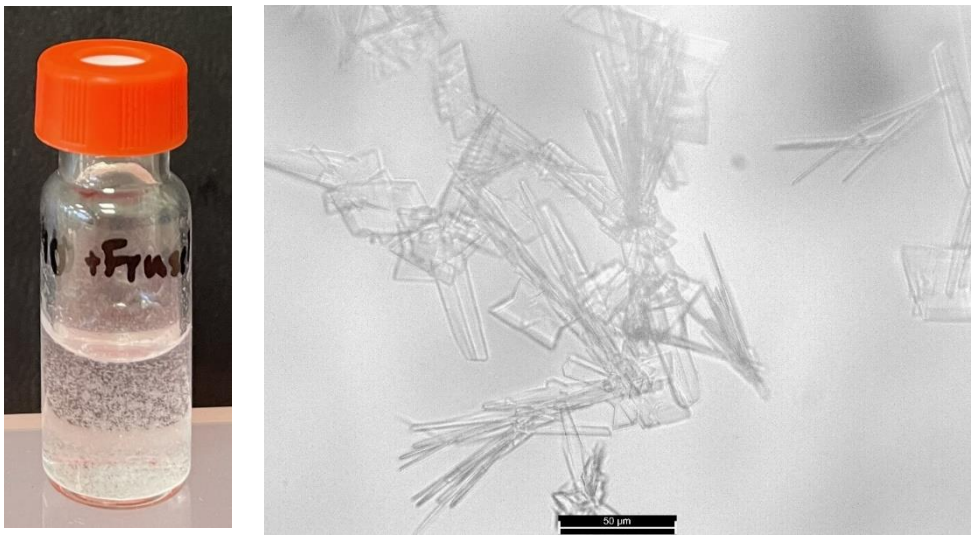


Photograph (left) of the increase in opacity in the combination of caffeine citrate (10 mg/mL) and amphotericin liposomal 2 mg/mL (C) compared to the control (D) (amphotericin + diluent); corresponding photomicrograph (right) (Leica MC190HD, objective x40) of test sample

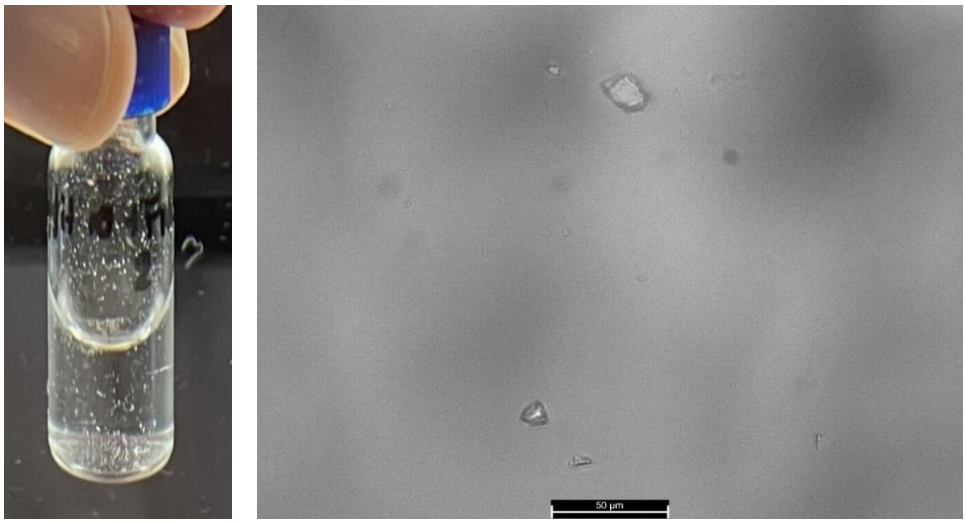
3. Caffeine citrate and furosemide



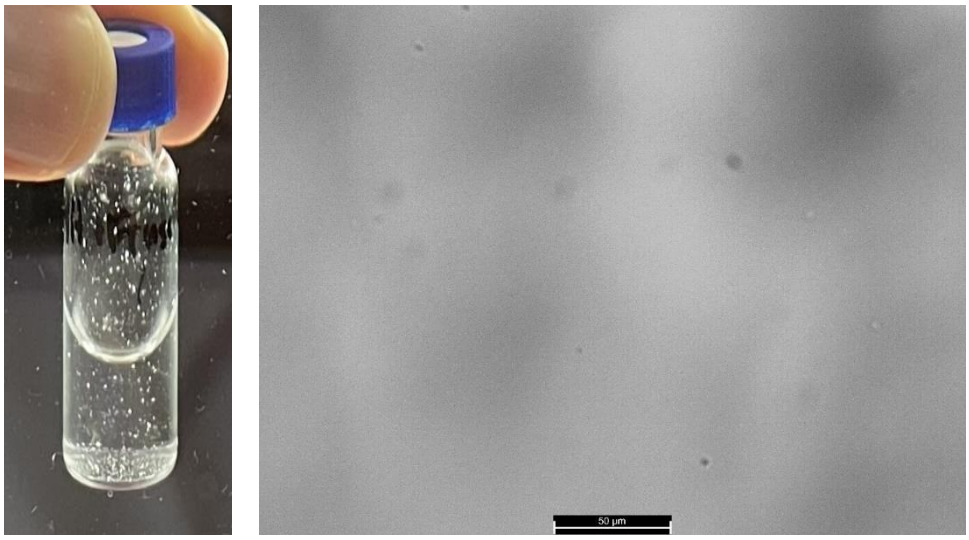
Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of caffeine citrate (20 mg/mL) and furosemide 1 mg/mL



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of caffeine citrate (10 mg/mL) and furosemide 1 mg/mL

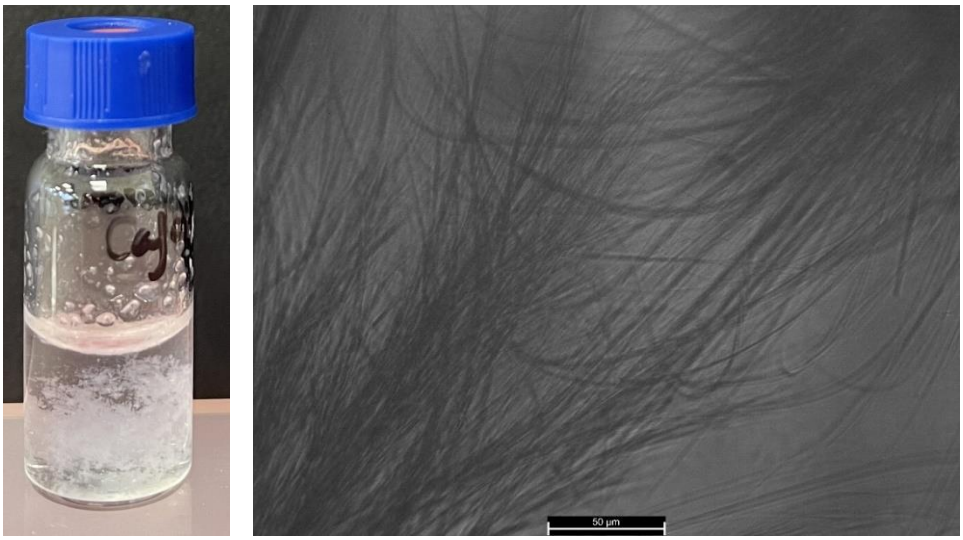


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles observed under polarized light) in the combination of caffeine citrate (20 mg/mL) and furosemide 0.2 mg/mL

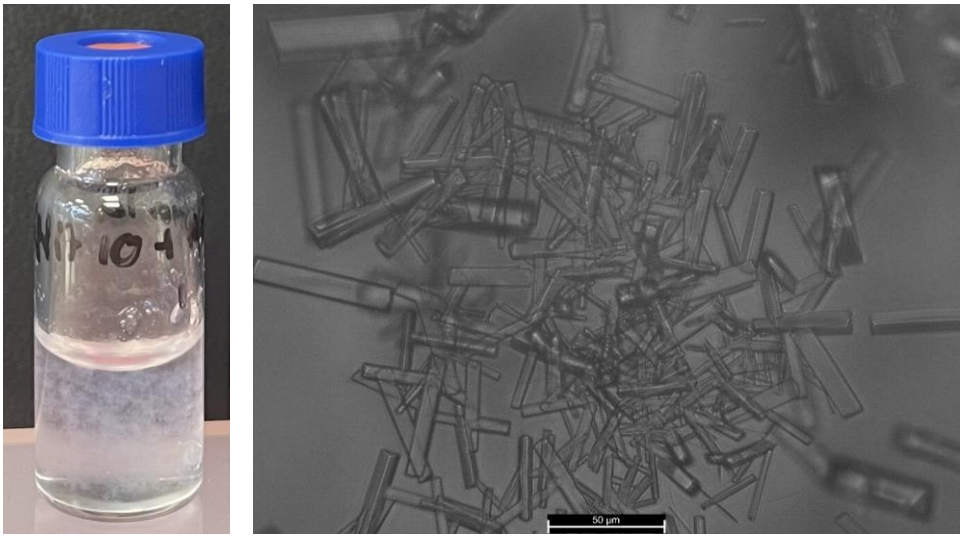


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (particles observed under polarized light) in the combination of caffeine citrate (10 mg/mL) and furosemide 0.2 mg/mL

4. Caffeine citrate and hydrocortisone

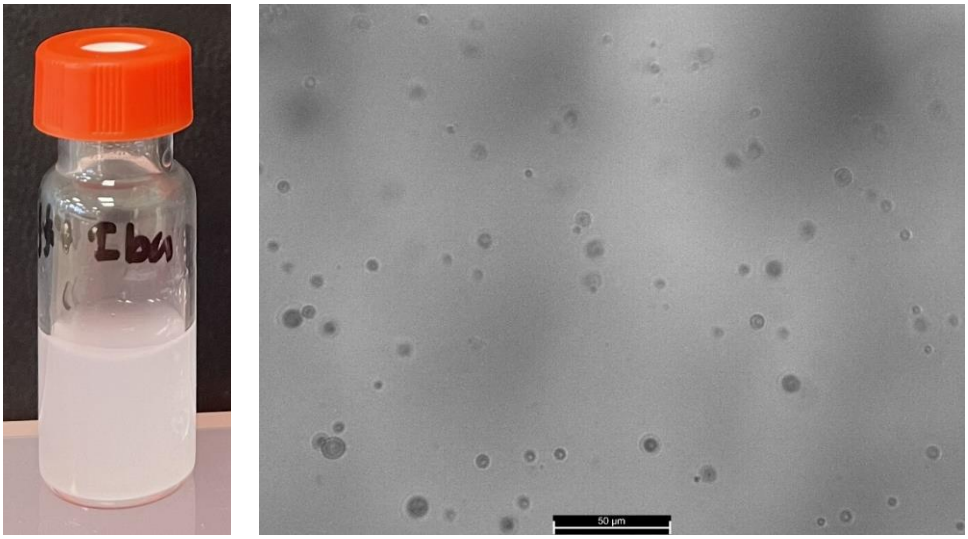


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of caffeine citrate (20 mg/mL) and hydrocortisone 10 mg/mL

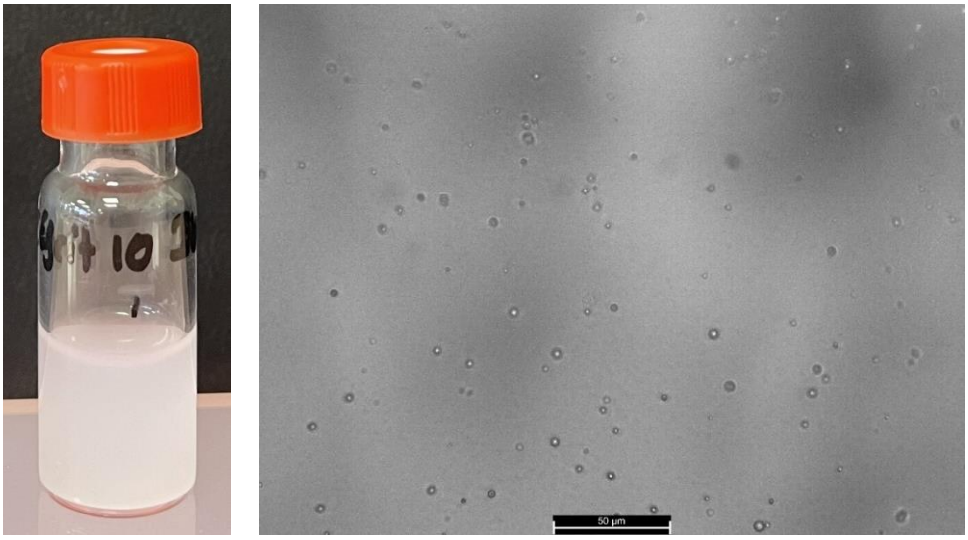


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (white precipitate) in the combination of caffeine citrate (10 mg/mL) and hydrocortisone 10 mg/mL

5. Caffeine citrate and ibuprofen

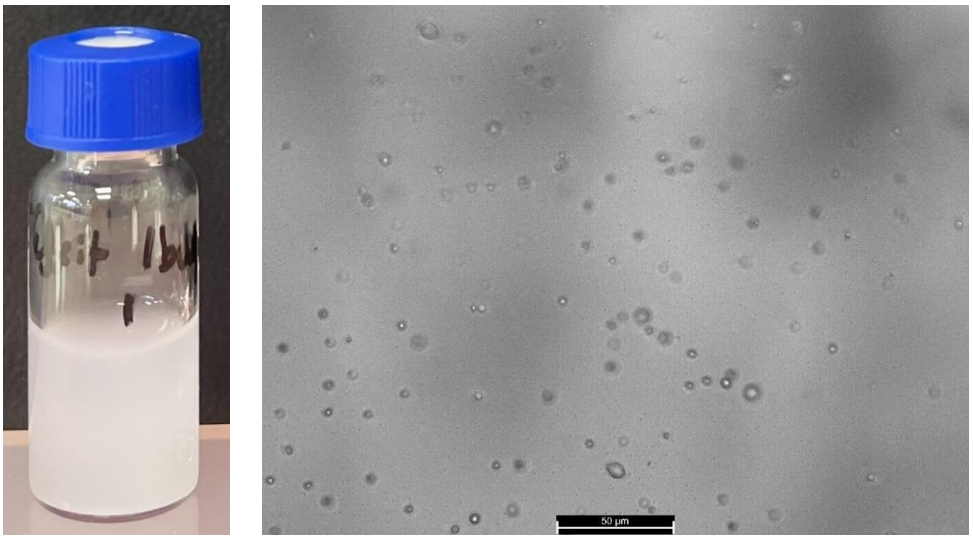


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of caffeine citrate (20 mg/mL) and ibuprofen 5 mg/mL

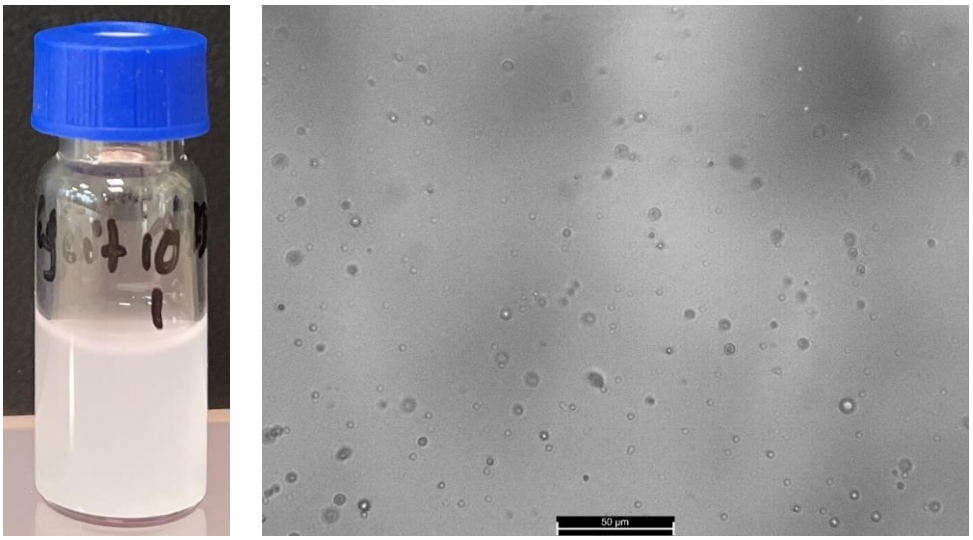


Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of caffeine citrate (10 mg/mL) and ibuprofen 5 mg/mL

6. Caffeine citrate and ibuprofen lysine



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of caffeine citrate (20 mg/mL) and ibuprofen lysine 4 mg/mL



Photograph (left) and corresponding photomicrograph (right) (Leica MC190HD, objective x40) of physical incompatibility (milky turbidity) in the combination of caffeine citrate (10 mg/mL) and ibuprofen lysine 4 mg/mL

Appendix 8

Incompatibilities of citrate buffer with secondary drugs

Figure 1. Precipitation in the combination of caffeine citrate 20 mg/mL + aciclovir 5 mg/mL (left); citrate buffer + aciclovir 5 mg/mL (right)

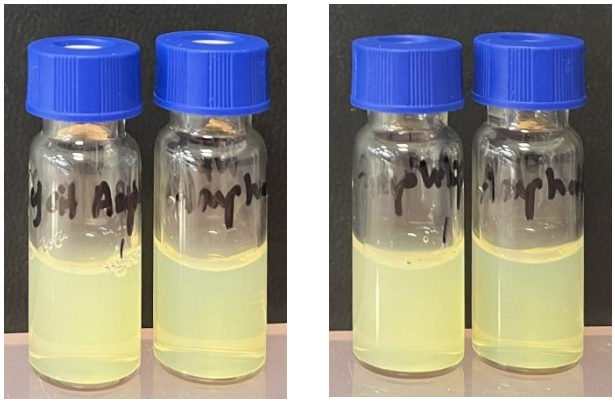


Figure 2. Increased opacity in the combination of caffeine citrate 20 mg/mL + amphotericin liposomal 2 mg/mL in comparison to the control sample (left); citrate buffer + amphotericin liposomal 2 mg/mL in comparison to the control sample; b, d – control samples

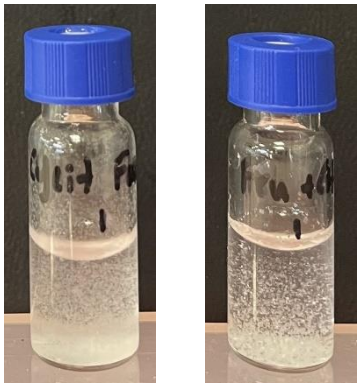


Figure 3. Precipitation in the combination of caffeine citrate 20 mg/mL + furosemide 1 mg/mL (left) and citrate buffer + + furosemide 1 mg/mL (right)

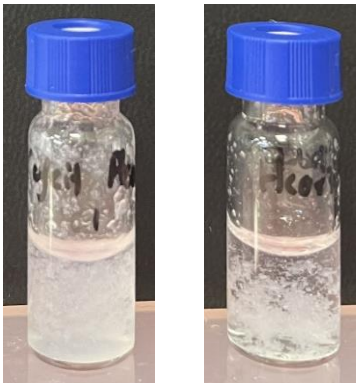


Figure 4. Precipitation in the combination of caffeine citrate 20 mg/mL + hydrocortisone 10 mg/mL (left) and citrate buffer + hydrocortisone 10 mg/mL (right)

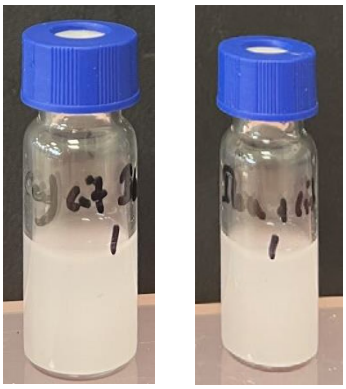


Figure 5. Milky turbidity in the combination of caffeine citrate 20 mg/mL + ibuprofen 5 mg/mL (left) and citrate buffer + ibuprofen 5 mg/mL (right)

Figure 6. Milky turbidity in the combination of caffeine citrate 20 mg/mL + ibuprofen lysine 4 mg/mL (left) and citrate buffer + ibuprofen lysine 4 mg/mL (right)

Appendix 9

Size distribution plots – Caffeine compatibility with lipid emulsions

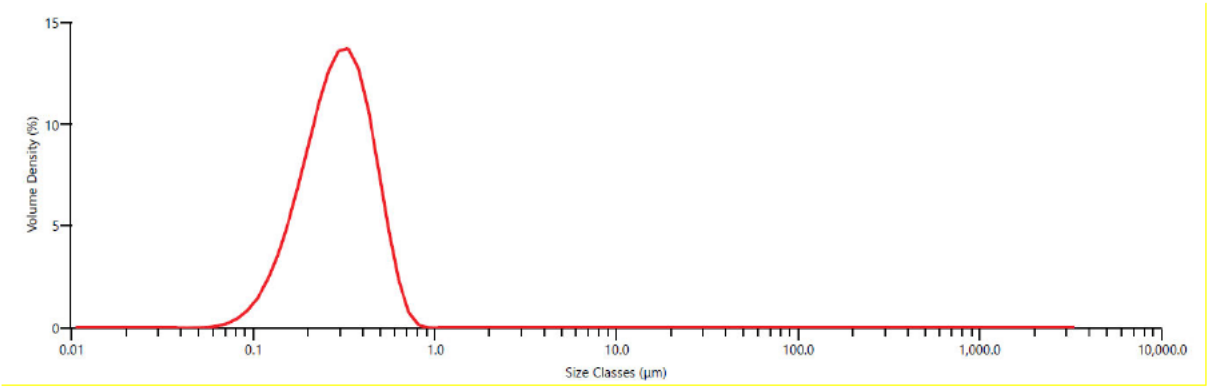


Figure 1. Caffeine citrate 20 mg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.310 µm; Dv50 = 0.294 µm; Dv10 = 0.158 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.2% and 8.8%, respectively (no droplets > 1 µm)

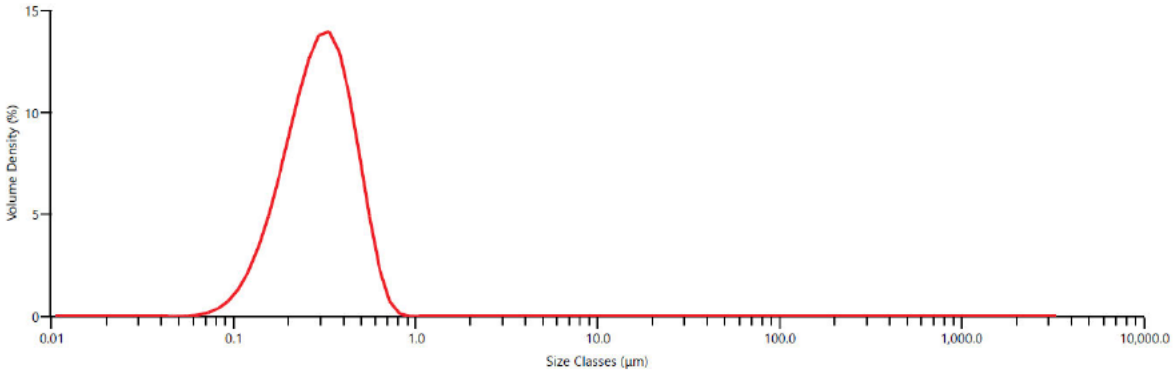


Figure 2. Caffeine citrate 20 mg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.313 µm; Dv50 = 0.297 µm; Dv10 = 0.161 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.1% and 8.9%, respectively (no droplets > 1 µm)

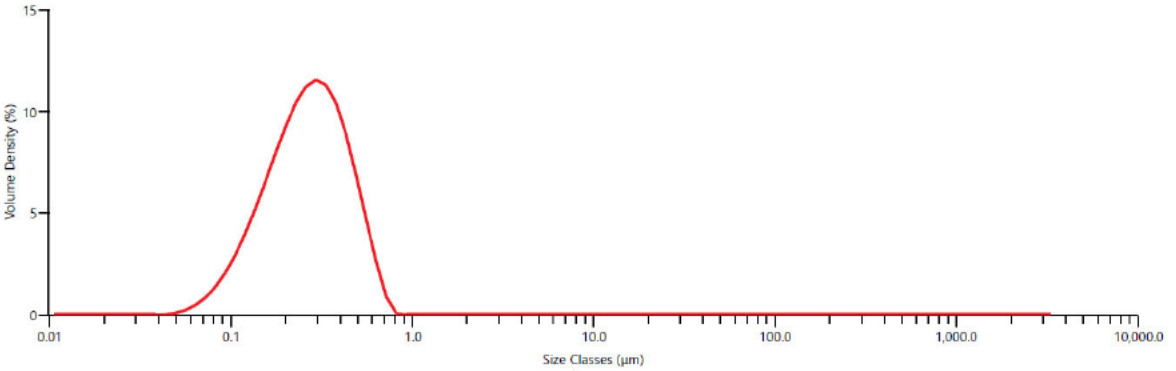


Figure 3. Caffeine citrate 10 mg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.290 µm; Dv50 = 0.268 µm; Dv10 = 0.129 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.2% and 8.8%, respectively (no droplets > 1 µm)

