Rapid analysis of iodinated x-ray contrast media in secondary and tertiary treated wastewater by direct injection liquid chromatography-tandem mass spectrometry

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Abstract

The iodinated x-ray contrast media (ICM) are the most widely administered intravascular pharmaceuticals and are known to persist in the aquatic environment. A rapid method using direct injection liquid chromatography-tandem mass spectrometry (DI-LC-MS/MS) has been developed to measure eight ICM. These include iopamidol, iothalamic acid, diatrizoic acid, iohexol, iomeprol, iopromide, plus both ioxaglic acid and iodipamide, which have not previously reported in the literature. The LC-MS/MS fragmentation patterns obtained for each of the compounds are discussed and the fragments lost for each transition are identified. Matrix effects in post-RO water, MQ water, tap water and secondary effluent have also been investigated. The DI-LC-MS/MS method was validated on both secondary and tertiary treated wastewater, and applied to samples from an advanced activated sludge wastewater treatment plant (WWTP) and a water recycling facility using microfiltration (MF) and reverse osmosis (RO) in Perth, Western Australia. As well as providing information of the efficacy for RO to remove specific ICM, these results also represent the first values of ICM published in the literature for Australia.

1. Introduction

The iodinated x-ray contrast media (ICM) are the most widely administered intravascular pharmaceuticals, used to aid visualisation of organs and vessels that otherwise would not absorb x-rays. Administered in very high doses (60-120g)[1], ICM are chemically inert, metabolically stable, and rapidly eliminated from the body via urine or faeces. While they are considered non-toxic to humans and wildlife[1-3], ICM are polar and persistent; properties that enable them to persist in the aquatic environment and leach through the subsoil into groundwater aquifers [4,5]. Studies have reported μg/l-level ICM concentrations in groundwater and bank filtrate samples [5-8] and also in raw and treated drinking water [6,7,9]. While bench scale and field studies have found anoxic or anaerobic conditions can promote biodegradation, little removal has been found under aerobic conditions[5,7].

Concentrations in the effluent from wastewater treatment plants (WWTP) can be between 5–40 μg I/l [5,10,11], particularly if these facilities receive waste from hospitals or radiological clinics. The persistence of ICM through conventional and activated sludge wastewater treatment plants is well documented [3,8,10,12]. Tertiary wastewater treatment has also been shown to be incapable of efficiently removing ICM. Removal through ozonation is slow and incomplete [5,13,14] and, while oxidation via UV/H_2O_2 is slightly more efficient, the ICM that was tested (iopromide) showed lower reactivity than any other pharmaceutical [15]. Reverse osmosis (RO) alone appears to effectively remove adsorbable organic iodine (AOI)[5], although the fate of individual ICM has not yet been studied.

Originally, ICM were only measured as a sum as AOI [3], but more recently researchers have utilised solid phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC-MS/MS), achieving detection limits in the tens of ng/L [10,11,16-19]. However, SPE methods for ICM can suffer from poor recovery [17], and are time consuming and expensive, particularly when sequential SPE columns are used [6,11]. Analysis by