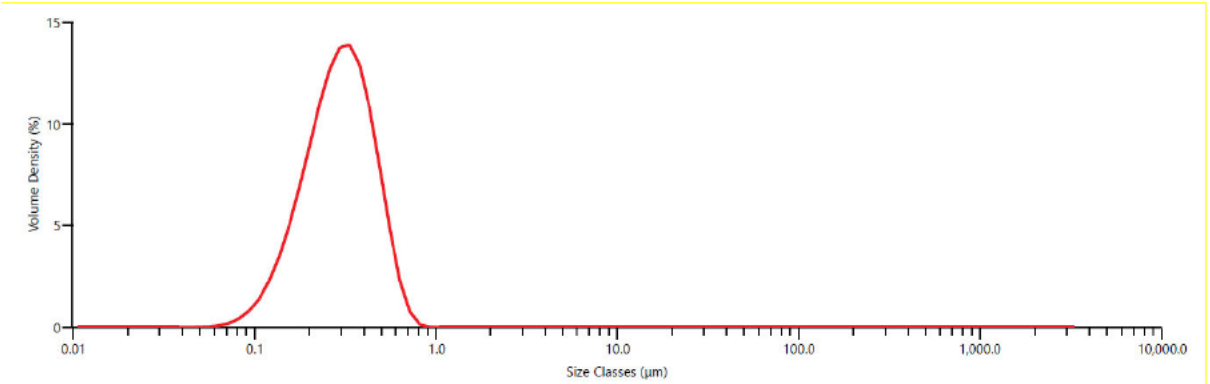


Figure 4. Caffeine citrate 10 mg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.311 µm; Dv50 = 0.295 µm; Dv10 = 0.160 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.3% and 8.7%, respectively (no droplets > 1 µm)

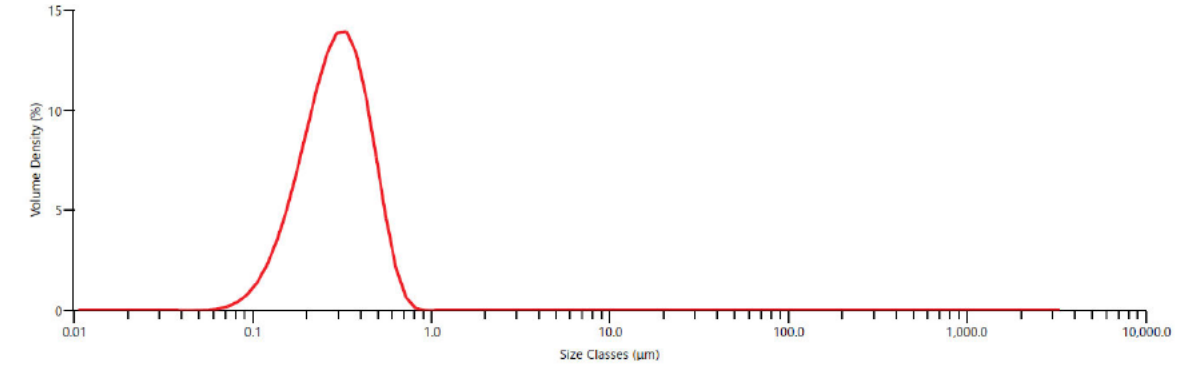


Figure 5. Caffeine base injection 10 mg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.309 µm; Dv50 = 0.293 µm; Dv10 = 0.159 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.7% and 8.3%, respectively (no droplets > 1 µm)

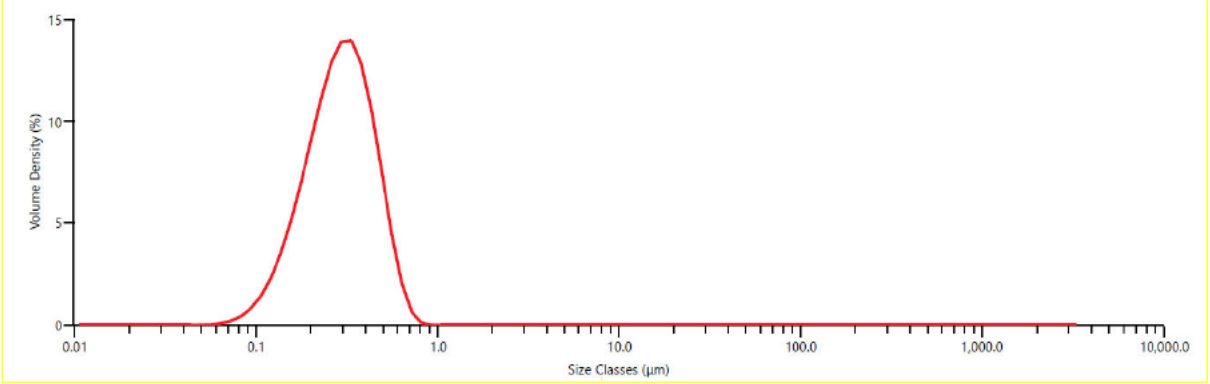


Figure 6. Caffeine base injection 10 mg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.309 µm; Dv50 = 0.293 µm; Dv10 = 0.159 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.8% and 8.2%, respectively (no droplets > 1 µm)

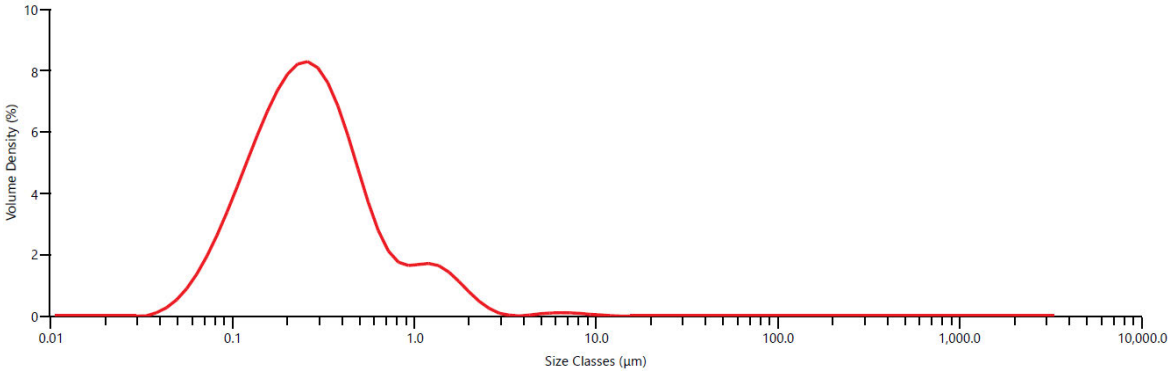


Figure 7. Gentamicin 2 mg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.415 µm; Dv50 = 0.257 µm; Dv10 = 0.102 µm; Proportional of droplets in diameter <0.5 µm and 0.5-5 µm was 80.4% and 19.6%, respectively (8.2% droplets > 1 µm)

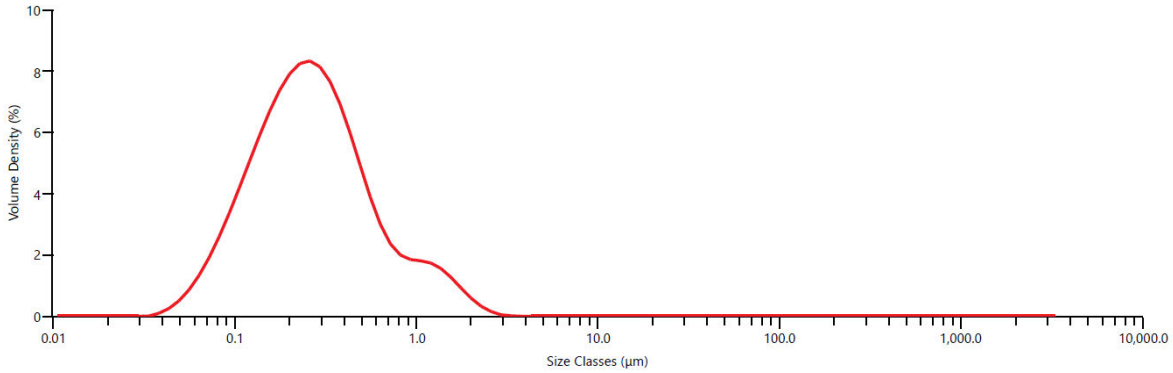


Figure 8. Gentamicin 2 mg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.372 µm; Dv50 = 0.257 µm; Dv10 = 0.103 µm; Proportional of droplets in diameter <0.5 µm and 0.5-5 µm was 80.6% and 19.4%, respectively (7% droplets > 1 µm)

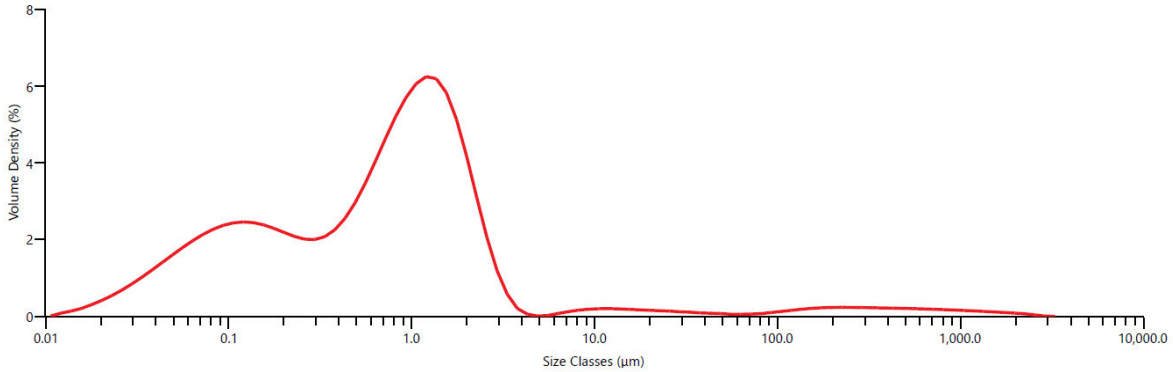


Figure 9. Gentamicin 10 mg/mL and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 22.9 µm; Dv50 = 0.743 µm; Dv10 = 0.067 µm; Proportional of droplets in diameter <0.5 µm and > 0.5 µm was 40.6% and 59.4%, respectively (38.9% droplets > 1 µm)

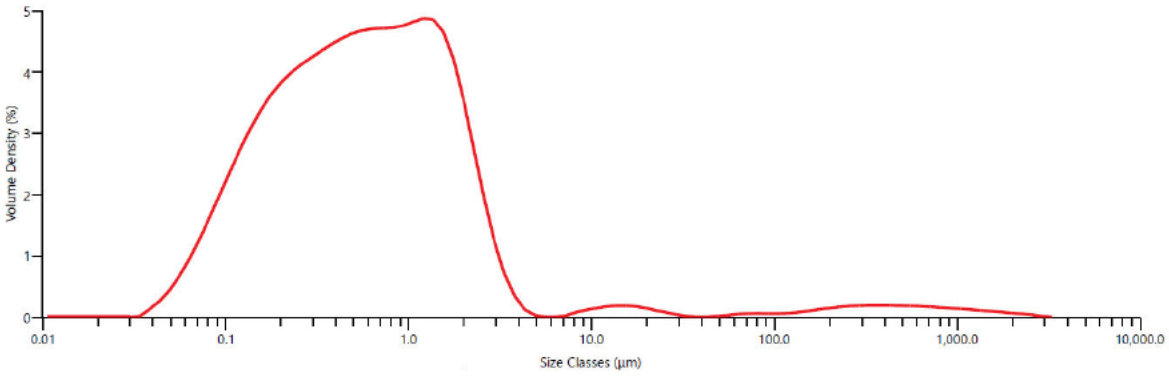


Figure 10. Gentamicin 10 mg/mL and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 20.3 µm; Dv50 = 0.568 µm; Dv10 = 0.125 µm; Proportional of droplets in diameter <0.5 µm and > 0.5 µm was 46.7% and 53.3 %, respectively (32% droplets > 1 µm)

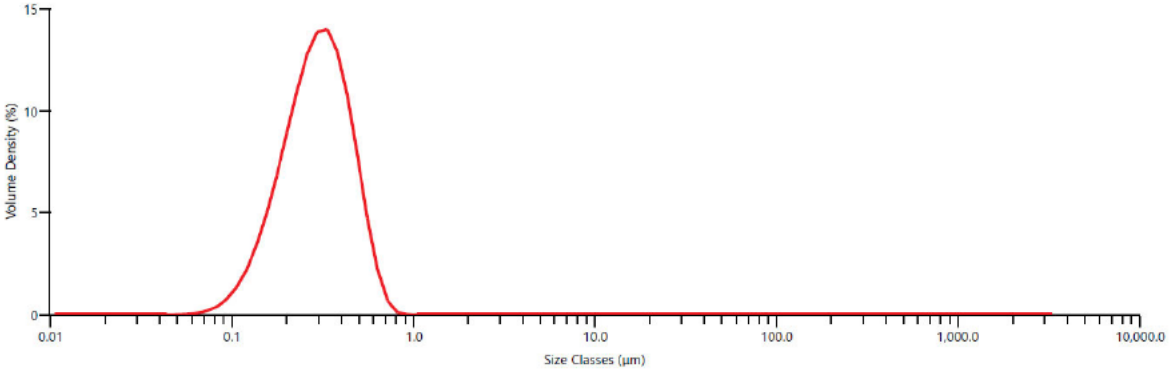


Figure 11. WFI and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.311 µm; Dv50 = 0.295 µm; Dv10 = 0.161 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.4% and 8.6%, respectively (no droplets > 1 µm)

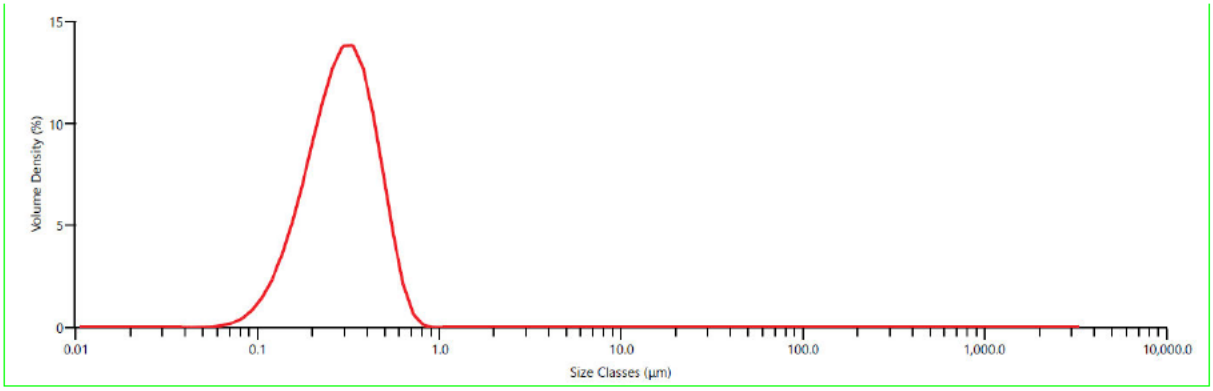


Figure 12. WFI and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.308 µm; Dv50 = 0.292 µm; Dv10 = 0.158 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.8% and 8.2%, respectively (no droplets > 1 µm)

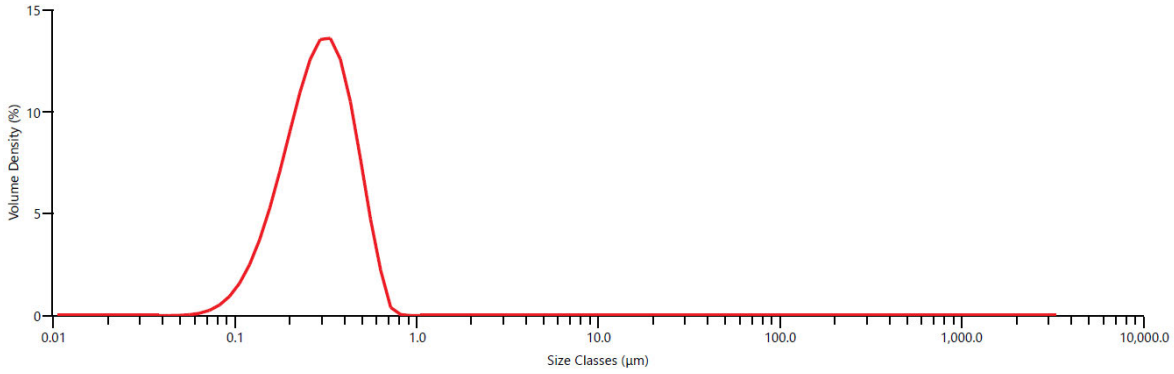


Figure 13. NS and SMOFlipid (20%) (1:1) at 0 h. Mean droplet diameter (MDD; D[4,3] = 0.306 µm; Dv50 = 0.291 µm; Dv10 = 0.155 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.8% and 8.2%, respectively (no droplets > 1 µm)

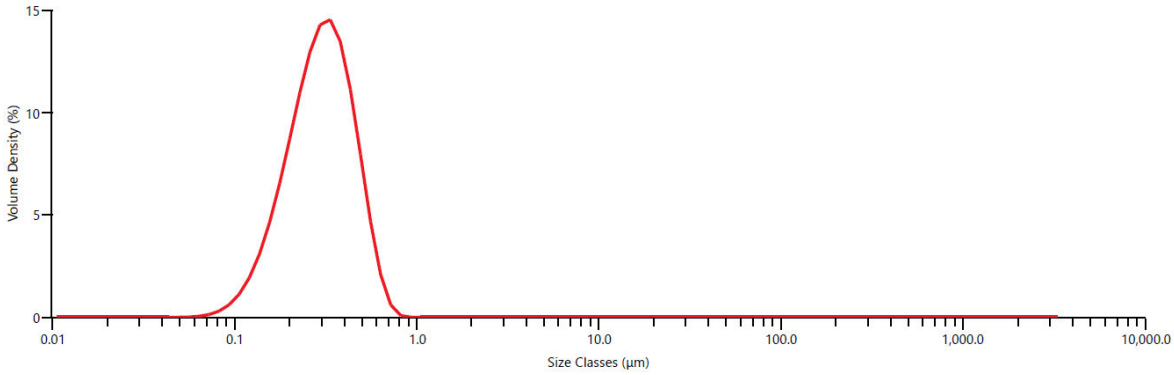


Figure 14. NS and SMOFlipid (20%) (1:1) at 2 h. Mean droplet diameter (MDD; D[4,3] = 0.314 µm; Dv50 = 0.299 µm; Dv10 = 0.167 µm; Proportional of droplets in diameter <0.5 µm and 0.5-1 µm was 91.6% and 8.4%, respectively (no droplets > 1 µm)

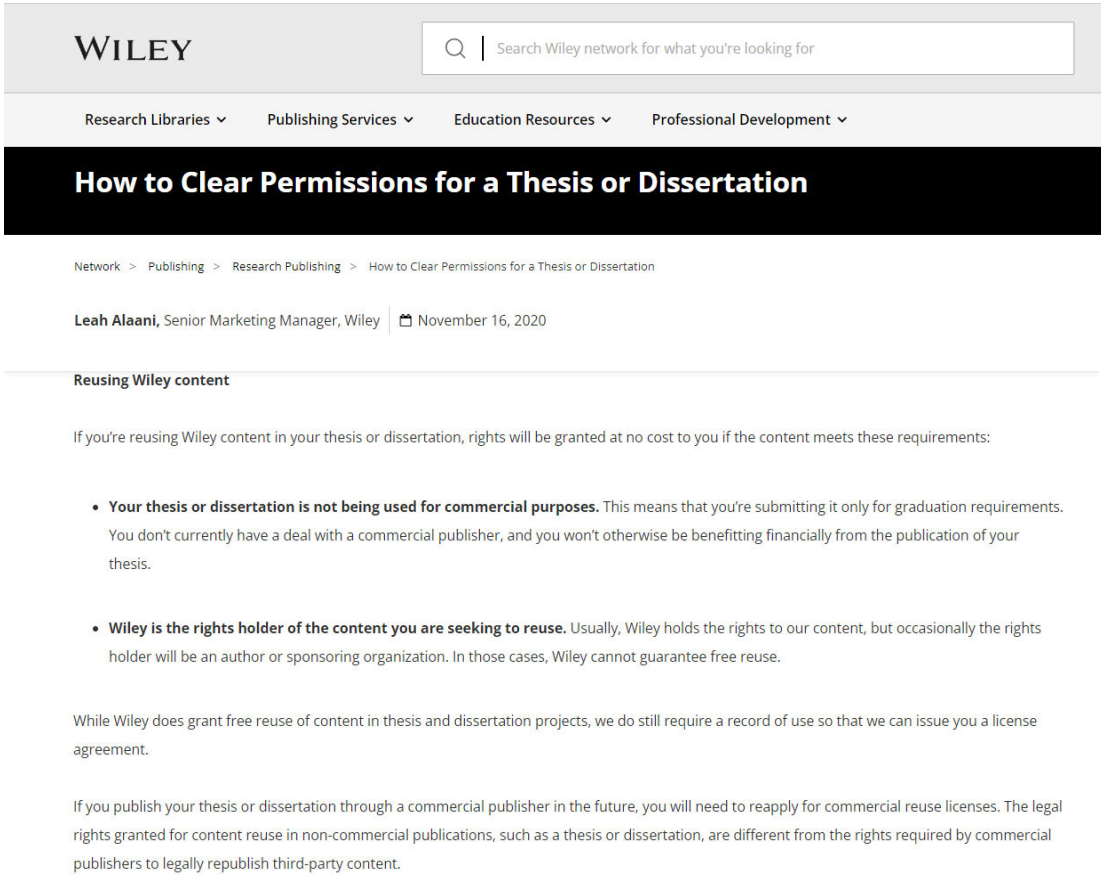
Appendix 10

Publications and corresponding attribution statements

Paper 1 – Publication waiver, attribution statement and paper

As per the publishing journal, Pharmacology Research and Perspectives, Wiley maintains a policy for which permissions are not required for further use of published material within a thesis or dissertation, as long as the thesis is not being used for commercial purposes. Full permission for inclusion of the published paper within this thesis has thereby been granted. Please see below for full details.

https://www.wiley.com/en-us/network/publishing/research-publishing/trending-stories/how-to-clear-permissions-for-a-thesis-or-dissertation



WILEY

Search Wiley network for what you're looking for

Research Libraries | Publishing Services | Education Resources | Professional Development

How to Clear Permissions for a Thesis or Dissertation

Network > Publishing > Research Publishing > How to Clear Permissions for a Thesis or Dissertation

Leah Alaani, Senior Marketing Manager, Wiley | November 16, 2020

Reusing Wiley content

If you're reusing Wiley content in your thesis or dissertation, rights will be granted at no cost to you if the content meets these requirements:

- **Your thesis or dissertation is not being used for commercial purposes.** This means that you're submitting it only for graduation requirements. You don't currently have a deal with a commercial publisher, and you won't otherwise be benefitting financially from the publication of your thesis.
- **Wiley is the rights holder of the content you are seeking to reuse.** Usually, Wiley holds the rights to our content, but occasionally the rights holder will be an author or sponsoring organization. In those cases, Wiley cannot guarantee free reuse.

While Wiley does grant free reuse of content in thesis and dissertation projects, we do still require a record of use so that we can issue you a license agreement.

If you publish your thesis or dissertation through a commercial publisher in the future, you will need to reapply for commercial reuse licenses. The legal rights granted for content reuse in non-commercial publications, such as a thesis or dissertation, are different from the rights required by commercial publishers to legally republish third-party content.

Attribution Statement

I, **D Thisuri N De Silva**, declare that I significantly contributed the following towards the published article "*Development of a pharmaceutical science systematic review process using a semi-automated machine learning tool: Intravenous drug compatibility in the neonatal intensive care setting*".

- Conception and design
- Acquisition of data and method
- Data conditioning and manipulation
- Analysis and statistical method
- Interpretation and discussion
- Final approval

[redacted] 25/03/2024

I, as a co-author, endorse that the stated level of contribution by the candidate (as above) is appropriate.

Brioni R. Moore	[redacted]
Tobias Strunk	[redacted]
Michael Petrovski	[redacted]
Vanessa Varis	[redacted]
Kevin Chai	[redacted]
Leo Ng	[redacted]
Kevin T. Batty	[redacted]

Research article: Development of a pharmaceutical science systematic review process using a semi-automated machine learning tool: Intravenous drug compatibility in the neonatal intensive care setting.

D Thisuri N De Silva[1], Brioni R Moore[1,2,3,4], Tobias Strunk[3,4,5], Michael Petrovski[6], Vanessa Varis[7], Kevin Chai[8], Leo Ng[9,10], Kevin T Batty[1,2]

[1] Curtin Medical School, Curtin University, Perth, Western Australia, Australia.
[2] Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia.
[3] Medical School, The University of Western Australia, Crawley, Western Australia, Australia.
[4] Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, Western Australia, Australia.
[5] Neonatal Directorate, King Edward Memorial Hospital, Child and Adolescent Health Service, Subiaco, Western Australia, Australia.
[6] Pharmacy Department, King Edward Memorial Hospital, Women and Newborn Health Service, Subiaco, Western Australia, Australia.
[7] University Library, Curtin University, Perth, Western Australia, Australia.
[8] School of Population Health, Curtin University, Perth, Western Australia, Australia.
[9] Curtin School of Allied Health, Curtin University, Perth, Western Australia, Australia.
[10] School of Health Sciences, Swinburne University of Technology, Hawthorn, Victoria, Australia.

	Conception and design	Acquisition of Data and Method	Data Conditioning and Manipulation	Analysis and Statistical Method	Interpretation and Discussion
Brioni R Moore	✓				✓
Co-author 1 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					
Tobias Strunk	✓				✓
Co-author 2 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: Signed: [redacted]					
Michael Petrovski	✓				✓
Co-author 3 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					

Vanessa Varis	✓				✓
Co-author 4 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version.  Signed: [redacted]					
Kevin Chai	✓		✓		✓
Co-author 5 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version.  Signed: [redacted]					
Leo Ng	✓				✓
Co-author 6 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version.  Signed:					
Kevin T Batty	✓	✓	✓		✓
Co-author 7 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version.  Signed:					

Received: 28 April 2023 | Accepted: 11 December 2023

DOI: 10.1002/prp2.1170

ORIGINAL ARTICLE

Development of a pharmaceutical science systematic review process using a semi-automated machine learning tool: Intravenous drug compatibility in the neonatal intensive care setting

D. Thisuri N. De Silva[1] | Brioni R. Moore[1,2,3,4] | Tobias Strunk[3,4,5] | Michael Petrovski[6] | Vanessa Varis[7] | Kevin Chai[8] | Leo Ng[9,10] | Kevin T. Batty[1,2]

[1]Curtin Medical School, Curtin University, Perth, Western Australia, Australia

[2]Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia

[3]Medical School, The University of Western Australia, Crawley, Western Australia, Australia

[4]Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, Western Australia, Australia

[5]Neonatal Directorate, King Edward Memorial Hospital, Child and Adolescent Health Service, Subiaco, Western Australia, Australia

[6]Pharmacy Department, King Edward Memorial Hospital, Women and Newborn Health Service, Subiaco, Western Australia, Australia

[7]University Library, Curtin University, Perth, Western Australia, Australia

[8]School of Population Health, Curtin University, Perth, Western Australia, Australia

[9]Curtin School of Allied Health, Curtin University, Perth, Western Australia, Australia

[10]School of Health Sciences, Swinburne University of Technology, Hawthorn, Victoria, Australia

Correspondence
Kevin T. Batty, Curtin Medical School, Curtin University, GPO Box U1987, Perth, WA 6845, Australia.
Email: kevin.batty@curtin.edu.au

Abstract

Our objective was to establish and test a machine learning-based screening process that would be applicable to systematic reviews in pharmaceutical sciences. We used the SPIDER (Sample, Phenomenon of Interest, Design, Evaluation, Research type) model, a broad search strategy, and a machine learning tool (Research Screener) to identify relevant references related to y-site compatibility of 95 intravenous drugs used in neonatal intensive care settings. Two independent reviewers conducted pilot studies, including manual screening and evaluation of Research Screener, and used the kappa-coefficient for inter-reviewer reliability. After initial deduplication of the search strategy results, 27 597 references were available for screening. Research Screener excluded 1735 references, including 451 duplicate titles and 1269 reports with no abstract/title, which were manually screened. The remainder (25 862) were subject to the machine learning screening process. All eligible articles for the systematic review were extracted from <10% of the references available for screening. Moderate

Abbreviations: NICU, neonatal intensive care unit; PICO, Population, Intervention, Comparison, Outcomes; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SPIDER, Sample Phenomenon of Interest, Design, Evaluation, Research type.

inter-reviewer reliability was achieved, with kappa-coefficient ≥0.75. Overall, 324 references were subject to full-text reading and 118 were deemed relevant for the systematic review. Our study showed that a broad search strategy to optimize the literature captured for systematic reviews can be efficiently screened by the semi-automated machine learning tool, Research Screener.

KEYWORDS
machine learning, pharmaceutical science, physicochemical compatibility, systematic review

1 | INTRODUCTION

Well-conducted systematic reviews and meta-analyses are considered to provide the highest level of evidence for informed decisions in policy and practice. The process for systematic reviews is typically defined by well-established models, such as PICO (Population, Intervention, Comparison, Outcomes)[1] and SPIDER (Sample, Phenomenon of Interest, Design, Evaluation, Research type).[2] PICO is commonly used for systematic reviews of clinical research, whereas SPIDER appears to offer advantages for other scientific disciplines.

The required methodological rigor of systematic reviews is associated with significant time and economic demands,[3] with screening of titles and abstracts considered to be the most time and labor-intensive component of the review process.[4] Hence, there is a growing interest for more automated solutions to facilitate systematic reviews.[5] However, despite the profusion of systemic reviews in recent years, there is a paucity of reviews in the pharmaceutical sciences disciplines which have used the SPIDER model and evaluated a machine learning screening tool to expedite the process.

The introduction of new technologies, such as web-based tools, for streamlining the screening process has provided promising results by substantially reducing the time for initial screening. Machine learning-based screening tools include Rayyan,[6] Abstrackr,[7] RobotAnalyst,[8] and ASReview.[9] Nevertheless, there are limitations and barriers to the widespread use of some screening tools, including the risk of missing articles (this could be improved by semi-automation), a requirement to "train" the program by initially screening a high number of articles (limiting the time savings), the use of a dedicated computer/server for installation of the screening tool, or failure to adapt to multiple platforms.[4] Research Screener (RS), a semi-automated machine learning tool, has the advantage of applying contemporary Natural Language Processing algorithms and is able to train itself for abstract ranking from a small selection of seed abstracts.[4] By contrast, other tools such as Rayyan may require numerous seed abstracts for training its model.[10] Research Screener also has practical advantages; for example, it can be used on a wide range of hardware platforms and it does not require a dedicated server.[4]

The Research Screener process is illustrated in Figure 1. Two items are initially provided to Research Screener by the researchers as separate files from the reference manager software: (i) All potentially eligible articles retrieved from the systematic review search strategy and (ii) at least one seed article assessed as highly relevant. Using the seed article(s) abstract, the Research Screener algorithm ranks articles by relevance and the screening process commences with presentation of the top 50 unread articles (cycle 1). Independently, members of the review team screen the abstracts of these 50 articles to flag those which are deemed relevant according to predetermined inclusion criteria for the systematic review. The titles are retained for full article screening and, in conjunction with the irrelevant (discarded) articles, are used to refine the Research Screener algorithm. Research

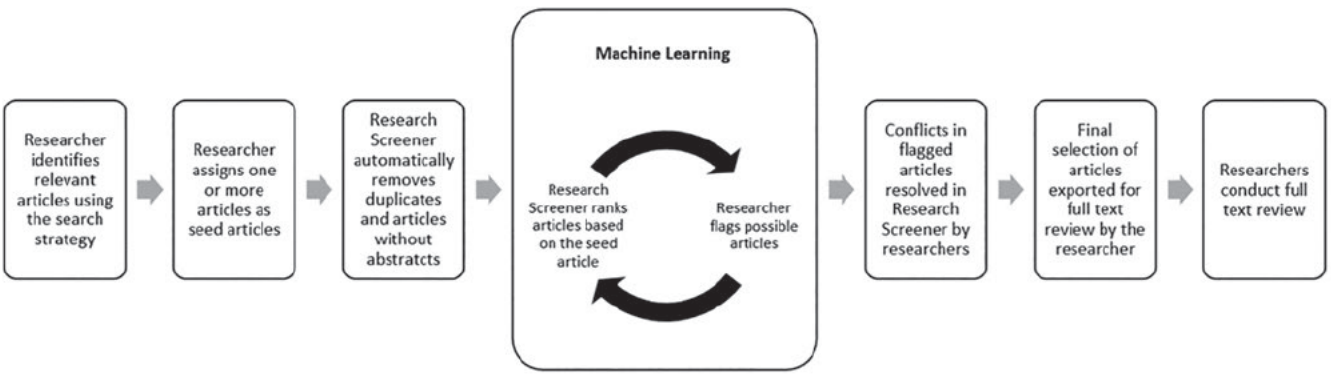


FIGURE 1 Research Screener assisted screening process (adapted from Chai et al.[4]).

Screener re-ranks articles in the set of records (references) available for screening to determine the next 50 most relevant articles (cycle 2) and the process continues in cycles of 50 articles. The screening process ceases when either all articles have been screened by the reviewers or the research team completes screening to a level of confidence that all relevant articles have been identified (e.g., several cycles with no article selected as relevant). Upon completion of the initial screen, the principal reviewer can access the combined results, including conflicts in the flagged articles (i.e., disagreements between the individual reviewers). The conflicts are resolved in Research Screener, by an open process of consideration by the reviewers and/or an independent third reviewer. The final selected articles (flagged by both reviewers and the resolved conflicts) are exported for full-text review.

We report the process of establishing and testing a robust literature search strategy in accordance with the SPIDER model and the use of Research Screener[4] in the reference selection process for a systematic review in pharmaceutical sciences. The aim of our systematic review was to collate the current evidence on intravenous drug compatibility as applicable to y-site administration in neonatal intensive care (NICU) settings. To the best of our knowledge, no systematic reviews have been conducted to evaluate peer-reviewed physicochemical compatibility studies in this context. Two systematic reviews with related objectives (drug compatibility in adult intensive care settings) have been reported previously[11,12] and were conducted by manual screening of up to 2000 citations.[11]

2 | METHODS

2.1 | Development of the search strategy

The research question, "In-vitro studies conducted to evaluate the physical and chemical compatibility of intravenous drugs used in NICUs," was defined in consultation with members of the research team (TDS, BRM, TS, MP, KTB). The SPIDER model (Sample, Phenomenon of Interest, Design, Evaluation, Research type) for systematic reviews[2] was adapted for the protocol of the present review, which was registered in Open Science Framework (https://doi.org/10.17605/OSF.IO/XGK6V). The search strategy (Table 1) was structured as three concepts (categories), the first of which focused on compatibility, incompatibility, and stability terms. The second concept focused on intravenous, injection, and y-site terms, and the third comprised a list of drugs based on expert panel review (TS, MP, KTB) of a compilation of neonatal drug protocols from seven health-care institutions (four different countries; TDS).

The search concepts were pilot tested (TDS, VV) in iterative stages, using the Embase database and various terms within concepts 1 and 2, and a panel of six drugs (aminophylline, indometacin, ketamine, pentoxifylline, caffeine, and sotalol). The six drugs were selected on the basis of their potential relevance to the planned systematic review and a total known list of 59 articles, which was determined from a standard reference source[13] and our own independent, manual literature search. The optimum search strategy captured 1622 articles and included all known articles of interest.

TABLE 1 Final search strategy for the systematic review of intravenous drug compatibility in the neonatal intensive care setting.

Concept 1	Concept 2	Concept 3
compatib*	intravenous*	NICU drugs
incompatib*	intra-venous*	(6 drugs in pilot study)
stability	iv	(95 drugs in full review)
instability	y-site	
	y- site	
	ysite	
	injection*	
	infusion*	
	parenteral	
	injectable*	
	mixture*	

The first stage of evaluating the screening process was to test the feasibility and reliability of title reading only. Two independent reviewers (TDS, KTB) manually screened a random selection of 400 titles from the set of 1622 references (25%) and the kappa coefficient[14] was calculated to determine the inter-reviewer reliability associated with title reading as a screening process for the systematic review.

As Research Screener had not previously been used in a pharmaceutical sciences systematic review, the full set of 1622 articles was then used to pilot test the tool. Three seed articles were used and two reviewers (TDS, KTB) conducted the screening process, with the kappa coefficient calculated to assess the inter-reviewer reliability.

2.2 | Application of search strategy and Research Screener tool

Based on the pilot study results, the search strategy was applied to all 95 drugs in concept 3 (Table 1) and five databases, comprising two inter-disciplinary (Proquest and Web of Science) and three intra-disciplinary databases (Embase, Medline, and Cinahl) to retrieve articles. The retrieved references were initially deduplicated using a validated deduplication tool "Systematic Review Accelerator" and the final library was entered into Research Screener. Eight articles were identified to provide seed abstracts for the screening process. Following exclusion of articles by Research Screener (comprising conference proceedings, duplicates, and articles with no abstracts), the reviewers proceeded with independent cyclical screening of the captured articles. The reviewers also manually screened (by title) the articles with no abstracts and the kappa coefficient was determined to quantify reviewer agreement for each relevant process.

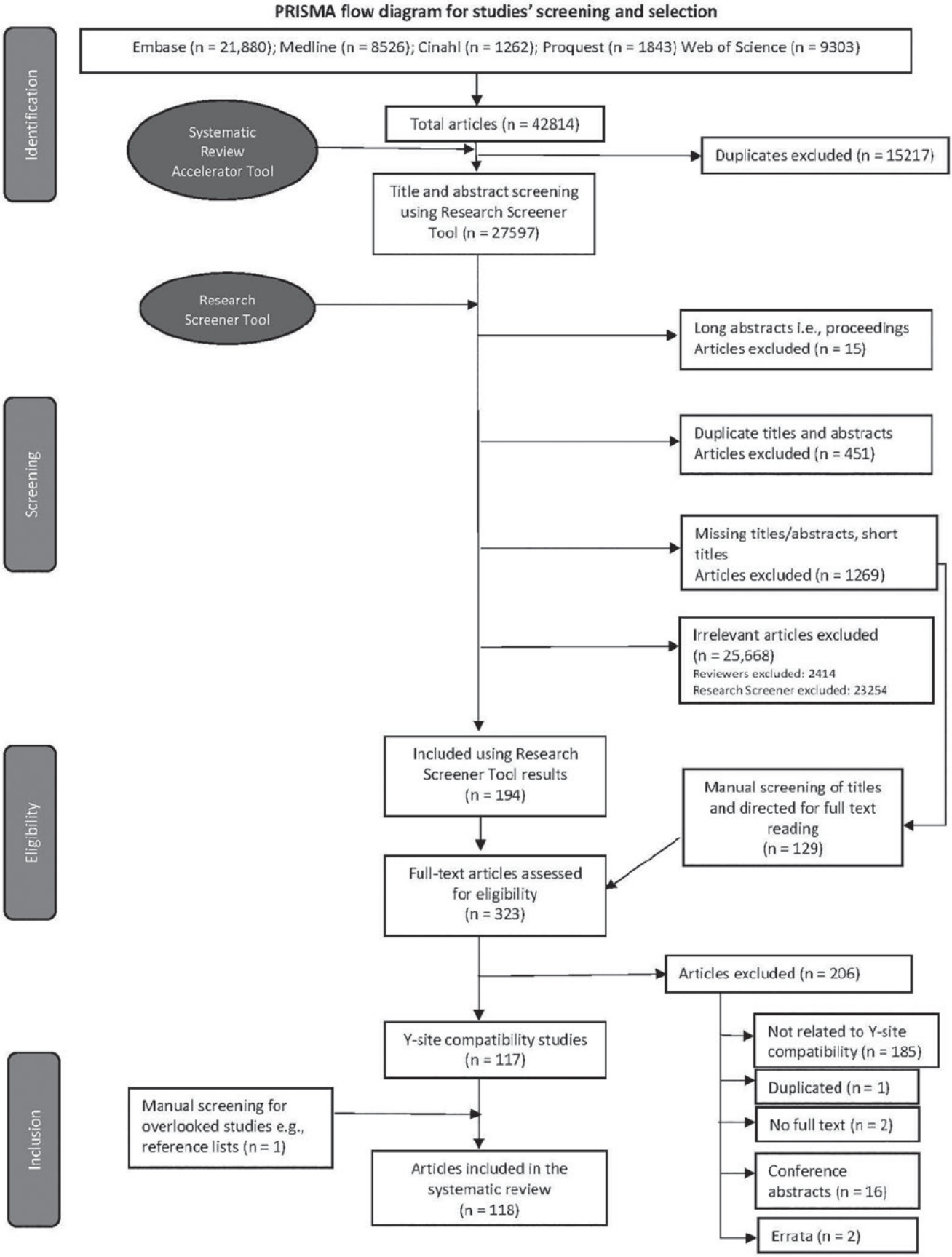


FIGURE 2 PRISMA* flow diagram for the systematic review search, screening, and selection process (*PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

3 | RESULTS

3.1 | Manual screening versus semi-automated screening (Research Screener)

The kappa coefficient from the manual screening pilot study of 400 titles was 0.75, which suggests "moderate agreement"[14] in the reviewers' title screening process. In the pilot study using Research Screener, 98 references (out of 1622) were removed because they did not contain abstracts (e.g., letters, editorials, and short communications, because abstracts are essential for the Research Screener machine learning cycles). These excluded titles were separately exported back to the reference manager software and saved in a separate group for manual screening. The remainder (1524) were directed for screening by Research Screener (TDS, KTB). Fifteen conflicts were subsequently resolved by the reviewers. The kappa coefficient following screening of the full set of pilot study articles via Research Screener was 0.86, which was indicative of "strong" agreement between the two reviewers.[14]

3.2 | Main review

A total of 42 814 results were retrieved from the selected databases (Embase—21 880, Medline—8526, Cinahl—1262, Proquest—1843, Web of Science—9303) and the Systematic Review Accelerator deduplication process retained 27 597 references for further screening (Figure 2).

Research Screener initially removed 15 long abstract articles (i.e., conference proceedings in which the reference manager record contains all conference abstracts combined), 451 duplicated titles/abstracts, and 1269 articles with missing abstracts/titles, from the full set of 27 597 records (Figure 2). The 1269 articles with no abstract/title included short reports, editorials, letters, and notes, and were directed for manual screening by the reviewers. The remainder (25 862) were subject to screening by the two independent reviewers in cycles of 50, as outlined above.

Reviewer 1 completed 52 cycles of screening via Research Screener, which comprised 10% of the references available for screening and concluded after 14 cycles with no abstracts selected (Figure 3). Reviewer 2 completed 35 cycles (6%) of screening and concluded after four cycles with no abstracts selected. As a result, 149 articles were flagged by both reviewers. A further 67 were selected by only one reviewer and classified as conflicts for resolution by the review team, from which 37 were considered potentially eligible and included in the full-text review. Including the eight seed abstracts, a total of 194 articles (0.75%) were directed for full-text consideration at this stage. The kappa coefficient was 0.80, indicating strong agreement.

The 1269 references without titles/abstracts were screened manually by the two reviewers to select potentially eligible reports for full-text read (most included a title and were only missing an abstract) and 129 were selected for progression to full-text review (kappa coefficient 0.78, indicating moderate agreement).

Overall, a total of 323 articles were subject to full-text reading, of which 117 were found to fully comply with the inclusion and exclusion criteria and were included in the formal systematic review (reported elsewhere). Screening of reference lists of the selected articles identified one further study which was not captured in the initial search strategy and was therefore included in the final total of 118 articles for systematic review (Figure 2).

Further insights to the value of Research Screener are shown in Figures 3 and 4. Of the 186 articles which were directed to full-text read (excluding the eight seed abstracts), 55 were eventually selected for inclusion in the systematic review. Reviewer 1 encountered all 55 articles by the 29th cycle of article flagging (1408

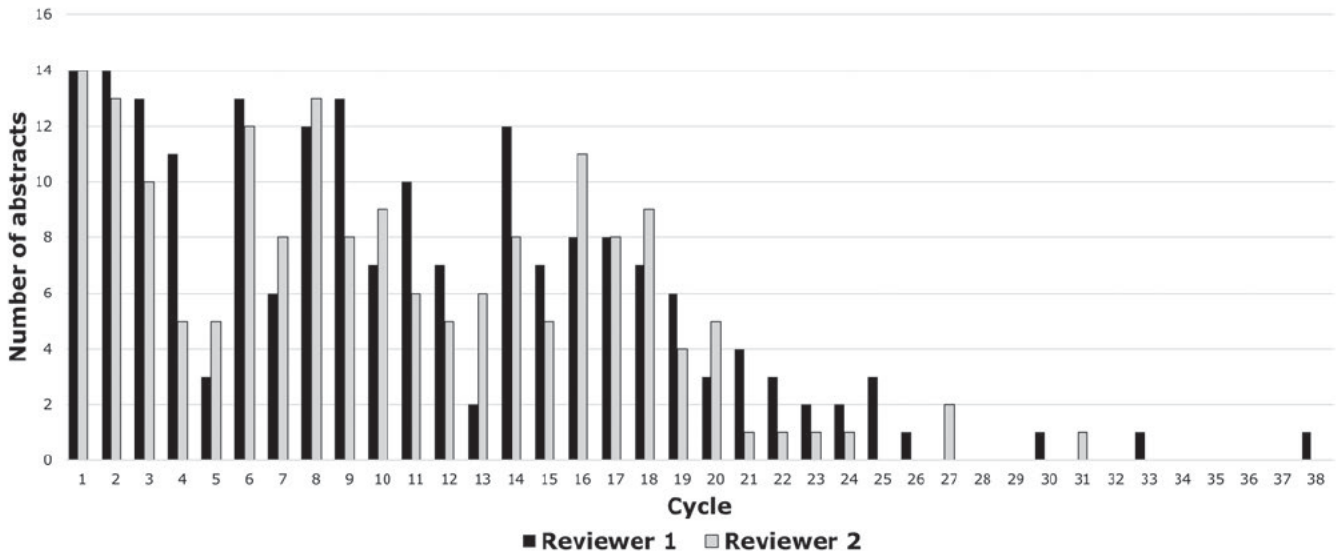


FIGURE 3 Number of abstracts flagged by each reviewer for full-text review in the Research Screener process. Reviewers 1 and 2 completed 52 and 35 cycles, respectively.

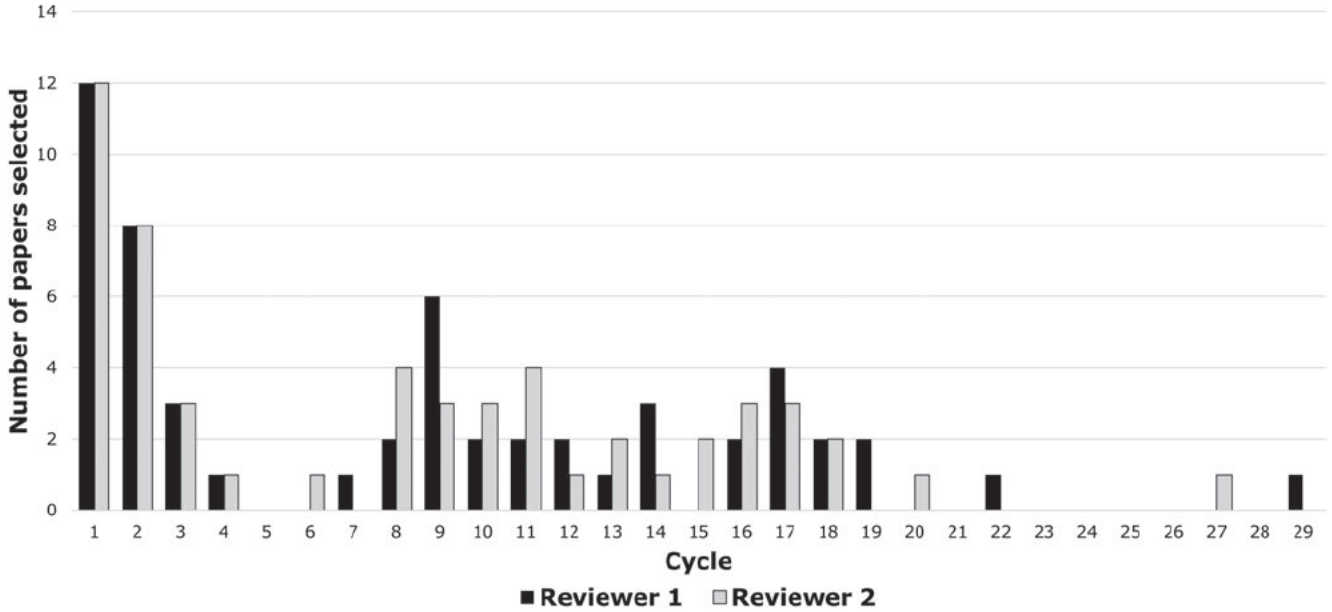


FIGURE 4 Number of papers selected for the review (after full-text read) from each screening cycle.

papers, 5.4%) and reviewer 2 by the 27th cycle (1304 papers; 5%). Similar results were observed in an acute pain systematic review, where all of the reviewed articles were identified after screening 5% of the search results.[4]

The cyclical trends in selection of studies for the systematic review (Figure 4) demonstrate that Research Screener presented 44% (24/55) of the articles to the reviewers in the first four cycles, ostensibly due to the effective use of the eight seed abstracts. Thereafter, selection rates varied between the two reviewers and became sporadic after 19 cycles.

In order to estimate the potential time saved by completing the screening process from <10% of the full search strategy results, screening time data for each reviewer were extracted from Research Screener and analyzed. The mean (95% confidence interval; range) time to screen each title/abstract in the final 20% of cycles screened by the two reviewers was 8.4 (6.8–10.1; 2–131) and 15.2 (12.6–17.9; 1–244) seconds, respectively. The final 20% of cycles was selected for this analysis because it represented a continuous series of cycles in which relatively few papers were potentially eligible, thus providing a plausible, conservative estimate of the time to screen subsequent cycles, if this had been required. Therefore, based on the >23 250 titles/abstracts that did not require screening, the potential time saving was at least 56 and 98 h for each reviewer.

4 | DISCUSSION

Our study has demonstrated the combined use of the SPIDER systematic review model, a broad search strategy to capture over 27 000 deduplicated articles and screening via the machine learning tool, Research Screener, to expedite the extraction of eligible articles for a pharmaceutical science systematic review. We tested the literature search and screening process using a pilot study and assessment of inter-reviewer reliability.

In the process of establishing the final search strategy, we found the large number of captured articles was unavoidable, since our endeavors in the pilot study to constrain the search had excluded essential references. It became apparent that our search strategy required several generic terms, such as "stability," "compatib*," "intravenous*," and "injection*" (Table 1), and we concluded this requirement to include common terms may be a broader issue for systematic reviews in pharmaceutical sciences and other scientific disciplines. Hence, the iterative process of the pilot study was an important evaluation step in developing our systematic review, to maximize the capture of relevant references, and we would encourage this course of action. The value of machine learning screening tools is that large databases from search strategies can be efficiently managed to extract articles for full-text review.

The pilot study indicated that 7.3% (119/1622) of the captured articles could be relevant to our systematic review, which was comparable to 7.5% in a previous study,[11] and therefore suggested approximately 2000 articles would be identified as potentially eligible for the systematic review. However, the proportion of articles selected for full-text review was lower than predicted from the pilot study and appeared to be related to at least two factors. Firstly, many of the selected articles included several drugs from concept 3 of the search strategy (Table 1), thus limiting the overall pool of eligible studies. Second, in retrospect, the pilot study included some intravenous drugs which are more commonly used in neonatal/pediatric settings than in adult patients, or for which there is a limited body of relevant, published literature (e.g., caffeine, pentoxifylline, indomethacin, and sotalol). The reviewers

noted (anecdotally) that some terms, such as stability and intravenous, are used in a wide range of contexts and a number of abstracts were easily and swiftly excluded. Importantly, due to the machine learning algorithms and user-friendly operation of Research Screener, the overall workload impact in the screening process was modest. Further investigation of the reasons behind the relatively low selection rate from the initial pool of articles was outside the scope and value of the present study, as the goal was to optimize capture of eligible papers.

There was an appreciable time saving associated with Research Screener. Recent reports indicate the time to screen abstracts for systematic reviews ranges from 30 to 60 s per abstract and varies according to the experience of the reviewer.[4,7,15,16] In the present study, the two reviewers noted that screening the cycles with a rich source of eligible papers was more time consuming than the latter cycles (after cycle 20), where most abstracts could be rapidly excluded. As a result of the Research Screener ranking and screening process, whereby the average title/abstract screening time from the final 20% of cycles for the two reviewers was 8.4 and 15.2 s, respectively, the overall time saving was at least 56 and 98 h, respectively, if screening the results of the full search strategy was necessary.

One limitation of Research Screener and similar tools is the preclusion of papers which do not contain an abstract. In our systematic review, the reviewers were required to manually screen 1269 such references; however, there was moderate inter-reviewer agreement, and this was an important pool of articles in the present study, contributing approximately half of the final body of literature for the systematic review.

Overall, we have shown the importance of testing the systematic review search strategy process and optimizing the literature captured. Semi-automated machine learning tools such as Research Screener may then be utilized to efficiently screen the results of the search strategy, providing a manageable workload and confidence in the outcomes and scientific rigor of the systematic review.

AUTHOR CONTRIBUTIONS

KTB, BRM, and TDS conceived the study, with advice from TS and MP. All authors contributed to the study design. TDS and KTB had principal responsibility for acquiring the data; BRM was the independent monitor and VV contributed to the pilot study. KTB and TDS conducted initial analysis and interpretation of the data, with advice from all authors. KTB and TDS prepared the first draft of the manuscript. Revision and additional contributions to the manuscript were provided by all authors. All authors approved the final manuscript.

ACKNOWLEDGMENTS

TDS is the recipient of a Sri Lankan AHEAD (Accelerating Higher Education Expansion and Development) program scholarship. Open access publishing facilitated by Curtin University, as part of the Wiley - Curtin University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

At the time of submission, KC and LN are the developers of the Research Screener software and receive financial remuneration to maintain the hosting platform and its related requirements. All other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data not provided in the manuscript are available on reasonable request to the authors.

ETHICS STATEMENT

Not applicable.

ORCID

D. Thisuri N. De Silva https://orcid.org/0000-0003-2508-9550
Brioni R. Moore https://orcid.org/0000-0001-9792-7838
Tobias Strunk https://orcid.org/0000-0001-7079-2339
Michael Petrovski https://orcid.org/0009-0008-4142-0411
Vanessa Varis https://orcid.org/0000-0001-7953-7254
Kevin Chai https://orcid.org/0000-0003-1645-0922
Leo Ng https://orcid.org/0000-0002-9814-0495
Kevin T. Batty https://orcid.org/0000-0003-3850-1778

REFERENCES

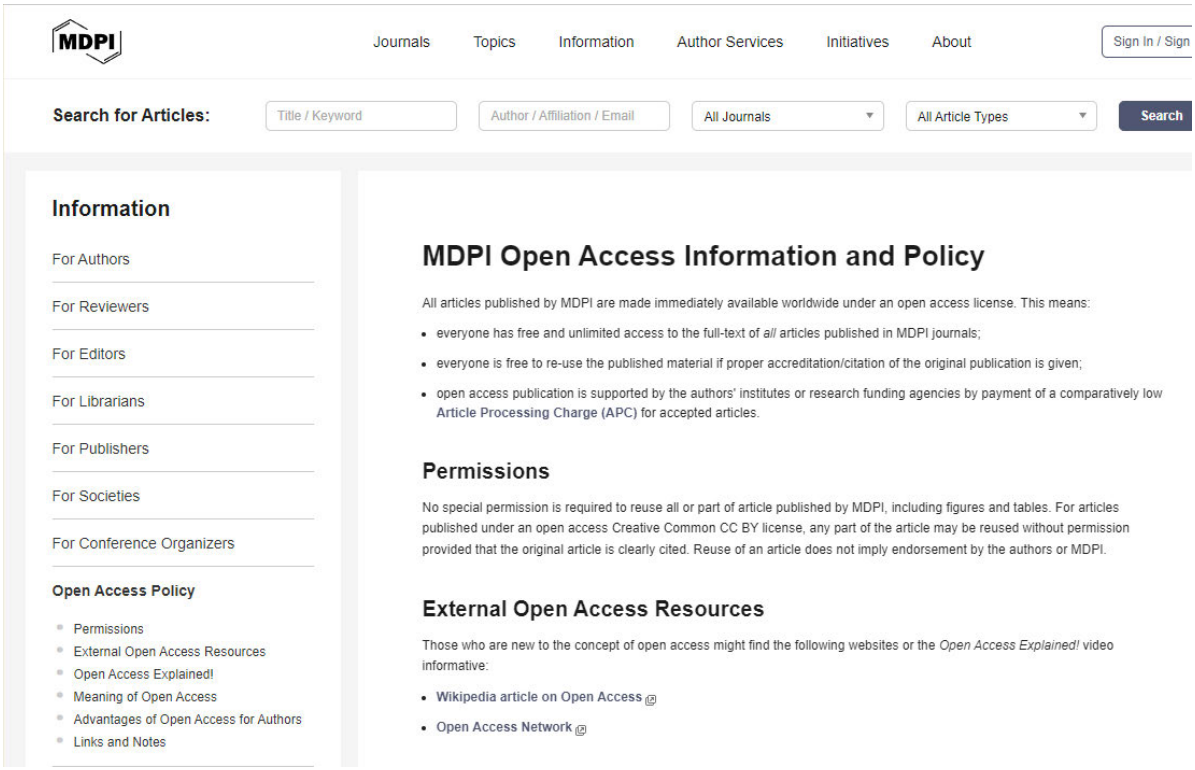
1. Eriksen MB, Frandsen TF. The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: a systematic review. *J Med Libr Assoc*. 2018;106(4):420-431. doi:10.5195/jmla.2018.345
2. Cooke A, Smith D, Booth A. Beyond PICO: the SPIDER tool for qualitative evidence synthesis. *Qual Health Res*. 2012;22(10):1435-1443. doi:10.1177/1049732312452938
3. Tawfik GM, Dila KAS, Mohamed MYF, et al. A step by step guide for conducting a systematic review and meta-analysis with simulation data. *Trop Med Health*. 2019;47:46. doi:10.1186/s41182-019-0165-6
4. Chai KEK, Lines RLJ, Gucciardi DF, Ng L. Research screener: a machine learning tool to semi-automate abstract screening for systematic reviews. *Syst Rev*. 2021;10(1):93. doi:10.1186/s13643-021-01635-3
5. Michelson M, Reuter K. The significant cost of systematic reviews and meta-analyses: a call for greater involvement of machine learning to assess the promise of clinical trials. *Contemp Clin Trials Commun*. 2019;16:100443. doi:10.1016/j.conctc.2019.100443
6. Olofsson H, Brolund A, Hellberg C, et al. Can abstract screening workload be reduced using text mining? User experiences of the tool Rayyan. *Res Synth Methods*. 2017;8(3):275-280. doi:10.1002/jrsm.1237
7. Gates A, Johnson C, Hartling L. Technology-assisted title and abstract screening for systematic reviews: a retrospective evaluation of the Abstrackr machine learning tool. *Syst Rev*. 2018;7(1):45. doi:10.1186/s13643-018-0707-8
8. Przybyla P, Brockmeier AJ, Kontonatsios G, et al. Prioritising references for systematic reviews with RobotAnalyst: a user study. *Res Synth Methods*. 2018;9(3):470-488. doi:10.1002/jrsm.1311
9. van de Schoot R, de Bruin J, Schram R, et al. An open source machine learning framework for efficient and transparent systematic reviews. *Nat Mach Intell*. 2021;3(2):125-133. doi:10.1038/s42256-020-00287-7
10. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev*. 2016;5(1):210. doi:10.1186/s13643-016-0384-4

11. Kanji S, Lam J, Johanson C, et al. Systematic review of physical and chemical compatibility of commonly used medications administered by continuous infusion in intensive care units. *Crit Care Med*. 2010;38(9):1890-1898. doi:10.1097/CCM.0b013e3181e8adcc
12. Castells Lao G, Rodríguez Reyes M, Roura Turet J, Prat Dot M, Soy Muner D, López CC. Compatibility of drugs administered as Y-site infusion in intensive care units: a systematic review. *Med Intensiva*. 2020;44(2):80-87. doi:10.1016/j.medin.2018.08.004
13. *ASHP injectable drug information: A comprehensive guide to compatibility and stability*. American Society of Health-System Pharmacists; 2022.
14. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med*. 2012;22(3):276-282.
15. Haddaway NR, Westgate MJ. Predicting the time needed for environmental systematic reviews and systematic maps. *Conserv Biol*. 2019;33(2):434-443. doi:10.1111/cobi.13231
16. Shemilt I, Khan N, Park S, Thomas J. Use of cost-effectiveness analysis to compare the efficiency of study identification methods in systematic reviews. *Syst Rev*. 2016;5(1):140. doi:10.1186/s13643-016-0315-4

How to cite this article: De Silva DTN, Moore BR, Strunk T, et al. Development of a pharmaceutical science systematic review process using a semi-automated machine learning tool: Intravenous drug compatibility in the neonatal intensive care setting. *Pharmacol Res Perspect*. 2024;12:e1170. doi:10.1002/prp2.1170

Paper 2 – Publication waiver, attribution statement and paper

As per communication with the publishing journal, Pharmaceutics, MDPI maintains an Open Access policy for which permissions are not required for further use or replication of published material. Full permission for inclusion of the published paper within this thesis has thereby been granted. Please see below for full details. www.mdpi.com/openaccess



Attribution Statement

I, **D Thisuri N De Silva,** declare that I significantly contributed the following towards the published article "*The Physicochemical Compatibility of Sildenafil Injection with Parenteral Medications Used in Neonatal Intensive Care Settings*".

- Conception and design
- Acquisition of data and method
- Data conditioning and manipulation
- Analysis and statistical method
- Interpretation and discussion
- Final approval

[redacted] 25/03/2024

I, as a co-author, endorse that the stated level of contribution by the candidate (as above) is appropriate.

Tobias Strunk	[redacted]
Michael Petrovski	[redacted]
Madhu Page-Sharp	[redacted]
Brioni R. Moore	[redacted]
Kevin T. Batty	[redacted]

Research article: The physicochemical compatibility of sildenafil injection with parenteral medications used in neonatal intensive care settings.

D Thisuri N De Silva[1], Tobias Strunk[2,3,4], Michael Petrovski[5], Madhu Page-Sharp[1], Brioni Moore[1,2,3,6], Kevin T Batty[1,6]

[1] Curtin Medical School, Curtin University, Perth, Western Australia, Australia.
[2] Medical School, The University of Western Australia, Crawley, Western Australia, Australia.
[3] Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, Western Australia, Australia.
[4] Neonatal Directorate, King Edward Memorial Hospital, Child and Adolescent Health Service, Subiaco, Western Australia, Australia.
[5] Pharmacy Department, King Edward Memorial Hospital, Women and Newborn Health Service, Subiaco, Western Australia, Australia.
[6] Curtin Health Innovation Research Institute, Curtin University, Bentley 6102, Australia

	Conception and design	Acquisition of Data and Method	Data Conditioning and Manipulation	Analysis and Statistical Method	Interpretation and Discussion
Tobias Strunk	✓				✓
Co-author 1 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: Signed: [redacted]					
Michael Petrovski	✓				✓
Co-author 2 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					
Madhu Page-Sharp	✓	✓			✓
Co-author 3 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					
Brioni R Moore	✓				✓
Co-author 4 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					

Kevin T Batty	✓	✓		✓	✓

Co-author 5 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version.

Signed: 

pharmaceutics

MDPI

Article

The Physicochemical Compatibility of Sildenafil Injection with Parenteral Medications Used in Neonatal Intensive Care Settings

D. Thisuri N. De Silva [1], Tobias Strunk [2,3,4], Michael Petrovski [5], Madhu Page-Sharp [1], Brioni R. Moore [1,2,3,6] and Kevin T. Batty [1,6,*]

[1] Curtin Medical School, Curtin University, Bentley 6102, Australia; d.desilva2@postgrad.curtin.edu.au (D.T.N.D.S.); m.page-sharp@curtin.edu.au (M.P.-S.); brioni.moore@curtin.edu.au (B.R.M.)
[2] Medical School, The University of Western Australia, Crawley 6009, Australia; tobias.strunk@health.wa.gov.au
[3] Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands 6009, Australia
[4] Neonatal Directorate, King Edward Memorial Hospital, Child and Adolescent Health Service, Subiaco 6008, Australia
[5] Pharmacy Department, King Edward Memorial Hospital, Women and Newborn Health Service, Subiaco 6008, Australia; michael.petrovski@health.wa.gov.au
[6] Curtin Health Innovation Research Institute, Curtin University, Bentley 6102, Australia
* Correspondence: kevin.batty@curtin.edu.au; Tel.: +61-(8)-9266-2535

Abstract: Sildenafil is used to treat pulmonary hypertension in neonatal intensive care unit (NICU) settings. As multiple intravenous (IV) medications are co-administered in NICU settings, we sought to investigate the physicochemical compatibility of sildenafil with a range of IV drugs. Sildenafil 600 mcg/mL or 60 mcg/mL was mixed 1:1 with the secondary drug solution to simulate Y-site co-administration procedures. Physical compatibility was evaluated by visual observation against a black and white background and under polarized light for two hours for changes in colour, precipitation, haze and evolution of gas. Chemical compatibility was determined from sildenafil concentrations, using a validated, stability-indicating high-performance liquid chromatography assay. Sildenafil 600 mcg/mL was physicochemically compatible with 29 of the 45 drugs tested at 'high-end' clinical concentrations and physically incompatible with 16 drugs and six '2-in-1' parenteral nutrition solutions. Sildenafil 600 mcg/mL was compatible with lower, clinically relevant concentrations of calcium gluconate, heparin and hydrocortisone. Aciclovir, amoxicillin, ampicillin, ibuprofen lysine, indometacin, phenobarbitone and rifampicin were incompatible with sildenafil 600 mcg/mL, however these IV medications were compatible with sildenafil 60 mcg/mL. Sildenafil 600 mcg/mL and 60 mcg/mL were incompatible with amphotericin, flucloxacillin, furosemide, ibuprofen, meropenem and sodium bicarbonate. Sildenafil compatibility with commonly used syringe filters was also investigated. Sildenafil solution was compatible with nylon syringe filters, however, absorption/adsorption loss occurred with polyethersulfone and cellulose ester filters.

Keywords: sildenafil; physical compatibility; chemical compatibility; neonates; syringe filters

Citation: De Silva, D.T.N.; Strunk, T.; Petrovski, M.; Page-Sharp, M.; Moore, B.R.; Batty, K.T. The Physicochemical Compatibility of Sildenafil Injection with Parenteral Medications Used in Neonatal Intensive Care Settings. *Pharmaceutics* **2024**, *16*, 419. https://doi.org/10.3390/pharmaceutics16030419

Academic Editor: Romána Zelkó

Received: 24 January 2024
Revised: 29 February 2024
Accepted: 13 March 2024
Published: 18 March 2024

1. Introduction

Sildenafil is a phosphodiesterase type 5 inhibitor used in the second-line management of persistent pulmonary hypertension (PPHN) of the newborn, with proven reduction in mortality and a favourable adverse effect profile [1]. Conventional first-line treatment for PPHN is inhaled nitric oxide, however, as this is an expensive treatment modality, it is not commonly used in all countries. In addition, up to 50% of infants with PPHN may not respond to nitric oxide, therefore sildenafil has become a well-established second line therapy [2]. The conventional treatment regimen of intravenous (IV) sildenafil for PPHN is a loading dose of 0.4 mg/kg administered over three hours, followed by a continuous

infusion of 1.6 mg/kg/day for up to seven days, with the sildenafil concentration typically in the order of 400–800 mcg/mL in glucose 5% *w*/*v* (D5W) injection [1]. In preterm infants, a lower loading dose of 0.1 mg/kg administered over 45 min and continuous infusion of 0.5 to 1.2 mg/kg/day is recommended, using sildenafil concentrations in the order of 60–100 mcg/mL in D5W injection [3].

In neonatal intensive care unit (NICU) settings, infants often require several concurrent IV medications, which may be at high concentrations due to fluid restrictions. Multiple IV access sites for these medications pose a threat of pain, risk of infection and thromboembolism to the patients [4]. Due to the limited vascular access in these patients, IV drug administration via a "Y-site" arrangement with three-way connectors is commonly used to infuse multiple drugs simultaneously [5]. Combined with low infusion flow rates and high drug concentrations, one risk with Y-site administration of IV drug combinations is physical and/or chemical drug incompatibilities in the IV apparatus [6]. Physical incompatibility can present as visible precipitates, haze, colour change or gas formation. Infusion of particulate matter of adequate size (i.e., larger than the 4–9 μm capillary diameter) into the vasculature of neonates can cause serious adverse embolic events and may be fatal [7]. Furthermore, chemical incompatibility may lead to sub-optimal clinical outcomes or adverse effects if toxic compounds are formed. Therefore, physicochemical compatibility should be carefully considered when IV drugs are co-administered via Y-sites, with due regard to concentrations and combinations that are applicable to the clinical setting, such as NICU [5].

Physicochemical compatibility of IV sildenafil with other drugs has been reported for pentoxifylline, epinephrine, norepinephrine, vasopressin, heparin, milrinone and dopamine. All drugs were found to be compatible with sildenafil at the concentrations tested, except heparin, which was compatible at 1 unit/mL and incompatible at 100 units/mL [8–10].

Against this background, we sought to investigate the physicochemical compatibility of sildenafil with a range of NICU drugs, at higher end clinically relevant concentrations, and with a selection of 2-in-1 parenteral nutrition (PN) solutions.

2. Materials and Methods

Sildenafil (sildenafil citrate; $C_{22}H_{30}N_6O_4S{\cdot}C_6H_8O_7$; MW 666.7; certified reference material), was purchased from Sigma-Aldrich Chemicals, St. Louis, MO, USA. HPLC grade acetonitrile was from Fisher Scientific, Fair Lawn, NJ, USA. All other laboratory chemicals were of analytical grade. All parenteral medications and solutions were of clinical grade (see online Supplementary File for list of medications and manufacturers—Table S1). The composition of the 2-in-1 PN solutions is provided in Table S2 of the online Supplementary File.

2.1. High Performance Liquid Chromatography (HPLC) Assay

The Agilent 1200 series HPLC system comprised a binary pump with degasser, autosampler, thermostated column oven and a dual wavelength UV detector (Agilent Technology, Waldbronn, Germany). Chemstation software (vRev. B.03.01.SR1; Agilent Technology) was used to acquire and process data.

A reversed phase HPLC column (Kinetex, 5μm, C_{18}; 100 × 4.6 mm; Phenomenex, Torrance, CA, USA) was maintained at 30 °C. The mobile phase was an isocratic mixture of 40% *v*/*v* acetonitrile and 60% *v*/*v* 50 mM potassium dihydrogen orthophosphate buffer (pH 6; HI 5221 pH Meter, Hanna Instruments, Woonsocket, RI, USA). The flow rate and UV detector were 1 mL/min and 240 nm, respectively. The injection volume was 5 μL, unless otherwise specified.

The stability-indicating HPLC method development was guided by previous studies [8,9,11] and validated in accordance with the International Council for Harmonization guidelines [12]. Sildenafil 600 mcg/mL was prepared by diluting sildenafil injection (Revatio; Viatris, Australia; Supplementary File—Table S1) with D5W and exposing it to forced degradation experiments with acidic, alkali and oxidative stress conditions.

Oxidative stress: Sildenafil 600 mcg/mL was mixed 1:1 with 20% *v*/*v* hydrogen peroxide (2 mL volume in 4 mL glass vials with impermeable caps, *n* = 3), and stored in a stability chamber at 45 °C (Fitoclima 600, Aralab, Rio de Mouro, Portugal). Samples (300 µL) were withdrawn at 0, 1, 2, 4 and 7 days and frozen (−80 °C) to arrest further degradation until assayed. At the time of assay, samples were thawed at ambient room temperature (22 °C), vortex mixed, diluted 1-in-50 with water, then analysed by HPLC as described above (injection volume 20 µL).

Acid stress: Sildenafil 600 mcg/mL was mixed with 4 M hydrochloric acid (1:1 *v*/*v*; 2 mL in 4 mL glass vials with impermeable caps, *n* = 3), and stored at 45 °C. Samples (300 µL) were withdrawn at 0, 1, 2, 4 and 7 days, neutralised with 4 M sodium hydroxide solution and frozen (−80 °C). At the time of assay, samples were thawed, vortex mixed, diluted 1-in-50 with water and then analysed by HPLC as described above (injection volume 20 µL).

Alkali stress: A similar process as described above for acid stress was followed, using 4 M sodium hydroxide solution and neutralisation with 4 M hydrochloric acid.

Heat stress: Sildenafil 600 mcg/mL was mixed with water (1:1 *v*/*v*; 2 mL in 4 mL glass vials with impermeable caps, *n* = 3), and stored at 60 °C (PURA 4 water bath, Julabo GmbH, Seelbach, Germany). Samples (500 µL) were withdrawn at 0 and 3 days and frozen (−80 °C). At the time of assay, samples were thawed, vortex mixed and analysed by HPLC as described above (injection volume 5 µL).

Light stress: Sildenafil 600 mcg/mL was mixed with water (1:1 *v*/*v*; 2 mL in 4 mL glass vials with impermeable caps, *n* = 3) and exposed to light (laboratory fluorescent lighting 24/7 and normal daylight (indirect sunlight) for approximately 12 h per day) at room temperature (22 °C). Samples (500 µL) were withdrawn at 0 and 7 days and frozen (−80 °C). At the time of assay, samples were thawed, vortex mixed and analysed by HPLC as described above (injection volume 5 µL).

To establish linearity and range for the HPLC assay, a calibration curve was constructed using sildenafil solutions at concentrations of 3, 10, 30, 100, 300 and 800 mcg/mL (*n* = 3). Calibration curve and analyte concentration data were analysed using Microsoft Excel (Version 2309 Build 16.0.16827.20166). The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were estimated using the formulae below, where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve [12]. LLOQ was confirmed by precision data.

$$\mathrm{LOD} = \frac{3.3 \times \sigma}{\mathrm{S}}$$

$$\mathrm{LLOQ} = \frac{10 \times \sigma}{\mathrm{S}}$$

Accuracy and precision of the HPLC assay was evaluated at sildenafil concentrations of 600, 100, 10 and 2.9 (LLOQ) mcg/mL (*n* = 5) using the sildenafil reference standard and the commercial sildenafil injection diluted with D5W. The concentrations of the two series were compared (expressed as a fraction of the nominal concentration). Intra-assay and inter-assay precision were determined by calculating percentage relative standard deviation (%RSD) for the same sildenafil concentrations.

2.2. Preparation of Samples for Physical and Chemical Compatibility Testing

Sildenafil injection (800 mcg/mL) was diluted using D5W to achieve clinically relevant concentrations of 60 and 600 mcg/mL. The higher sildenafil concentration is consistent with a high-end dosage regimen for infants ≥37 weeks gestational age, and the lower sildenafil concentration is consistent with a low-end dosage regimen for pre-term infants <37 weeks gestational age [13]. Secondary test drugs and 2-in-1 PN solutions were prepared/diluted in accordance with the manufacturer's instructions or standard local neonatal clinical protocols at King Edward Memorial Hospital. Drug concentrations were based on the recommendations for a patient weighing 2 kg (see Table 1 and Table 2 for secondary drug concentrations used in the present study). Medications that were originally contained

in glass ampoules or required reconstitution were filtered immediately prior to mixing (33 mm × 0.22 µm Polyethersulfone (PES) membrane, Millex-GP, Merck Millipore Ltd., Carrigtwohill, Co., Cork, Ireland).

A panel of 45 drugs and 6 PN solutions were selected and endorsed by local clinical experts (TS, MP). Five drugs were included in the study as positive (compatible: epinephrine, norepinephrine, milrinone and dopamine) or negative (incompatible: heparin 100 units/mL) controls, and the remaining forty drugs were previously untested against sildenafil. Epinephrine, norepinephrine, milrinone and dopamine were tested in the present study at different concentrations to previous reports [8,9].

Drug combinations (sildenafil and the test drug or PN solution) were mixed at a 1:1 (*v*/*v*) ratio to simulate Y-site administration, consistent with established methods [8,14–17]. Drug preparation, mixing and testing was carried out at room temperature (22 °C).

The first stage of compatibility tests comprised a combination of sildenafil 600 mcg/mL and the secondary drug at clinically relevant high-end concentrations, consistent with the standard NICU protocols and expert advice. If incompatibility was detected, the drug combination was then tested using sildenafil 600 mcg/mL and the secondary drug at a low-end clinically relevant concentration, if applicable. The third and fourth stages of tests comprised sildenafil 60 mcg/mL and the secondary drug at high- and low-end concentrations, respectively, as applicable. The 'up to four-way' combination design optimised the scope for clinically relevant information on incompatible combinations.

Twelve 2 mL clear glass HPLC vials with impermeable screw cap lids were used for each binary combination of drugs/fluids and the respective control solutions. Sildenafil, secondary drug combinations and the control samples were prepared as described below:

- Set 1—Sildenafil injection solution (0.4 mL of 60 or 600 mcg/mL) and secondary test drug solution/fluid (0.4 mL); $n = 4$.
- Set 2—Sildenafil injection solution (0.4 mL of 60 or 600 mcg/mL) diluted with 0.4 mL of the diluent of the secondary test drug ($n = 4$) as the reference control solution for the purpose of visual comparison and HPLC assay of sildenafil concentration. The diluent was D5W for PN solutions.
- Set 3—The test drug solution/fluid (0.4 mL) was diluted with 0.4 mL of D5W ($n = 4$) for the purpose of visual comparison.

2.3. Physical Compatibility Testing

All vials were gently mixed and inspected with an unaided eye against a black and white background for any change in colour, haze or precipitation. The observations were carried out immediately after mixing, and 5, 15, 60 and 120 min. Samples were also observed under a polarized light viewer (Apollo I Liquid Viewer with a LED light source and 1.7× Magnifier, Adelphi Manufacturing Company Ltd., Haywards Heath, West Sussex, UK) for any visible precipitation or particulate matter. Physical compatibility was based on the visual appearance of the drug combination (set 1) in comparison to control solutions (set 2 and 3). Any inconclusive observation was confirmed by a second independent observer and all physical incompatibilities were photographed. If precipitation or particles were observed in the drug combination vials, an aliquot was examined under light microscopy (Leica MC190HD, 40× magnification, Leica Microsystems (Switzerland) Ltd., CH—9435, Heerbrugg, Switzerland).

2.4. Chemical Compatibility Testing

The HPLC assay was used to evaluate chemical compatibility if the combination was physically compatible. If any physical incompatibility was observed, such combinations were not chemically tested to avoid contamination of the HPLC system. At 2 h after mixing, the sildenafil concentration in the four vials of sildenafil plus test drug (set 1) was measured by HPLC and compared to the four sildenafil reference solution vials (set 2). The ratio of the mean peak areas was determined and the 95% CI of the ratio was calculated using the confidence limits from a two-sided *t*-test ($\alpha = 0.05$; SigmaPlot V.15; Inpixon

GmbH, Düsseldorf, Germany). Consistent with previous studies, incompatibilities of sildenafil:drug combinations were defined as a ratio of the mean peak area outside the range of 90–110% [8,9,18–20].

2.5. Evaluation of Absorption/Adsorption Loss of Sildenafil by Syringe Filters

The compatibility of sildenafil injection with conventional syringe filters has not previously been reported but is clinically relevant information and was required for subsequent tests in the present study. Six types of syringe filters and two inline filters composed of different filter membranes (cellulose esters, nylon, polyvinylidene fluoride, polyethersulfone and polypropylene; online Supplementary File Table S5) were tested to evaluate the absorption/adsorption loss of sildenafil during the process of filtration.

Sildenafil 60 mcg/mL and 600 mcg/mL solutions were used for filter testing and the drug recovery in the filtrate was determined by HPLC assay. The peak area values obtained with and without filtration were compared and data were reported as percent recovery according to the following formula:

$$\text{Recovery of sildenafil } (\%) = \frac{\text{sildenafil concentration(filtered; peak area of the chromatogram)}}{\text{sildenafil concentration of the unfiltered solution}} \times 100$$

A pilot test was carried out using the eight filter types (online Supplementary File Table S5) and the two concentrations of sildenafil solution in D5W. Filtrate was collected as five separate, consecutive 1 mL portions of solution to examine the influence of the volume of filtrate on the drug recovery. Testing was carried out in triplicate and a new filter unit was used for each sample.

Based on the pilot study results, four filters were selected for further testing due to the recovery data and/or clinical relevance of the filters (see online Supplementary File Table S5): nylon (NY, 15 mm × 0.2 µm); Millex-GP (PES, polyethersulfone, 33 mm × 0.22 µm); Millex-GS (MCE, mixed cellulose esters, 33 mm × 0.22 µm); inline filter (polyethersulfone 25 mm × 0.2 µm). Sildenafil commercial injection solution (60 and 600 mcg/mL in D5W) was tested in a similar manner using a test volume of 4 mL (n = 3).

3. Results

3.1. HPLC Method Validation

The HPLC chromatograms revealed the sildenafil peak was well resolved from the solvent and degradation product peaks in all stress conditions tested. Sildenafil eluted at approximately 4.2 min whereas all degradation products eluted at less than 3 min (online Supplementary File Figures S1–S5). Oxidation of sildenafil resulted in the most extensive degradation profile, with a loss of 14.9% at the seventh day of exposure. Degradation products were detected at 1.5, 1.7 and 2.9 min. Alkali degradation of sildenafil was found to be 11.4% at the seventh day of exposure, with one degradation product detected at 0.9 min. Exposure of sildenafil to acid, heat and light showed no detectable degradation peaks, with post-exposure sildenafil drug concentrations of 98.5%, 103.6% and 99.2%, respectively.

The assay was linear for sildenafil in aqueous solution (n = 3) within the concentration range 3–800 mcg/mL ($r^2 > 0.999$) (online Supplementary File Figure S6). The LOD and LLOQ for sildenafil were 0.96 and 2.9 mcg/mL, respectively. The HPLC method was accurate and precise according to standard definitions [12], with accuracy being 100–105% for all samples and precision (%RSD) being <4.2% for inter- and intra-assay samples (online Supplementary File Table S3).

3.2. Sildenafil Compatibility

3.2.1. Sildenafil 600 mcg/mL

Sildenafil 600 mcg/mL was physically and chemically compatible with 29 of the 45 drugs tested at high-end clinical concentrations in the present study: alprostadil, liposomal amphotericin, benzylpenicillin, caffeine (base), caffeine citrate, cefotaxime, ciprofloxacin, clonidine, cloxacillin, dexmedetomidine, dobutamine, dopamine, epinephrine, fentanyl, fluconazole, gentamicin, insulin, levetiracetam, linezolid, metronidazole, midazo-

lam, milrinone, morphine hydrochloride, morphine sulfate, norepinephrine, paracetamol, piperacillin/tazobactam, vancomycin and vecuronium (Table 1). However, sildenafil 600 mcg/mL was physically incompatible with 16 drugs and all 6 of the 2-in-1 PN solutions, with precipitates and haziness occurring almost immediately (Table 1). In the first series of re-testing sildenafil 600 mcg/mL with secondary drugs at lower, clinically relevant concentrations, three of the combinations were found to be compatible (calcium gluconate 50 mg/mL; heparin 2 units/mL; hydrocortisone 1 mg/mL; Table 1). However, sildenafil 600 mcg/mL was incompatible with amoxicillin (100 mg/mL and 50 mg/mL), ampicillin (100 mg/mL and 50 mg/mL) and meropenem (50 mg/mL and 25 mg/mL) (Table 1). All physical incompatibilities were visible to the naked eye, except for the combination with calcium gluconate (100 mg/mL) which required polarized light for clear visualisation. Photographs of selected incompatible drug combinations and their corresponding photomicrographs can be found in the online Supplementary File (Figures S9–S12).

Table 1. Physicochemical compatibility of sildenafil 600 mcg/mL with secondary drugs and 2-in-1 parenteral nutrition solutions (see online Supplementary File Table S2 for details).

Secondary Drug	Test Concentration	Diluent	P/C *	SIL Ratio	95% CI of Ratio
Aciclovir	5 mg/mL	D5W	I [a]	-	-
Alprostadil	20 mcg/mL	NS	C	99.9	99.4–100.4
Amoxicillin	100 mg/mL	WFI	I [b]	-	-
Amoxicillin	50 mg/mL	WFI	I [b]	-	-
Amphotericin (Fungizone)	100 mcg/mL	D5W	I [b]	-	-
Amphotericin liposomal	2 mg/mL	D5W	C	99.9	99.0–100.8
Ampicillin	100 mg/mL	WFI	I [b]	-	-
Ampicillin	50 mg/mL	WFI	I [b]	-	-
Benzylpenicillin	100 mg/mL	WFI	C	101.2	99.7–102.7
Caffeine (base)	10 mg/mL	U	C	101.0	100.1–101.9
Caffeine citrate	20 mg/mL	U	C	100.4	99.6–101.2
Calcium gluconate	100 mg/mL	U	I [c]	-	-
Calcium gluconate	50 mg/mL	NS	C	100.0	99.1–100.8
Cefotaxime	100 mg/mL	WFI	C	102.1	99.9–104.3
Ciprofloxacin	2 mg/mL	U	C	101.3	100.3–102.2
Clonidine	2 mcg/mL	NS	C	99.7	99.1–100.4
Cloxacillin	100 mg/mL	WFI	C	101.1	100.2–102.0
Dexmedetomidine	1 mcg/mL	NS	C	100.0	98.9–101.1
Dobutamine hydrochloride	7.2 mg/mL	NS	C	99.9	99.1–100.7
Dobutamine hydrochloride	7.2 mg/mL	D5W	C	100.4	99.5–101.3
Dopamine	7.2 mg/mL	NS	C	100.5	99.9–101.0
Dopamine	7.2 mg/mL	D5W	C	100.7	100.2–101.3
Epinephrine	64 mcg/mL	D5W	C	99.9	99.3–100.5
Fentanyl	50 mcg/mL	U	C	98.2	95.4–100.9
Flucloxacillin	50 mg/mL	D5W	I [d]	-	-
Fluconazole	2 mg/mL	U	C	100.2	99.4–100.9
Furosemide	1 mg/mL	D5W	I [b]	-	-
Furosemide	0.2 mg/mL	D5W	I [b]	-	-
Gentamicin	10 mg/mL	WFI	C	101.9	101.3–102.5
Gentamicin	10 mg/mL	NS	C	102.2	100.4–104.0
Heparin	100 units/mL	NS	I [d]	-	-
Heparin	2 units/mL	NS	C	99.1	98.3–100.0
Hydrocortisone	10 mg/mL	NS	I [a]	-	-
Hydrocortisone	1 mg/mL	NS	C	99.7	93.2–106.1
Ibuprofen	5 mg/mL	NS	I [e]	-	-
Ibuprofen lysine	4 mg/mL	NS	I [e]	-	-
Indometacin	200 mcg/mL	NS	I [e]	-	-
Insulin	0.2 units/mL	NS	C	100.5	98.6–102.4
Levetiracetam	5 mg/mL	NS	C	99.7	98.8–100.6
Linezolid	2 mg/mL	U	C	98.8	97.8–99.8
Meropenem	50 mg/mL	NS	I [b]	-	-
Meropenem	25 mg/mL	NS	I [b]	-	-
Metronidazole	5 mg/mL	U	C	99.2	98.3–100.1
Midazolam	1 mg/mL	U	C	100.3	99.9–100.8
Midazolam	120 mcg/mL	NS	C	99.9	98.6–101.2
Midazolam	120 mcg/mL	D5W	C	100.5	99.6–101.4
Midazolam	500 mcg/mL	NS	C	100.5	98.4–102.7
Milrinone	400 mcg/mL	D5W	C	100.5	99.5–101.4
Morphine hydrochloride	200 mcg/mL	D5W	C	100.4	99.6–101.2
Morphine sulfate	200 mcg/mL	D5W	C	99.9	99.4–100.3
Norepinephrine	64 mcg/mL	D5W	C	100.1	99.2–101.0
Paracetamol	10 mg/mL	U	C	100.0	99.4–100.6
Phenobarbitone	20 mg/mL	WFI	I [b]	-	-
Piperacillin/tazobactam	200 mg/mL	WFI	C	101.6	101.0–102.2
Rifampicin	6 mg/mL	NS	I [f]	-	-

Table 1. *Cont.*

Secondary Drug	Test Concentration	Diluent	P/C *	SIL Ratio	95% CI of Ratio
Sodium bicarbonate	4.2% *w*/*v*	WFI	I [b]	-	-
Vancomycin	10 mg/mL	D5W	C	100.4	99.4–101.4
Vecuronium	1 mg/mL	WFI	C	101.4	100.7–102.1
Parenteral nutrition PN 1	-	-	I [a]	-	-
Parenteral nutrition PN 2	-	-	I [a]	-	-
Parenteral nutrition PN 3	-	-	I [a]	-	-
Parenteral nutrition PN 4	-	-	I [a]	-	-
Parenteral nutrition PN 5	-	-	I [a]	-	-
Parenteral nutrition PN 6	-	-	I [a]	-	-

* P/C—Physicochemical compatibility; SIL—Sildenafil; C—Compatible; I—Incompatible; D5W—Glucose 5% *w*/*v*; WFI—Water for injection; NS—Normal saline/sodium chloride 0.9% *w*/*v*; U—Undiluted. [a]—White precipitate appeared 5–10 min after mixing; [b]—White precipitate appeared immediately after mixing; [c]—Particles observed under polarized light; [d]—Haze developed after mixing; [e]—Milky turbidity appeared immediately after mixing; [f]—Heavy precipitate appeared immediately after mixing—Colour could not be determined as the solution was coloured.

3.2.2. Sildenafil 60 mcg/mL

Sildenafil 60 mcg/mL was physically compatible with all drug and PN fluid combinations except furosemide, meropenem and sodium bicarbonate (Table 2). The only combination shown to be physically compatible and chemically incompatible was ibuprofen. By contrast, sildenafil 60 mcg/mL was physically and chemically compatible with ibuprofen lysine.

Table 2. Physicochemical compatibility of secondary drugs and 2-in-1 parenteral nutrition solutions tested with sildenafil 60 mcg/mL, their concentrations and diluents.

Secondary Drug	Test Concentration	Diluent	P/C *	SIL * Ratio	95% CI of Ratio
Aciclovir	5 mg/mL	D5W	R	105.8	105.2–106.4
Amoxicillin	100 mg/mL	WFI	R	105.9	105.4–106.4
Amphotericin (Fungizone)	100 mcg/mL	D5W	R	104.2	102.8–105.7
Ampicillin	100 mg/mL	WFI	R	105.8	105.0–106.5
Flucloxacillin	50 mg/mL	D5W	R	105.7	104.9–106.5
Furosemide	1 mg/mL	D5W	I [a]	-	-
Furosemide	0.2 mg/mL	D5W	I [b]	-	-
Heparin	100 units/mL	NS	C	99.3	98.7–99.9
Hydrocortisone	10 mg/mL	NS	C	99.8	99.5–100.0
Hydrocortisone	1 mg/mL	NS	C	99.8	99.3–100.3
Ibuprofen	5 mg/mL	NS	I	**74.0**	72.9–75.1
Ibuprofen lysine	4 mg/mL	NS	C	99.4	98.9–99.9
Indometacin	200 mcg/mL	NS	C	99.1	98.7–99.5
Meropenem	50 mg/mL	NS	I [b]	-	-
Meropenem	25 mg/mL	NS	I [b]	-	-
Phenobarbitone	20 mg/mL	WFI	R	104.3	103.1–105.6
Rifampicin	6 mg/mL	NS	R	102.4	101.5–103.3
Sodium bicarbonate	4.2% *w*/*v*	WFI	I [c]	-	-
Sodium bicarbonate	4.2% *w*/*v*	NS	I [c]	-	-
Sodium bicarbonate	4.2% *w*/*v*	D5W	I [c]	-	-
Parenteral nutrition PN 1	-	-	R	103.9	103.3–104.6
Parenteral nutrition PN 2	-	-	R	105.4	104.2–106.6
Parenteral nutrition PN 3	-	-	R	105.7	104.9–106.4
Parenteral nutrition PN 4	-	-	R	104.8	103.8–105.9
Parenteral nutrition PN 5	-	-	R	105.5	104.8–106.2
Parenteral nutrition PN 6	-	-	R	106.6	105.3–107.8

* P/C—Physicochemical compatibility; SIL—Sildenafil; C—Compatible; I—Incompatible; R—Re-test by filtration (see Table 3); D5W—Glucose 5% *w*/*v*; WFI—Water for injection; NS—Normal saline/sodium chloride 0.9% *w*/*v*; U—Undiluted. Bold SIL ratio shows chemical incompatibility. [a]—White precipitate appeared 1 h after mixing; [b]—Particles observed under polarized light; [c]—Haze developed after mixing.

Thirteen drug combinations with sildenafil 60 mcg/mL, including the six PN solutions, resulted in sildenafil ratios >102% (Table 2). These combinations were re-tested, after filtering the combinations and control samples using nylon filters (Table 3). Apart from aciclovir and rifampicin (which were classified as compatible), all re-tested combinations of sildenafil with secondary drugs and PN solutions produced a significantly lower sildenafil ratio after filtration. The sildenafil ratio (filtered) was in the range of 90–110% for amoxicillin, ampicillin, phenobarbitone and three PN solutions; hence these combinations also were classified as compatible (Table 3). However, as the sildenafil ratio (filtered) was <90% for amphotericin, flucloxacillin and three PN solutions, possibly due to a sub-visible precipitate

being filtered by the nylon filters (personal communication, C Locher and EKY Tang), these combinations were classified as incompatible (Table 3).

Table 3. Re-testing of drug combinations with sildenafil 60 mcg/mL in which SIL ratio (Table 2) was > 102%. Combinations considered compatible if sildenafil filtered ratio was in range of 90–110% (nylon filters; see methods for further details).

Secondary Drug	Test Concentration	SIL * Ratio (Unfiltered)	95% CI of Ratio (Unfiltered)	SIL * Ratio (Filtered)	95% CI of Ratio (Filtered)	P/C *
Aciclovir	5 mg/mL	107.1	106.3–108.0	106.1	104.2–108.0	C
Amoxicillin	100 mg/mL	105.9	103.6–108.1	98.3	95.4–101.3	C
Amphotericin (Fungizone)	100 mcg/mL	105.8	104.8–106.8	78.3	75.1–81.5	I
Ampicillin	100 mg/mL	102.6	100.0–105.2	94.4	92.2–96.5	C
Flucloxacillin	50 mg/mL	106.1	104.4–107.9	84.9	82.0–87.8	I
Phenobarbitone	20 mg/mL	102.8	100.8–104.8	95.5	92.7–98.3	C
Rifampicin	6 mg/mL	102.7	100.5–104.8	108.6	106.0–111.2	C
Parenteral nutrition PN 1	-	107.0	106.0–108.1	87.5	86.5–88.6	I
Parenteral nutrition PN 2	-	105.5	104.6–106.3	91.2	89.3–93.2	C
Parenteral nutrition PN 3	-	105.9	105.1–106.7	94.1	92.0–96.1	C
Parenteral nutrition PN 4	-	106.9	106.1–107.6	77.9	72.4–83.5	I
Parenteral nutrition PN 5	-	105.8	105.1–106.6	94.0	92.5–95.5	C
Parenteral nutrition PN 6	-	106.2	105.1–107.4	88.9	87.2–90.6	I

* SIL—Sildenafil; P/C—Physicochemical compatibility; C—Compatible; I—Incompatible.

3.3. Absorption/Adsorption Loss of Sildenafil by Filter Material

The pilot study using 8 filters and 5 mL sildenafil solution showed the lowest drug recovery was in the first millilitre of the filtrate in all filters studied. For sildenafil 600 mcg/mL solution, the first millilitre had a drug recovery >90% in all filters tested (online Supplementary File Figure S7). In the second to fifth millilitres, drug recovery was >98%. However, for sildenafil 60 mcg/mL solution, only the nylon, polypropylene and inline 'lipid' filters showed a drug recovery of >90% in the first millilitre of the filtrate. All filter types showed a drug recovery >94% in the remainder of the sildenafil 60 mcg/mL filtrate (online Supplementary File Figure S8).

The filter test results obtained using the sildenafil commercial injection solution (600 mcg/mL) revealed that all filter types tested (NY, PES, MCE and Inline PES) showed a drug recovery >90% in the first millilitre of the filtrate (Figure 1). One way ANOVA results showed a statistically significant difference in drug recovery in the first millilitre compared to the remainder of the filtrate ($p < 0.05$).

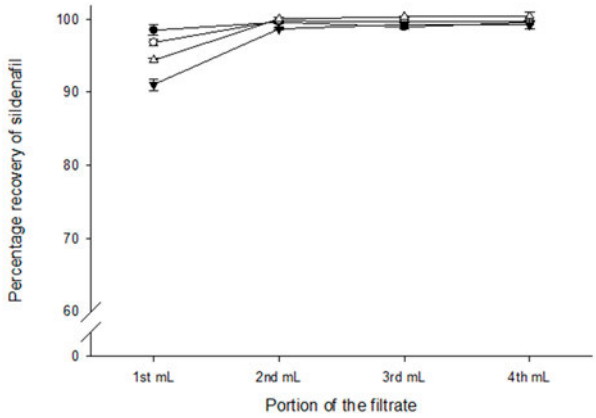


Figure 1. Recovery (%) of sildenafil 600 mcg/mL injection solution from sterilising filters. Sildenafil concentration was determined from each of four successive millilitres of solution passed through filters (● nylon; ○ polyethersulfone; ▼ mixed cellulose esters; △ inline polyethersulfone; see online Supplementary File Table S5 for further details). Data are mean ± SD (n = 3).

However, in the sildenafil 60 mcg/mL solution PES, MCE and inline filters showed a drug recovery <80% in the first millilitre of the filtrate (Figure 2). The drug recovery was >97% in all millilitre portions of the filtrate when the nylon filters were used and no statistically significant difference in drug recovery was observed between any millilitre portions. The first millilitre of the filtrate had a statistically significantly lower drug recovery (

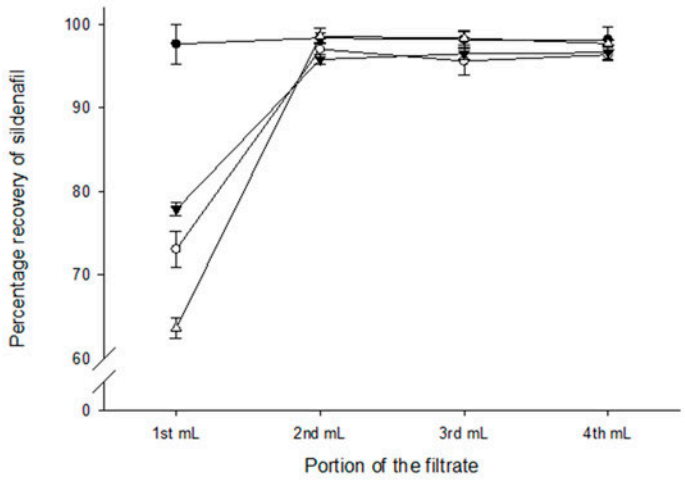


Figure 2. Recovery (%) of sildenafil 60 mcg/mL injection solution from sterilising filters. Sildenafil concentration was determined from each of four successive millilitres of solution passed through filters (● nylon; ○ polyethersulfone; ▼ mixed cellulose esters; △ inline polyethersulfone; see online Supplementary File Table S5 for further details). Data are mean ± SD (n = 3).

4. Discussion

Our study has demonstrated that sildenafil 600 mcg/mL injection was physically and chemically compatible with 29 IV drugs at high-end, clinically relevant concentrations for NICU settings (Table 1). None of these drugs were tested at lower concentrations or against sildenafil 60 mcg/mL in the present study. Rather, it was concluded that lower drug concentrations would also be compatible.

Sixteen of the secondary drugs (at their standard or high-end clinically relevant concentration), and all six 2-in-1 PN solutions, were physically incompatible with sildenafil 600 mcg/mL (Table 1). Nine of these sixteen drugs were evaluated at only one relevant concentration and subsequently tested against sildenafil 60 mcg/mL. A further four were evaluated at lower, clinically relevant concentrations and found to be physically incompatible (amoxicillin, ampicillin, furosemide and meropenem); hence, sildenafil 600 mcg/mL was deemed incompatible with 13 of the 45 IV drugs at concentrations relevant to NICU settings. However, sildenafil 600 mcg/mL was found to be compatible with three drugs at low concentrations (calcium gluconate 50 mg/mL, heparin 2 units/mL and hydrocortisone 1 mg/mL; Table 1), which could be co-administered at these lower, clinically relevant concentrations if required. The results for heparin align with previous data indicating that heparin was incompatible at higher concentration (100 units/mL) [8] and compatible at a lower concentration (1 unit/mL) [9]. Furthermore, the calcium gluconate concentration used for urgent correction of hypocalcaemia is 50 mg/mL [13] and this concentration was found to be physicochemically compatible with sildenafil 600 mcg/mL. Hence, calcium gluconate was not tested with sildenafil 60 mcg/mL.

Fifteen drugs and the six 2-in-1 PN solutions were tested against the lower sildenafil concentration of 60 mcg/mL, which is used in preterm infants [3]. Four drugs showed physical and chemical compatibility, three were physically incompatible and one (ibuprofen) was chemically incompatible (Table 2). The remaining seven drugs and the PN solutions were found to have sildenafil ratios >102%. Although there was no visible or microscopic evidence of precipitation (including Tyndall beam and magnified polarised light obser-

vation), we were aware of unpublished data suggesting sub-visible precipitates for other drug combinations (personal communication, C Locher and EKY Tang). Therefore, a series of filter validation studies were conducted which identified 0.2 μm nylon filters as the most suitable, and these combinations were investigated before and after filtering (Table 3). Based on pre-determined criteria for the 90–110% sildenafil ratio (filtered), it was concluded that aciclovir, amoxicillin, ampicillin, phenobarbitone and rifampicin were compatible with sildenafil 60 mcg/mL, but amphotericin and flucloxacillin were incompatible. Three of the PN solutions were also classified as compatible, however, there were no notable features of these three formulations (#2, #3 and #5) compared to the incompatible formulations and further investigation of this finding was beyond the scope of the present study.

Physical incompatibilities in the present study ranged from florid precipitation to hazy fluids and potential sub-visible precipitation. The former were generally visible to the naked eye, where the limit of detection is approximately 100 μm for discrete particles and 10 μm for hazy or cloudy fluids [21], the observation of which may be enhanced by polarised light [10] or Tyndall beam [22]. Sub-visible particles in the order of 1–2 μm also may be detected by the visual enhancement techniques or light microscopy, however, it has been postulated that incompatible drug combinations could cause nano- or micro-precipitation, ostensibly <1 μm (personal communication, C Locher and EKY Tang). In the present study, sub-detectable precipitation may explain the substantially lower sildenafil ratio after 0.2 μm filtration for amphotericin, flucloxacillin and three PN solutions. Although the clinical impact of injection of particulate matter <1 μm is unclear, the pre-determined criteria for the sildenafil ratio (outside the range of 90–110%) was applied in the present study to define incompatible drug combinations and recommend avoidance in NICU clinical settings.

The present study included some potential limitations that are consistent with previous investigations of physicochemical compatibility. For example, due to the resource constraints and unclear interpretation or clinical significance of pH changes [23], the determination of pH was not performed (the volume of drug solutions required for pH determination would be >5 mL, and placing a wet pH probe into consecutive samples would reduce the drug concentration and may produce false results). As pH changes may contribute to chemical reaction [24] or altered drug solubility [25], the use of HPLC analysis in the present physicochemical study would likely counter the need for pH analysis. Another potential issue was conducting HPLC analysis only for the primary drug (sildenafil). This is consistent with previous IV physicochemical compatibility studies where a large number of secondary drugs have been tested [26–29]. However, there are some reports where both the primary and secondary drugs have been assayed, typically in studies where a modest range of secondary drugs have been tested [8,16,18]. HPLC analysis of both the primary and secondary IV drugs would have significant cost and complexity implications, to ensure validated HPLC assays were developed for each secondary drug. Consequently, we assumed that physicochemical incompatibility would cause a decline in the concentration of both IV drugs and be detected by HPLC assay of the primary drug. Nevertheless, there may be situations where quantifying the secondary drug concentration is of potential value if chemical incompatibility is suspected or inconclusive results require further investigation.

A potential limitation related to clinical interpretation of the present study was the drug combination contact time of 2 h, which was based on a previous report that 60 min was a plausible maximum contact time for two drug solutions in the IV tubing from the Y-site to the tip of a cannula in NICU settings [30]. By comparison, a four hour study duration is commonly used for drug compatibility studies and may be applicable to other clinical settings [14–17,31–35]. A further clinical consideration is that the present study and most IV compatibility research has been conducted at room temperature [31,32,34–36], which is comparable to the ambient temperature in the majority of clinical settings, including NICU. However, whilst the IV drugs in syringes (or other delivery devices) and a proportion of the IV tubing in NICU will most likely be at room temperature, part of the IV tubing may

be inside a humidicrib at up to 37 °C and some recent IV compatibility studies have been conducted at elevated temperature to simulate the humidicrib environment [8,37].

5. Conclusions

Sildenafil 600 mcg/mL was physicochemically compatible with approximately 70% of the 45 clinically relevant IV drugs used in NICU settings that were tested in the present study. A further seven drugs were compatible with sildenafil 60 mcg/mL. Six drugs (amphotericin, flucloxacillin, furosemide, ibuprofen, meropenem and sodium bicarbonate) were incompatible with sildenafil and should not be co-administered via Y-site infusions. Six 2-in-1 PN solutions were incompatible with sildenafil 600 mcg/mL; however, three appeared to be compatible with sildenafil 60 mcg/mL and three were deemed incompatible. Sildenafil solution was compatible with nylon syringe filters; however, absorption/adsorption loss from the first millilitre of filtrate occurred with polyethersulfone and cellulose ester filters, which should be avoided for small volumes and/or low concentrations of sildenafil solution.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmaceutics16030419/s1, Figure S1: Sildenafil 600 mcg/mL exposure to 20% v/v hydrogen peroxide; Figure S2: Sildenafil 600 mcg/mL exposure to 4 M NaOH; Figure S3: Sildenafil 600 mcg/mL exposure to 4 M HCl; Figure S4: Sildenafil 600 mcg/mL in water (1:1 v/v) exposure to heat; Figure S5: Sildenafil 600 mcg/mL in water (1:1 v/v) exposure to laboratory fluorescent lighting 24/7 and normal daylight; Figure S6: Linearity curve for sildenafil solution in aqueous solution within the concentration range 3–800 mcg/mL (n = 3); Figure S7: Sildenafil percentage recovery by different filters using the 600 mcg/mL solution; Figure S8: Sildenafil percentage recovery by different filters using the 60 mcg/mL solution; Figure S9: Photograph and corresponding photomicrograph of physical incompatibility (white precipitate) of sildenafil 600 mcg/mL with furosemide 1 mg/mL; Figure S10: Photograph and corresponding photomicrograph of physical incompatibility (haze) of sildenafil 600 mcg/mL with heparin 100 units/mL; Figure S11: Photograph and corresponding photomicrograph of physical incompatibility (precipitate in an originally coloured solution) of sildenafil 600 mcg/mL with rifampicin 6 mg/mL; Figure S12: Photograph and corresponding photomicrograph of physical incompatibility (particles under polarized light) of sildenafil 600 mcg/mL with calcium gluconate 100 mg/mL Table S1: Manufacturers/suppliers of injectable products used for compatibility studies; Table S2: Composition of the 2-in-1 parenteral nutrition solutions, manufactured at King Edward Memorial Hospital; Table S3: Accuracy, intra-assay and inter-assay precision data for selected sildenafil concentrations; Table S4: Robustness test results for deliberate changes in method parameters; Table S5: Syringe filter types tested, the membrane and mesh size description.

Author Contributions: D.T.N.D.S., T.S., M.P. and K.T.B. conceived the study, with advice from M.P.-S. and B.R.M. All authors contributed to the study design. D.T.N.D.S. and K.T.B. had principal responsibility for acquiring the data, with assistance from M.P.-S. KTB and D.T.N.D.S. conducted initial analysis and interpretation of the data, with advice from all authors. K.T.B. and D.T.N.D.S. prepared the first draft of the manuscript. Revision and additional contributions to the manuscript were provided by all authors. All authors approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data not provided in the manuscript are available upon reasonable request to the authors.

Acknowledgments: The authors gratefully acknowledge the assistance of Nabeelah Mukadam, Karen Donn, Sarah Woodland, Stephanie Teoh, Caitlyn Byrne and Jack Lee (King Edward Memorial Hospital for Women), and Giuseppe Luna and Jorge Martinez (Curtin University). D.T.N.D.S. is the recipient of a Sri Lankan AHEAD (Accelerating Higher Education Expansion and Development) program scholarship.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Steinhorn, R.H.; Kinsella, J.P.; Pierce, C.; Butrous, G.; Dilleen, M.; Oakes, M.; Wessel, D.L. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. *J. Pediatr.* **2009**, *155*, 841–847. [CrossRef]
2. Yaseen, H.; Darwich, M.; Hamdy, H. Is sildenafil an effective therapy in the management of persistent pulmonary hypertension? *J. Clin. Neonatol.* **2012**, *1*, 171–175. [CrossRef]
3. Steiner, M.; Salzer, U.; Baumgartner, S.; Waldhoer, T.; Klebermass-Schrehof, K.; Wald, M.; Langgartner, M.; Berger, A. Intravenous sildenafil i.v. as rescue treatment for refractory pulmonary hypertension in extremely preterm infants. *Klin. Padiatr.* **2014**, *226*, 211–215. [CrossRef]
4. Pettit, J. Assessment of infants with peripherally inserted central catheters: Part 1. Detecting the most frequently occurring complications. *Adv. Neonatal Care* **2002**, *2*, 304–315. [CrossRef] [PubMed]
5. O'Brien, F.; Clapham, D.; Krysiak, K.; Batchelor, H.; Field, P.; Caivano, G.; Pertile, M.; Nunn, A.; Tuleu, C. Making medicines baby size: The challenges in bridging the formulation gap in neonatal medicine. *Int. J. Mol. Sci.* **2019**, *20*, 2688. [CrossRef] [PubMed]
6. Sherwin, C.M.T.; Medlicott, N.J.; Reith, D.M.; Broadbent, R.S. Intravenous drug delivery in neonates: Lessons learnt. *Arch. Dis. Child.* **2014**, *99*, 590–594. [CrossRef] [PubMed]
7. Parikh, M.J.; Dumas, G.; Silvestri, A.; Bistrian, B.R.; Driscoll, D.F. Physical compatibility of neonatal total parenteral nutrient admixtures containing organic calcium and inorganic phosphate salts. *Am. J. Health Syst. Pharm.* **2005**, *62*, 1177–1183. [CrossRef] [PubMed]
8. AlSalman, F.; Howlett, M.; Breatnach, C.; Kelly, H.; O'Brien, F. Supporting the use of sildenafil infusions in paediatric and neonatal intensive care—A compatibility study. *Eur. J. Pharm. Biopharm.* **2020**, *151*, 153–161. [CrossRef] [PubMed]
9. Luu, Y.; Thigpen, J.; Brown, S.D. Stability of sildenafil in combination with heparin and dopamine. *Am. J. Health Syst. Pharm.* **2017**, *74*, e64–e71. [CrossRef]
10. Campbell, A.L.; Petrovski, M.; Senarathna, S.; Mukadam, N.; Strunk, T.; Batty, K.T. Compatibility of pentoxifylline and parenteral medications. *Arch. Dis. Child.* **2020**, *105*, 395–397. [CrossRef] [PubMed]
11. Atipairin, A.; Woradechakul, C.; Chee, K.S.; Sawatdee, S.; Yoon, A.S. Method validation for determination of sildenafil citrate in extemporaneous oral suspension. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 131–136.
12. European Medicines Agency. *ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology*; European Medicines Agency: London, UK, 1995.
13. King Edward Memorial Hospital—Clinical Neonatal Medication Protocols. Available online: https://www.kemh.health.wa.gov.au/For-Health-Professionals/Clinical-Guidelines/Neonatal (accessed on 16 July 2023).
14. Allen, L.V., Jr.; Levinson, R.S.; Phisutsinthop, D. Compatibility of various admixtures with secondary additives at Y-injection sites of intravenous administration sets. *Am. J. Hosp. Pharm.* **1977**, *34*, 939–943. [CrossRef] [PubMed]
15. Akkerman, S.R.; Zhang, H.; Mullins, R.E.; Yaughn, K. Stability of milrinone lactate in the presence 29 critical care drugs and 4 i.v. solutions. *Am. J. Health Syst. Pharm.* **1999**, *56*, 63–68. [CrossRef] [PubMed]
16. Anderson, C.; Boehme, S.; Ouellette, J.; Stidham, C.; Mackay, M. Physical and chemical compatibility of injectable acetaminophen during simulated Y-site administration. *Hosp. Pharm.* **2014**, *49*, 42–47. [CrossRef] [PubMed]
17. Bell, M.S.; Nolt, D.H. Visual compatibility of doxapram hydrochloride with drugs commonly administered via a Y-site in the intensive care nursery. *Am. J. Health Syst. Pharm.* **2003**, *60*, 193–194. [CrossRef] [PubMed]
18. Knudsen, L.; Eisend, S.; Haake, N.; Kunze, T. Physicochemical compatibility of commonly used analgesics and sedatives in the intensive care medicine. *Eur. J. Hosp. Pharm.* **2014**, *21*, 161–166. [CrossRef]
19. Koller, A.K.; Krebs, S.; Dorje, F. Medication Safety in Intravenous Therapy: A Compatibility Study of Clonidine with Drugs Frequently Used in Intensive Care. *Pharmaceutics* **2020**, *13*, 21. [CrossRef]
20. Johnson, C.E.; Bhatt-Mehta, V.; Mancari, S.C.; McKown, J.A. Stability of midazolam hydrochloride and morphine sulfate during simulated intravenous coadministration. *Am. J. Hosp. Pharm.* **1994**, *51*, 2812–2815. [CrossRef]
21. United States Pharmacopeia and National Formulary. General chapters <1790> Visual Inspection of Injections. In *United States Pharmacopeial Convention*; USP 40–NF 35; USP-NF: North Bethesda, MD, USA, 2017.
22. Chandler, S.W.; Trissel, L.A.; Weinstein, S.M. Combined administration of opioids with selected drugs to manage pain and other cancer symptoms: Initial safety screening for compatibility. *J. Pain Symptom Manag.* **1996**, *12*, 168–171. [CrossRef]
23. Kanji, S.; Lam, J.; Johanson, C.; Singh, A.; Goddard, R.; Fairbairn, J.; Lloyd, T.; Monsour, D.; Kakal, J. Systematic review of physical and chemical compatibility of commonly used medications administered by continuous infusion in intensive care units. *Crit. Care Med.* **2010**, *38*, 1890–1898. [CrossRef]
24. Staven, V.; Iqbal, H.; Wang, S.; Grønlie, I.; Tho, I. Physical compatibility of total parenteral nutrition and drugs in Y-site administration to children from neonates to adolescents. *J. Pharm. Pharmacol.* **2017**, *69*, 448–462. [CrossRef] [PubMed]
25. Humbert-Delaloye, V.; Berger-Gryllaki, M.; Voirol, P.; Testa, B.; Pannatier, A. Screening for physicochemical incompatibilities of intravenous drugs in intensive care units: The case of monobasic potassium phosphate and furosemide. *Eur. J. Hosp. Pharm.* **2015**, *22*, 56–58. [CrossRef]

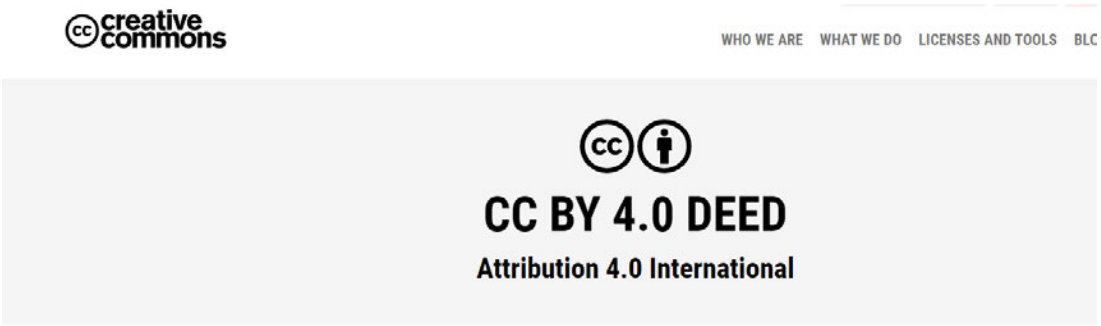
26. Semark, A.J.; Venkatesh, K.; McWhinney, B.C.; Pretorius, C.; Roberts, J.A.; Cohen, J.; Venkatesh, B. The compatibility of a low concentration of hydrocortisone sodium succinate with selected drugs during a simulated Y-site administration. *Crit. Care Resusc.* **2013**, *15*, 63–66. [CrossRef]
27. Baririan, N.; Chanteux, H.; Viaene, E.; Servais, H.; Tulkens, P.M. Stability and compatibility study of cefepime in comparison with ceftazidime for potential administration by continuous infusion under conditions pertinent to ambulatory treatment of cystic fibrosis patients and to administration in intensive care units. *J. Antimicrob. Chemother.* **2003**, *51*, 651–658. [CrossRef] [PubMed]
28. Servais, H.; Tulkens, P.M. Stability and compatibility of ceftazidime administered by continuous infusion to intensive care patients. *Antimicrob. Agents Chemother.* **2001**, *45*, 2643–2647. [CrossRef]
29. Inagaki, K.; Takagi, J.; Lor, E.; Lee, K.J.; Nii, L.; Gill, M.A. Stability of fluconazole in commonly used intravenous antibiotic solutions. *Am. J. Hosp. Pharm.* **1993**, *50*, 1206–1208. [CrossRef] [PubMed]
30. Senarathna, S.M.D.K.G.; Strunk, T.; Petrovski, M.; Batty, K.T. Physical compatibility of pentoxifylline and intravenous medications. *Arch. Dis. Child.* **2019**, *104*, 292–295. [CrossRef]
31. Allen, L.V., Jr.; Stiles, M.L. Compatibility of various admixtures with secondary additives at Y-injection sites of intravenous administration sets. Part 2. *Am. J. Hosp. Pharm.* **1981**, *38*, 380–381. [CrossRef]
32. Bashaw, E.D.; Amantea, M.A.; Minor, J.R.; Gallelli, J.F. Visual compatibility of zidovudine with other injectable drugs during simulated Y-site administration. *Am. J. Hosp. Pharm.* **1988**, *45*, 2532–2533. [CrossRef]
33. Benedict, M.K.; Roche, V.F.; Banakar, U.V.; Hilleman, D.E. Visual compatibility of amiodarone hydrochloride with various antimicrobial agents during simulated Y-site injection. *Am. J. Hosp. Pharm.* **1988**, *45*, 1117–1118. [CrossRef]
34. Chiu, M.F.; Schwartz, M.L. Visual compatibility of injectable drugs used in the intensive care unit. *Am. J. Health Syst. Pharm.* **1997**, *54*, 64–65. [CrossRef] [PubMed]
35. De Basagoiti, A.; Katsumiti, A.; Abascal, S.; Bustinza, A.; Lopez-Gimenez, L.R.; Pascual, P.; De Miguel, M.; Campino, A. Physical compatibility of alprostadil with selected drugs commonly used in the neonatal intensive care units. *Eur. J. Pediatr.* **2021**, *180*, 1169–1176. [CrossRef] [PubMed]
36. Foushee, J.A.; Meredith, P.; Fox, L.M.; Wilder, A.G. Y-site physical compatibility of hydrocortisone continuous infusions with admixtures used in critically ill patients. *Am. J. Health Syst. Pharm.* **2020**, *77*, 1144–1148. [CrossRef]
37. Kirupakaran, K.; Mahoney, L.; Rabe, H.; Patel, B.A. Understanding the Stability of Dopamine and Dobutamine Over 24 h in Simulated Neonatal Ward Conditions. *Pediatr. Drugs* **2017**, *19*, 487–495. [CrossRef] [PubMed]

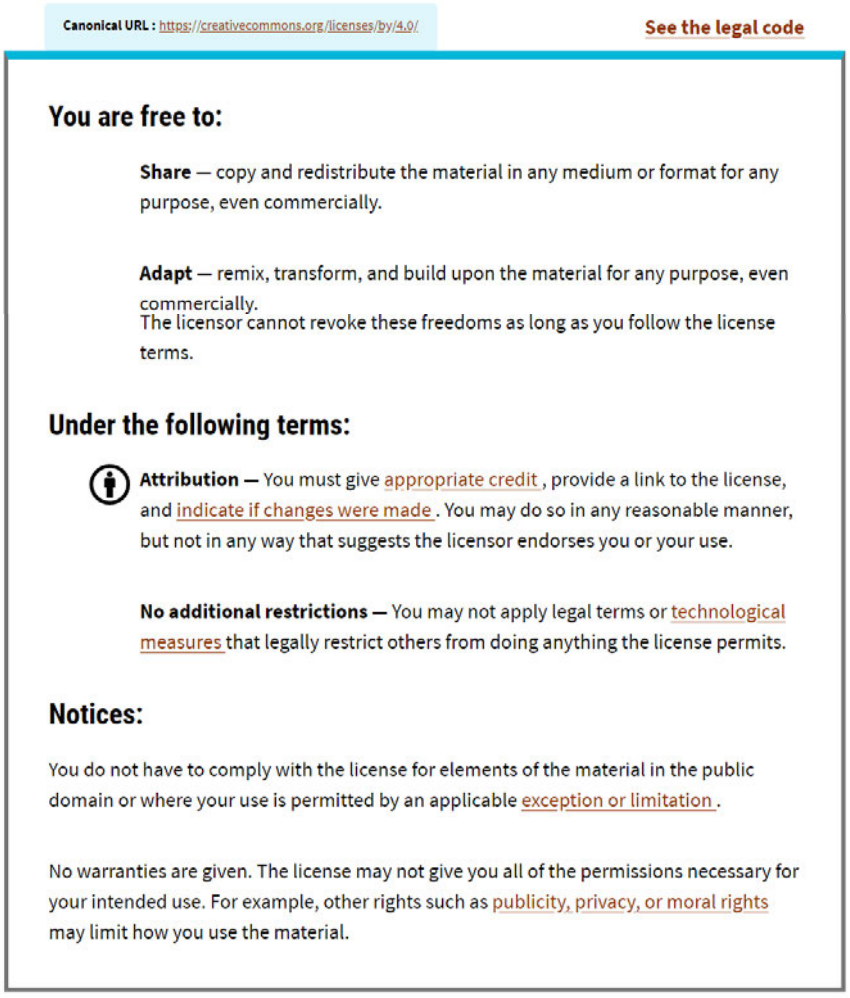
Paper 3 – Publication waiver, attribution statement and paper

As per communication with the publishing journal, European Journal of Clinical Pharmacology, Springer Nature provides waiver for inclusion of published material with a thesis or dissertation as per Springer Nature journal permissions advice and Creative Commons licence.

"Authors have the right to reuse their article's Version of Record, in whole or in part in their own thesis. Additionally, they may reproduce and make available their thesis, including Springer Nature content, as required by their awarding academic institution." (journalpermissions@springernature.com)

Full permission for inclusion of the published paper within this thesis has thereby been granted.





Attribution Statement

I, **D Thisuri N De Silva**, declare that I significantly contributed the following towards the published article "*Physicochemical compatibility of caffeine citrate and caffeine base injections with parenteral medications used in neonatal intensive care settings*".

- Conception and design
- Acquisition of data and method
- Data conditioning and manipulation
- Analysis and statistical method
- Interpretation and discussion
- Final approval

[redacted] 25/03/2024

I, as a co-author, endorse that the stated level of contribution by the candidate (as above) is appropriate.

Michael Petrovski	[redacted]
Tobias Strunk	[redacted]
Nabeela Mukadam	[redacted]
Madhu Page-Sharp	[redacted]
Brioni R. Moore	[redacted]
Kevin T. Batty	[redacted]

Research article: Physicochemical compatibility of caffeine citrate and caffeine base injections with parenteral medications used in neonatal intensive care settings.

D Thisuri N De Silva[1], Michael Petrovski[2], Tobias Strunk[3,4,5], Nabeelah Mukadam[6], Madhu Page-Sharp[1], Brioni Moore[1,3,4,7], Kevin T Batty[1,7]

[1] Curtin Medical School, Curtin University, Perth, Western Australia, Australia.
[2] Pharmacy Department, Sir Charles Gardiner Hospital, North Metropolitan Health Service, Nedlands, Western Australia, Australia.
[3] Medical School, The University of Western Australia, Crawley, Western Australia, Australia.
[4] Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, Western Australia, Australia.
[5] Neonatal Directorate, King Edward Memorial Hospital, Child and Adolescent Health Service, Subiaco, Western Australia, Australia.
[6] Pharmacy Department, King Edward Memorial Hospital, Women and Newborn Health Service, Subiaco, Western Australia, Australia.
[7] Curtin Health Innovation Research Institute, Curtin University, Bentley 6102, Australia

	Conception and design	Acquisition of Data and Method	Data Conditioning and Manipulation	Analysis and Statistical Method	Interpretation and Discussion
Michael Petrovski	✓				✓
Co-author 1 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					
Tobias Strunk	✓				✓
Co-author 2 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: Signed:__ [redacted]					
Nabeelah Mukadam	✓				✓
Co-author 3 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					

Madhu Page-Sharp	✓				✓
Co-author 4 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					
Brioni R Moore	✓				✓
Co-author 5 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					
Kevin T Batty	✓	✓	✓		✓
Co-author 6 Acknowledgement: I acknowledge that these represent my contribution to the above research output and I have approved the final version. Signed: [redacted]					

European Journal of Clinical Pharmacology (2024) 80:1079–1087
https://doi.org/10.1007/s00228-024-03678-6

RESEARCH

Physicochemical compatibility of caffeine citrate and caffeine base injections with parenteral medications used in neonatal intensive care settings

D. Thisuri N. De Silva[1] · Michael Petrovski[2] · Tobias Strunk[3,4,5] · Nabeelah Mukadam[6] · Madhu Page-Sharp[1] · Brioni R. Moore[1,3,4,7] · Kevin T. Batty[1,7]

Received: 18 February 2024 / Accepted: 19 March 2024 / Published online: 28 March 2024

Abstract

Purpose To investigate the physicochemical compatibility of caffeine citrate and caffeine base injections with 43 secondary intravenous (IV) drugs used in Neonatal Intensive Care Unit (NICU) settings.

Methods Caffeine citrate (20 mg/mL or 10 mg/mL) or caffeine base injection (10 mg/mL) were mixed in a volume ratio of 1:1 with the secondary drug solution to simulate Y-site co-administration procedures in NICUs. Physical compatibility was evaluated based on visual observation for 2 h, against a black and white background and under polarised light, for changes in colour, precipitation, haze and evolution of gas. Chemical compatibility was determined from caffeine concentration measurements, using a validated high-performance liquid chromatography assay.

Results Six of the 43 secondary drugs tested (aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine) were physically incompatible with caffeine citrate undiluted injection (20 mg/mL), at their high-end, clinically relevant concentrations for NICU settings. However, when tested at lower concentrations, hydrocortisone (1 mg/mL) was physicochemically compatible, whereas furosemide (0.2 mg/mL) was physically incompatible with caffeine citrate. The six drugs which showed physical incompatibility with caffeine citrate 20 mg/mL injection were also physically incompatible with caffeine citrate 10 mg/mL solution. All 43 secondary drugs tested were physicochemically compatible with caffeine base injection.

Conclusions Most secondary test drugs, except aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine, were physicochemically compatible with caffeine citrate injection. Caffeine base injection was physicochemically compatible with all 43 test drugs tested.

Keywords Caffeine citrate · Caffeine base · Physical compatibility · Chemical compatibility · Neonates

Introduction

Caffeine is a respiratory stimulant used to treat apnoea of prematurity in neonates [1, 2]. The benefits of caffeine include a reduction in both the frequency of apnoea events and the requirement for mechanical ventilation in premature neonates [3–6]. Caffeine is also known to offer advantages over other medications used for apnoea (e.g. theophylline), including fewer serum concentration measurements (due to wider therapeutic index) and less frequent dosing (due to long elimination half-life) [7, 8].

✉ Kevin T. Batty
Kevin.Batty@curtin.edu.au

1 Curtin Medical School, Curtin University, Bentley, WA, Australia

2 Pharmacy Department, Sir Charles Gardiner Hospital, North Metropolitan Health Service, Nedlands, WA, Australia

3 Medical School, The University of Western Australia, Crawley, WA, Australia

4 Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, WA, Australia

5 Neonatal Directorate, King Edward Memorial Hospital, Child and Adolescent Health Service, Subiaco, WA, Australia

6 Pharmacy Department, King Edward Memorial Hospital, Women and Newborn Health Service, Subiaco, WA, Australia

7 Curtin Health Innovation Research Institute, Curtin University, Bentley, WA, Australia

In accordance with international treatment guidelines [9], the intravenous (IV) dosage regimen for caffeine (expressed as caffeine base) in neonates comprises a loading dose of 20 mg/kg (once only) and a maintenance dose of 5 to 7.5 mg/kg once daily (maximum 10 mg/kg/day) commencing 24 h after the loading dose. For typical loading doses, a caffeine concentration of 10 mg/mL (undiluted product) is administered by IV infusion over 30 min and for the maintenance dose, 5 mg/mL caffeine injection is infused over 10 min [10].

In neonatal intensive care unit (NICU) settings, multiple IV medications are often co-administered at high concentrations and low flow rates via Y-site (three-way) connectors [11, 12]. As these drugs are mixed in the IV tubing, physicochemical compatibility of co-administered drugs is an important consideration to avoid adverse clinical outcomes [12–14].

Compatibility information for caffeine citrate is mostly related to visually observable physical changes [15, 16]. Chemical compatibility for caffeine citrate is limited to very few drugs, reported in compendia from the manufacturer's product information, including dopamine, fentanyl, heparin and calcium gluconate [17]. Stability data for caffeine base injection with parenteral nutrition (PN) solutions, IV fluids and admixtures have been reported [18]; however, there is a paucity of comprehensive physicochemical compatibility studies of caffeine base injection with other IV drugs. Caffeine base injection (10 mg/mL) is generally not commercially available, and this product is typically prepared by pharmaceutical compounding facilities as an isotonic formulation with a pH similar to caffeine citrate injection.

Our objective was to investigate the physicochemical compatibility of caffeine citrate and caffeine base injection with a range of NICU drugs, at higher-end, clinically relevant concentrations and with selected 2-in-1 PN solutions.

Materials and methods

Caffeine ($C_8H_{10}N_4O_2$; MW 194.2; certified reference material) was purchased from Sigma-Aldrich Chemicals, St Louis, MO, USA. High-performance liquid chromatography (HPLC) grade acetonitrile was from Fisher Scientific, Fair Lawn, NJ, USA. All other laboratory chemicals were of analytical grade.

Caffeine citrate injection (20 mg/mL; equivalent to 10 mg/mL of caffeine base; Phebra Pty Ltd, Australia) and caffeine base injection (10 mg/mL; Perth Children's Hospital, Australia) were tested against 43 secondary drugs and six 2-in-1 PN solutions, all of clinical grade (see Online Resource 1 for the list of drug manufacturers and composition of the PN solutions — Tables S1 and S2). Secondary drugs were prepared as per local NICU drug administration guidelines [10], using preferred diluents. Drug concentrations were based on the standard IV infusions for a patient weighing 2 kg.

The stability-indicating, HPLC assay method developed by Oliphant and colleagues [19] was modified and validated in accordance with the International Council for Harmonization (ICH) guidelines [20], for the determination of caffeine concentration in the present study (see Online Resource 1, Section 2, for details).

Preparation of samples for physicochemical compatibility testing

Caffeine citrate and caffeine base injections were initially used undiluted (20 and 10 mg/mL concentrations respectively). Secondary test drugs and 2-in-1 PN solutions were prepared/diluted in accordance with the manufacturer's instructions or standard neonatal clinical protocols [10]. Medications originally contained in glass ampoules and medications requiring reconstitution were filtered with a 0.22-µm syringe filter, before mixing (33 mm × 0.22 µm Polyethersulfone membrane, Millex-GP, Merk Millipore Ltd, Carrigtwohill, Co. Cork, Ireland).

A total of 43 drugs and 6 PN solutions were selected and endorsed by local clinical experts. These included drugs which were previously tested for physical compatibility, as compatible/incompatible controls.

Drug combinations were mixed at 1:1 volume ratio, to simulate Y-site administration, consistent with previously reported methods [15, 16, 21–24]. Drug preparation, mixing and testing were carried out at room temperature (22 °C).

The first stage of compatibility testing comprised a combination of caffeine citrate 20 mg/mL and caffeine base injection 10 mg/mL (separately) with the secondary drug at clinically relevant 'high-end' concentrations consistent with NICU protocols and expert advice. If incompatibility was detected, the secondary drug was then tested using caffeine citrate 10 mg/mL solution (diluted in water for injection), which is the recommended concentration for maintenance doses of caffeine [10]. If this combination also was incompatible, the next set of testing comprised caffeine citrate 20 mg/mL with the secondary drug at its 'low-end' concentration (if clinically applicable). Finally, the 'lower-end' caffeine concentration (caffeine citrate 10 mg/mL) was tested with the secondary drug 'lower-end' concentration, if previous results indicated this could be relevant.

Clear glass HPLC vials (2 mL) with impermeable screw cap lids were used for each binary combination of drugs/fluids and the respective control solutions. Initially, caffeine citrate and secondary drug combinations, and the control samples, were prepared as described below.

Set 1 — Caffeine citrate injection solution (0.4 mL of 20 mg/mL) and secondary test drug solution (0.4 mL); $n=3$.
Set 2 — Caffeine citrate injection solution (0.4 mL of 20 mg/mL) diluted with 0.4 mL of the diluent of the secondary test drug ($n=3$) as the reference control solution for the purpose of visual comparison and HPLC assay of caffeine concentration.
Set 3 — The test drug solution (0.4 mL) diluted with 0.4 mL of water for injection ($n=3$) for the purpose of visual comparison.

The same experimental procedure was followed for caffeine base injection (10 mg/mL) and conducted as a parallel experiment.

Physical compatibility testing

All combinations were observed with an unaided eye against a black and white background for any change in colour, haze, precipitation and evolution of gas. The observations were carried out at time 0 (immediately after mixing), 5, 15, 60 and 120 min after mixing. Further, at time 0 and after 120 min, the samples were observed under a polarised light viewer (Apollo I Liquid Viewer with a LED light source and 1.7 × Magnifier, Adelphi Manufacturing Company Ltd, West Sussex, UK) for any precipitation or particulate matter.

Physical incompatibility was based on the visual appearance in comparison to control solutions (sets 2 and 3). Inconclusive observations were confirmed by a second independent observer and all physically incompatible combinations were photographed. If precipitation or particles were observed in the drug combination vials, an aliquot was examined under light microscopy (Leica MC190HD, 40 × magnification, Leica Microsystems Ltd, Heerbrugg, Switzerland).

Chemical compatibility testing

If any physical incompatibility was observed (e.g. precipitate), the combinations were not subject to chemical compatibility testing, to avoid contamination of the HPLC system. Samples from sets 1 and 2 were analysed by HPLC after 2 h of observation. The ratio of the mean peak areas was determined, and the 95% confidence interval (CI) of the ratio was calculated using the confidence limits from a two-sided t-test ($\alpha=0.05$; SigmaPlot V.15; Inpixon GmbH, Düsseldorf, Germany). Consistent with previous studies, incompatibility of caffeine to drug combinations was defined as a ratio of the mean peak area outside the range of 90–110% [25–28].

Results

Six of the 43 secondary drugs tested (aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine) were physically incompatible with caffeine citrate undiluted injection, at their 'high-end' clinically relevant concentrations (Table 1). Two of the incompatible drugs were also tested at 'low-end' clinically relevant concentrations: hydrocortisone (1 mg/mL) was physicochemically compatible with caffeine citrate; however, furosemide (0.2 mg/mL) was physically incompatible (Table 1). All of the drugs which showed physical incompatibility with caffeine citrate undiluted injection were also physically incompatible with caffeine citrate 10 mg/mL solution (Table 2).

Most of the physical incompatibilities were visible to the unaided eye (Online Resource 1 for photographs, Figs. S3–S8), except the combinations with furosemide 0.2 mg/mL, which required observation under polarised light. As amphotericin (liposomal) was originally a pale-yellow hazy mixture, the incompatibility observed was an increase in the opacity in comparison to the control mixtures (Online Resource1; Fig. S4).

Further investigation of the incompatibility findings was conducted by mixing the six secondary drugs (separately, as described in the "Preparation of samples for physicochemical compatibility testing" section) with citrate buffer pH 4.5 (citric acid monohydrate 5 mg/mL and sodium citrate dihydrate 8.3 mg/mL in water). The same physical incompatibility characteristics (precipitation/haze) were observed with all six secondary drugs (Online Resource 1; Fig. S9), therefore indicating the citrate buffer was the cause of the incompatibility with caffeine citrate injection.

In contrast to the caffeine citrate data, all 43 secondary drugs and 6 PN solutions tested were physicochemically compatible with caffeine base injection (Table 3).

To complement the above results, the osmolality of the caffeine citrate 20 mg/mL and caffeine base 10 mg/mL injections was tested and found to be 142 and 269 mOsm/kg, respectively (Osmomat 030 Cryoscopic Osmometer; Gonotec GmbH, Berlin, Germany). By comparison, a recent report indicated that caffeine citrate 20 mg/mL oral solution had an osmolality of 150 mOsm/kg [29].

Discussion

The present study has shown that 37 IV drugs tested in a simulated Y-site study design at 'high-end', clinically relevant concentrations for NICU settings were physically and chemically compatible with caffeine citrate 20 mg/mL

Table 1 Physicochemical compatibility of caffeine citrate 20 mg/mL (10 mg/mL caffeine base) with secondary drugs 2-in-1 parenteral nutrition solutions (see Table S2 for details)

Secondary drug	Test concentration	Diluent	PC	CAF ratio	95% CI of ratio
Aciclovir	5 mg/mL	D5W	I[a]	-	-
Alprostadil	20 mcg/mL	NS	C	99.9	98.1–101.7
Amoxicillin	100 mg/mL	WFI	C	99.9	99.1–100.8
Amphotericin (Fungizone)	100 mcg/mL	D5W	C	98.8	97.1–100.6
Amphotericin liposomal	2 mg/mL	D5W	I[b]	-	-
Ampicillin	100 mg/mL	WFI	C	99.8	98.9–100.7
Benzylpenicillin	100 mg/mL	WFI	C	100.6	99.4–101.7
Calcium gluconate	100 mg/mL	U	C	99.5	98.2–100.9
Cefotaxime	100 mg/mL	WFI	C	100.2	99.3–101.2
Ciprofloxacin*	2 mg/mL	U	C	100.2	99.5–100.9
Clonidine	2 mcg/mL	NS	C	99.6	98.2–101.0
Cloxacillin	100 mg/mL	WFI	C	100.1	98.2–101.9
Dobutamine	7.2 mg/mL	NS	C	100.1	99.3–100.9
Dobutamine	7.2 mg/mL	D5W	C	100.0	98.8–101.3
Dopamine	7.2 mg/mL	NS	C	100.0	99.4–100.6
Dopamine	7.2 mg/mL	D5W	C	100.6	99.3–101.8
Dexmedetomidine	1 mcg/mL	NS	C	99.7	98.4–101.1
Epinephrine	64 mcg/mL	D5W	C	100.1	99.5–100.7
Fentanyl	50 mcg/mL	U	C	99.8	98.7–101.0
Flucloxacillin	50 mg/mL	D5W	C	99.2	96.9–101.5
Fluconazole	2 mg/mL	U	C	99.5	98.6–100.5
Furosemide	1 mg/mL	D5W	I[a]	-	-
Furosemide	0.2 mg/mL	D5W	I[c]	-	-
Gentamicin	10 mg/mL	NS	C	99.6	99.1–100.1
Heparin	100 units/mL	NS	C	99.7	98.5–100.8
Hydrocortisone	10 mg/mL	NS	I[a]	-	-
Hydrocortisone	1 mg/mL	NS	C	99.6	97.5–101.7
Indometacin	200 mcg/mL	NS	C	99.5	98.7–100.3
Ibuprofen	5 mg/mL	NS	I[d]	-	-
Ibuprofen lysine	4 mg/mL	NS	I[d]	-	-
Insulin	0.2 units/mL	NS	C	99.7	98.8–100.6
Levetiracetam	5 mg/mL	NS	C	99.5	99.0–100.0
Linezolid	2 mg/mL	U	C	99.9	98.8–100.9
Meropenem	50 mg/mL	NS	C	98.9	97.1–100.8
Metronidazole	5 mg/mL	U	C	99.9	98.9–101.0
Midazolam	1 mg/mL	U	C	99.5	97.8–101.3
Milrinone	400 mcg/mL	D5W	C	100.2	99.7–100.8
Morphine hydrochloride	200 mcg/mL	D5W	C	99.8	98.4–101.2
Morphine sulfate	200 mcg/mL	D5W	C	100.6	99.5–101.8
Norepinephrine	64 mcg/mL	D5W	C	100.3	99.7–101.0
Paracetamol	10 mg/mL	U	C	100.2	99.3–101.1
Phenobarbitone	20 mg/mL	WFI	C	99.8	99.0–100.6
Piperacillin/tazobactam	200 mg/mL	WFI	C	99.8	98.8–100.7
Rifampicin	6 mg/mL	NS	C	100.4	98.6–102.1
Sodium bicarbonate	4.2% w/v	D5W	C	99.4	98.9–99.8
Vancomycin	10 mg/mL	D5W	C	99.3	97.8–100.8
Vecuronium	1 mg/mL	WFI	C	99.7	99.4–100.1
Parenteral nutrition PN 1	-	-	C	99.2	97.7–100.8
Parenteral nutrition PN 2	-	-	C	100.5	99.1–102.0
Parenteral nutrition PN 3	-	-	C	99.6	98.9–100.2
Parenteral nutrition PN 4	-	-	C	99.9	98.9–101.0

Table 1 (continued)

Secondary drug	Test concentration	Diluent	PC	CAF ratio	95% CI of ratio
Parenteral nutrition PN 5	-	-	C	100.5	99.4–101.6
Parenteral nutrition PN 6	-	-	C	99.7	98.8–100.6

PC physical compatibility, *CAF* caffeine, *C* compatible, *I* incompatible, *D5W* glucose 5%, *WFI* water for injection, *NS* normal saline/ 0.9% sodium chloride, *U* undiluted

*Ciprofloxacin was also tested at 4 h to obtain a caffeine ratio of 99.6% and a 95% CI of ratio 98.1–101.1%

[a]A white precipitate appeared 10–15 min after mixing

[b]A higher opacity observed in the combination samples in comparison to controls

[c]Particles observed under polarised light after 30 min of mixing

[d]A milky turbidity appeared immediately after mixing

injection (Table 1). The apparent cause of the incompatibility of caffeine citrate injection with aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine injections was found to be the citrate buffer component. By comparison, all 43 drugs were compatible with caffeine base 10 mg/mL injection (Table 3). Caffeine citrate and base injections were also compatible with six 2-in-1 parenteral nutrition solutions.

Although physical compatibility information for caffeine citrate with a range of IV drugs has been reported, a modest compilation of chemical compatibility data from manufacturers' information (for dopamine, fentanyl, heparin and calcium gluconate) is available in contemporary guidelines [17]. Consistent with these data, our study demonstrated physicochemical compatibility of caffeine citrate injection with calcium gluconate, dopamine, fentanyl and heparin, albeit at different concentrations and/or experimental conditions. For example, a mixture of caffeine citrate 20 mg/mL and calcium gluconate 100 mg/mL was previously found to be physically compatible for 4 [16] and 24 h [17] at room temperature, and chemically stable for 24 h at room temperature [17]. These findings provide useful confirmation of our results that caffeine citrate and calcium gluconate injections were physicochemically compatible for 2 h at room temperature.

Table 2 Physicochemical compatibility of caffeine citrate 10 mg/mL (5 mg/mL caffeine base) with secondary drugs

Secondary drug	Test concentration	Diluent	PC
Aciclovir	5 mg/mL	D5W	I[a]
Amphotericin liposomal	2 mg/mL	D5W	I[b]
Furosemide	1 mg/mL	D5W	I[a]
Furosemide	0.2 mg/mL	D5W	I[c]
Hydrocortisone	10 mg/mL	NS	I[a]
Ibuprofen	5 mg/mL	NS	I[d]
Ibuprofen lysine	4 mg/mL	NS	I[d]

PC physical compatibility, *I* incompatible, *D5W* glucose 5% w/v, *NS* normal saline (sodium chloride 0.9% w/v)

[a]A white precipitate appeared 10–15 min after mixing

[b]A higher opacity observed in the combination samples in comparison to controls

[c]Particles observed under polarised light after 30 min of mixing

[d]A milky turbidity appeared immediately after mixing

Heparin has previously been investigated at 1 unit/mL (in glucose 5% w/v; D5W), 10 units/mL and 1000 units/mL in combination with caffeine citrate and shown to be physically compatible [15–17]. The present study complements these reports by demonstrating that heparin 100 units/mL was physicochemically compatible with caffeine citrate, for 2 h at room temperature (Table 1).

Fentanyl 10 mcg/mL (in D5W) was reported to be compatible and stable with caffeine citrate for 24 h at room temperature [17], and two studies have confirmed that fentanyl 50 mcg/mL was physically compatible for 4 h at room temperature [15, 16]. Furthermore, meropenem 50 mg/mL was recently found to be physically compatible with caffeine citrate injection for 4 h [30]. Hence, these results also are complemented by the present study, whereby fentanyl 50 mcg/mL and meropenem 50 mg/mL (separately) were found to be physically and chemically compatible with caffeine citrate injection (Table 1).

The present study also provides evidence of incompatibility between caffeine citrate injection (10 mg/mL and 20 mg/mL) and both ibuprofen (5 mg/mL) and ibuprofen lysine (4 mg/mL), the combinations of which resulted in turbidity immediately after mixing (Figs. S7 and S8). Although ibuprofen has not been studied previously for physicochemical compatibility, ibuprofen lysine 20 mg/mL was reported to be physically incompatible due to milky white precipitation upon mixing [31].

A range of inconsistent caffeine citrate compatibility data have been reported, some of which may be concentration-dependent or related to the experimental procedures (e.g. duration of admixture or physical methods used to determine compatibility), or the composition of the IV drug formulation [16]. For example, dopamine 0.6 mg/mL (in D5W) was reported to be compatible and stable with caffeine citrate for 24 h at room temperature [17], and a higher concentration (80 mg/mL) was found to be visually compatible for

Table 3 Physicochemical compatibility of caffeine base injection 10 mg/mL with secondary drugs 2-in-1 parenteral nutrition solutions (see Table S2 for details)

Secondary drug	Test concentration	Diluent	PC	CAF ratio	95% CI of ratio
Aciclovir	5 mg/mL	D5W	C	99.9	98.4–101.4
Alprostadil	20 mcg/mL	NS	C	99.4	97.8–101.1
Amoxicillin	100 mg/mL	WFI	C	100.3	99.6–100.9
Amphotericin (Fungizone)	100 mcg/mL	D5W	C	101.2	98.9–103.5
Amphotericin liposomal	2 mg/mL	D5W	C	100.2	98.8–101.6
Ampicillin	100 mg/mL	WFI	C	100.3	98.5–102.1
Benzylpenicillin	100 mg/mL	WFI	C	100.2	98.7–101.6
Calcium gluconate	100 mg/mL	U	C	100.3	99.1–101.5
Cefotaxime	100 mg/mL	WFI	C	99.4	96.9–101.8
Ciprofloxacin	2 mg/mL	U	C	99.7	99.1–100.3
Clonidine	2 mcg/mL	NS	C	99.6	98.2–101.1
Cloxacillin	100 mg/mL	WFI	C	100.7	99.6–101.7
Dobutamine	7.2 mg/mL	NS	C	100.8	98.4–103.2
Dobutamine	7.2 mg/mL	D5W	C	100.0	99.2–100.8
Dopamine	7.2 mg/mL	NS	C	100.6	99.6–101.5
Dopamine	7.2 mg/mL	D5W	C	100.7	99.3–102.1
Dexmedetomidine	1 mcg/mL	NS	C	100.9	99.8–102.0
Epinephrine	64 mcg/mL	D5W	C	99.9	99.3–100.4
Fentanyl	50 mcg/mL	U	C	100.0	98.7–101.4
Flucloxacillin	50 mg/mL	D5W	C	99.8	98.4–101.3
Fluconazole	2 mg/mL	U	C	99.6	98.6–100.6
Furosemide	1 mg/mL	D5W	C	100.4	99.2–101.7
Gentamicin	10 mg/mL	NS	C	99.8	98.9–100.7
Heparin	100 units/mL	NS	C	100.1	99.4–100.8
Hydrocortisone	10 mg/mL	NS	C	100.6	99.2–101.9
Indometacin	200 mcg/mL	NS	C	100.4	99.6–101.2
Ibuprofen	5 mg/mL	NS	C	99.8	98.5–101.2
Ibuprofen lysine	4 mg/mL	NS	C	99.9	98.7–101.0
Insulin	0.2 units/mL	NS	C	101.2	98.3–104.2
Levetiracetam	5 mg/mL	NS	C	101.1	100.2–102.0
Linezolid	2 mg/mL	U	C	100.4	97.8–103.0
Meropenem	50 mg/mL	NS	C	99.8	98.8–100.7
Metronidazole	5 mg/mL	U	C	100.4	99.5–101.3
Midazolam	1 mg/mL	U	C	99.5	98.8–100.2
Milrinone	400 mcg/mL	D5W	C	99.4	98.2–100.5
Morphine hydrochloride	200 mcg/mL	D5W	C	99.9	98.8–101.0
Morphine sulfate	200 mcg/mL	D5W	C	99.6	98.3–101.0
Norepinephrine	64 mcg/mL	D5W	C	99.7	99.1–100.4
Paracetamol	10 mg/mL	U	C	100.0	99.6–100.5
Phenobarbitone	20 mg/mL	WFI	C	100.5	98.9–102.0
Piperacillin/tazobactam	200 mg/mL	WFI	C	100.0	99.0–101.0
Rifampicin	6 mg/mL	NS	C	101.4	99.1–103.6
Sodium bicarbonate	4.2% w/v	D5W	C	99.3	98.4–100.3
Vancomycin	10 mg/mL	D5W	C	99.9	98.1–101.6
Vecuronium	1 mg/mL	WFI	C	100.3	98.6–102.0
Parenteral nutrition PN 1	-	-	C	100.1	99.1–101.1
Parenteral nutrition PN 2	-	-	C	100.3	98.2–102.4
Parenteral nutrition PN 3	-	-	C	99.3	98.0–100.7
Parenteral nutrition PN 4	-	-	C	99.7	98.2–101.2
Parenteral nutrition PN 5	-	-	C	100.6	99.3–101.9
Parenteral nutrition PN 6	-	-	C	99.3	97.6–101.1

PC physical compatibility, *CAF* caffeine, *C* compatible, *D5W* glucose 5% w/v, *WFI* water for injection, *NS* normal saline (sodium chloride 0.9% w/v), *U* undiluted

4 h at 25 °C [15]. By contrast, Audet and colleagues [16] reported that dopamine 3.2 mg/mL was physically incompatible with caffeine citrate, due to a 'yellowish tint' colour change immediately after mixing. However, in the present study, dopamine 7.2 mg/mL (in both D5W and 0.9% sodium chloride; NS) was physically and chemically compatible with caffeine citrate for 2 h after mixing (Table 1). Furthermore, for direct comparison with the previous report [16], we investigated the combinations of caffeine citrate 20 mg/mL injection with dopamine 3.2 and 1.2 mg/mL (in NS) and found no evidence of physicochemical incompatibility (physically compatible with no observed colour change and caffeine ratios of 99.4% and 99.1%, respectively).

Conflicting data regarding the compatibility of caffeine citrate with furosemide 10 mg/mL and aciclovir 50 mg/mL (separately) also have been reported, with one study finding the combinations were physically compatible [16], and an earlier study indicating they were physically incompatible, due to immediate precipitation [15]. By comparison, the present study has shown that lower, clinically relevant concentrations of these drugs (furosemide 1 and 0.2 mg/mL, and aciclovir 5 mg/mL) were physically incompatible with caffeine citrate, as the combinations produced a white precipitate within 15 min of mixing (Table 1 and Figs. S3 and S5). These results may indicate concentration-dependent physical incompatibility for mixtures of caffeine citrate and furosemide or aciclovir, which could be evaluated in clinical settings, based on the presence/absence of a visible white precipitate.

In regard to amphotericin (liposomal) and hydrocortisone, at concentrations of 4 mg/mL and 250 mg/mL respectively, Audet et al. [16] found these two drugs were physically compatible with caffeine citrate for 4 h at room temperature. By contrast, results in the present study showed that amphotericin (liposomal) and hydrocortisone, at lower clinically relevant NICU concentrations (2 mg/mL and 10 mg/mL respectively), were physically incompatible with caffeine citrate at 10 mg/mL and 20 mg/mL (Table 1 and Figs. S4 and S6). However, hydrocortisone at a concentration of only 1 mg/mL was physicochemically compatible with caffeine citrate 20 mg/mL (Table 1). This finding suggests the lower hydrocortisone IV infusion concentration (1 mg/mL) used in NICU settings may be safely co-administered with caffeine citrate through Y-sites, where required.

Audet et al. [16] also reported that midazolam 5 mg/mL was physically incompatible with caffeine citrate, due to the formation of a white precipitate at the time of mixing; however, our study showed that a lower concentration (1 mg/mL) was physicochemically compatible with caffeine citrate (Table 1).

Further contradictory studies regarding vancomycin 50 mg/mL or dobutamine 12.5 mg/mL mixed (separately) with caffeine citrate have reported the combinations to be physically compatible [15] and physically incompatible [16], resulting in white precipitate and colour change, respectively, at the time of mixing in the latter study. By comparison, we found that vancomycin and dobutamine, at the lower concentrations of 10 mg/mL and 7.2 mg/mL, respectively (in both D5W and NS), were physicochemically compatible with caffeine citrate 20 mg/mL (Table 1).

One directly conflicting result from the present study relates to the recent report that ciprofloxacin 2 mg/mL was physically incompatible with caffeine citrate 20 mg/mL due to crystal formation at 4 h after mixing [16]. By contrast, our data indicate the combination is physicochemically compatible for 2 h at the same concentrations. Hence, to clarify this discrepancy and formally compare our study with the previous report [16], we retested the combination after 4 h of mixing and confirmed its physicochemical compatibility in our laboratory, with no physical evidence of precipitate or crystal formation and a caffeine concentration ratio (by HPLC) of 99.6% (Table 1). As outlined above, similar inexplicable discrepancies are evident in specific studies [16] and compendia [17], and may require prudent clinical judgement to avoid adverse clinical outcomes.

Compared to the studies of caffeine citrate compatibility, there are no previous comprehensive physical or chemical compatibility studies of caffeine base injection with other IV drugs. However, the stability of caffeine base in a range of sodium chloride, potassium chloride and glucose IV solutions and PN fluids for up to 24 h has been reported [18]. The present investigation has shown that caffeine base injection was physicochemically compatible with all 43 secondary drugs and the six PN solutions tested (Table 3). Hence, in the absence of commercial preparations, a locally prepared caffeine base injection may be a useful alternative to caffeine citrate injection for Y-site co-administration with otherwise incompatible IV drugs.

One potential limitation of the present study was the well-established, fixed 1:1 mixing ratio of the two components for simulated Y-site compatibility studies [16, 21, 26, 32]. Recent reports have included other ratios (e.g. 1:4 or 1:10) to simulate extremes of high/low infusion rates of the individual components [27, 33–35]; however, in the NICU setting, the range of drug concentrations may be a more significant variable than the IV infusion rates. Nevertheless, contemporary IV compatibility study designs could include a balanced range of clinically relevant concentrations and mixing ratios, as appropriate. A further consideration in our study was the 2-h mixing duration, which is based on the typical contact time of two components in neonatal infusions (via Y-site mixing) being up to 1 h [36, 37], but accounts for potentially slower infusion rates that may occur in NICU settings [16]. Finally, some recent physical and physicochemical compatibility investigations have included turbidity and/or pH tests as part of the suite of physical tests [26, 28, 38]; however, due to resource implications for these tests, including the

large sample volumes (typically > 10 mL), turbidity and pH were not evaluated in the present study. Furthermore, recent reports have noted the intrinsic value, interpretation and specification limits of some physical compatibility tests are unclear or inconsistent [26, 28, 38, 39]. Hence, based on the range of well-accepted physical tests and validated HPLC assay for determination of chemical compatibility, we conclude the present study provides sufficiently robust evidence of physicochemical compatibility (or otherwise) for caffeine citrate and caffeine base injections in the context of simulated Y-site co-administration in NICU settings.

Conclusion

Most secondary test drugs and 2-in-1 PN solutions investigated in the present study, except aciclovir, amphotericin (liposomal), furosemide, hydrocortisone, ibuprofen and ibuprofen lysine, were physicochemically compatible with caffeine citrate injection (20 mg/mL). By comparison, caffeine base injection (10 mg/mL) was physicochemically compatible with all 43 test drugs and six PN solutions tested.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00228-024-03678-6.

Acknowledgements The authors gratefully acknowledge the assistance of Karen Donn, Stephanie Teoh, Caitlyn Byrne and Jack Lee (King Edward Memorial Hospital for Women), and Giuseppe Luna and Jorge Martinez (Curtin University). TDS was the recipient of a Sri Lankan AHEAD (Accelerating Higher Education Expansion and Development) program scholarship.

Author contribution TDS, MP, TS and KTB conceived the study, with advice from NM, MPS and BRM. All authors contributed to the study design. TDS and KTB had principal responsibility for acquiring the data. KTB and TDS conducted initial analysis and interpretation of the data, with advice from all authors. KTB and TDS prepared the first draft of the manuscript. Revision and additional contributions to the manuscript were provided by all authors. All authors approved the final manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions

Availability of data and material All data supporting the findings of this study are available within the paper or its Supplementary Information or are available on reasonable request to the authors.

Declarations

Ethics approval Not applicable as the study design involved no human or animal subjects.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Mathew OP (2011) Apnea of prematurity: pathogenesis and management strategies. J Perinatol 31:302–310. https://doi.org/10.1038/jp.2010.126
2. Bhatt-Mehta V, Schumacher RE (2003) Treatment of apnea of prematurity. Paediatr Drugs 5:195–210. https://doi.org/10.2165/00128072-200305030-00006
3. Bairam A, Boutroy MJ, Badonnel Y, Vert P (1987) Theophylline versus caffeine: comparative effects in treatment of idiopathic apnea in the preterm infant. J Pediatr 110:636–639. https://doi.org/10.1016/s0022-3476(87)80569-3
4. Brouard C, Moriette G, Murat I, Flouvat B, Pajot N, Walti H, de Gamarra E, Relier J-P (1985) Comparative efficacy of theophylline and caffeine in the treatment of idiopathic apnea in premature infants. Am J Dis Child 139:698–700. https://doi.org/10.1001/archpedi.1985.02140090060028
5. Erenberg A, Leff RD, Haack DG, Mosdell KW, Hicks GM, Wynne BA (2000) Caffeine citrate for the treatment of apnea of prematurity: a double-blind, placebo-controlled study. Pharmacotherapy 20:644–652. https://doi.org/10.1592/phco.20.7.644.35167
6. Moschino L, Zivanovic S, Hartley C, Trevisanuto D, Baraldi E, Roehr CC (2020) Caffeine in preterm infants: where are we in 2020? ERJ Open Res 6:00330–02019. https://doi.org/10.1183/23120541.00330-2019
7. Aranda JV, Cook CE, Gorman W, Collinge JM, Loughnan PM, Outerbridge EW, Aldridge A, Neims AH (1979) Pharmacokinetic profile of caffeine in the premature newborn infant with apnea. J Pediatr 94:663–668. https://doi.org/10.1016/s0022-3476(79)80047-5
8. Davis J, Spitzer A, Stefano J, Bhutani V, Fox W (1987) Use of caffeine in infants unresponsive to theophylline in apnea of prematurity. Pediatr Pulmonol 3:90–93. https://doi.org/10.1002/ppul.1950030210
9. Dobson NR, Hunt CE (2013) Pharmacology review: caffeine use in neonates: indications, pharmacokinetics, clinical effects, outcomes. NeoReviews 14:e540–e550. https://doi.org/10.1542/neo.14-11-e540
10. King Edward Memorial Hospital - Clinical Neonatal Medication Protocols. https://www.kemh.health.wa.gov.au/For-Health-Professionals/Clinical-Guidelines/Neonatal. Accessed 16 Jul 2023
11. Pettit J (2002) Assessment of infants with peripherally inserted central catheters: part 1. Detecting the most frequently occurring complications. Adv Neonatal Care 2:304–315. https://doi.org/10.1053/adnc.2002.36826
12. O'Brien F, Clapham D, Krysiak K, Batchelor H, Field P, Caivano G, Pertile M, Nunn A, Tuleu C (2019) Making medicines baby size: the

challenges in bridging the formulation gap in neonatal medicine. Int J Mol Sci 20:2688. https://doi.org/10.3390/ijms20112688
13. Sherwin CMT, Medlicott NJ, Reith DM, Broadbent RS (2014) Intravenous drug delivery in neonates: lessons learnt. Arch Dis Child 99:590–594. https://doi.org/10.1136/archdischild-2013-304887
14. Parikh MJ, Dumas G, Silvestri A, Bistrian BR, Driscoll DF (2005) Physical compatibility of neonatal total parenteral nutrient admixtures containing organic calcium and inorganic phosphate salts. Am J Health Syst Pharm 62:1177–1183. https://doi.org/10.1093/ajhp/62.11.1177
15. Mitchell A, Gailey R (1999) Compatibility of caffeine citrate with other medications commonly used in a neonatal intensive care unit. J Pediatr Pharm Pract 4:239–242
16. Audet M-A, Forest E, Friciu M, Forest J-M, Leclair G (2017) Compatibilité du citrate de caféine injectable avec plusieurs autres médicaments. Pharmactuel 50:27–33
17. American Society of Health-System Pharmacists (2022) ASHP injectable drug information: a comprehensive guide to compatibility and stability. American Society of Health-System Pharmacists: Bethesda (MD), pp 230–232
18. Nahata MC, Zingarelli JR, Durrell DE (1989) Stability of caffeine injection in intravenous admixtures and parenteral nutrition solutions. DICP 23:466–467. https://doi.org/10.1177/106002808902300606
19. Oliphant EA, Purohit TJ, Alsweiler JM, McKinlay CJ, Hanning SM (2022) Validation and application of a simple and rapid stability-indicating liquid chromatographic assay for the quantification of caffeine from human saliva. J Liq Chromatogr Relat Technol 45:10–17. https://doi.org/10.1080/10826076.2022.2095402
20. European Medicines Agency (1995) ICH Topic Q 2 (R1) Validation of analytical procedures: text and methodology. European Medicines Agency: London (UK)
21. Allen LV Jr, Levinson RS, Phisutsinthop D (1977) Compatibility of various admixtures with secondary additives at Y-injection sites of intravenous administration sets. Am J Hosp Pharm 34:939–943. https://doi.org/10.1093/ajhp/34.9.939
22. Akkerman SR, Zhang H, Mullins RE, Yaughn K (1999) Stability of milrinone lactate in the presence 29 critical care drugs and 4 i.v. solutions. Am J Health Syst Pharm 56:63–68. https://doi.org/10.1093/ajhp/56.1.63
23. Anderson C, Boehme S, Ouellette J, Stidham C, Mackay M (2014) Physical and chemical compatibility of injectable acetaminophen during simulated Y-site administration. Hosp Pharm 49:42–47. https://doi.org/10.1310/hpj4901-42
24. Bell MS, Nolt DH (2003) Visual compatibility of doxapram hydrochloride with drugs commonly administered via a Y-site in the intensive care nursery Am J Health Syst Pharm 60:193–194. https://doi.org/10.1093/ajhp/60.2.193
25. Luu Y, Thigpen J, Brown SD (2017) Stability of sildenafil in combination with heparin and dopamine. Am J Health Syst Pharm 74:e64–e71. https://doi.org/10.2146/ajhp150853
26. AlSalman F, Howlett M, Breatnach C, Kelly H, O'Brien F (2020) Supporting the use of sildenafil infusions in paediatric and neonatal intensive care – a compatibility study. Eur J Pharm Biopharm 151:153–161. https://doi.org/10.1016/j.ejpb.2020.04.008
27. Knudsen L, Eisend S, Haake N, Kunze T (2014) Physicochemical compatibility of commonly used analgesics and sedatives in the intensive care medicine. Eur J Hosp Pharm 21:161–166. https://doi.org/10.1136/ejhpharm-2014-000444
28. Koller AK, Krebs S, Dorje F (2020) Medication safety in intravenous therapy: a compatibility study of clonidine with drugs frequently used in intensive care. Pharmaceutics 13:21. https://doi.org/10.3390/pharmaceutics13010021
29. Latheef F, Wahlgren H, Lilja HE, Diderholm B, Paulsson M (2021) The risk of necrotizing enterocolitis following the administration of hyperosmolar enteral medications to extremely preterm infants. Neonatology 118:73–79. https://doi.org/10.1159/000513169
30. Lessard J-J, Caron E, Scherer H, Forest J-M, Leclair G (2020) Compatibility of Y-site injection of meropenem trihydrate with 101 other injectable drugs. Hosp Pharm 55:332–337. https://doi.org/10.1177/0018578719844168
31. Holt RJ, Siegert SWK, Krishna A (2008) Physical compatibility of ibuprofen lysine injection with selected drugs during simulated Y-site injection. J Pediatr Pharmacol Ther 13:156–161. https://doi.org/10.5863/1551-6776-13.3.156
32. Veltri MA, Conner KG (2002) Physical compatibility of milrinone lactate injection with intravenous drugs commonly used in the pediatric intensive care unit. Am J Health Syst Pharm 59:452–454. https://doi.org/10.1093/ajhp/59.5.452
33. Humbert-delaloye V, Berger-gryllaki M, Voirol P, Gattlen L, Pannatier A (2013) In vitro compatibility of various cardioactive drugs during simulated Y-site administration. Eur J Hosp Pharm 20:110–116. https://doi.org/10.1136/ejhpharm-2012-000239
34. Humbert-Delaloye V, Berger-Gryllaki M, Voirol P, Testa B, Pannatier A (2015) Screening for physicochemical incompatibilities of intravenous drugs in intensive care units: the case of monobasic potassium phosphate and furosemide. Eur J Hosp Pharm 22:56–58. https://doi.org/10.1136/ejhpharm-2013-000431
35. Voirol P, Berger-Gryllaki M, Pannatier A, Eggimann P, Sadeghipour F (2015) Visual compatibility of insulin aspart with intravenous drugs frequently used in ICU. Eur J Hosp Pharm 22:123–124. https://doi.org/10.1136/ejhpharm-2014-000478
36. Senarathna SMDKG, Strunk T, Petrovski M, Batty KT (2019) Physical compatibility of pentoxifylline and intravenous medications. Arch Dis Child 104:292–295. https://doi.org/10.1136/archdischild-2018-315376
37. Veltri M, Lee CKK (1996) Compatibility of neonatal parenteral nutrient solutions with selected intravenous drugs. Am J Health Syst Pharm 53:2611–2613. https://doi.org/10.1093/ajhp/53.21.2611
38. De Basagoiti A, Katsumiti A, Abascal S, Bustinza A, Lopez-Gimenez LR, Pascual P, De Miguel M, Campino A (2021) Physical compatibility of alprostadil with selected drugs commonly used in the neonatal intensive care units. Eur J Pediatr 180:1169–1176. https://doi.org/10.1007/s00431-020-03854-7
39. Kanji S, Lam J, Johanson C, Singh A, Goddard R, Fairbairn J, Lloyd T, Monsour D, Kakal J (2010) Systematic review of physical and chemical compatibility of commonly used medications administered by continuous infusion in intensive care units. Crit Care Med 38:1890–1898. https://doi.org/10.1097/CCM.0b013e3181e8adcc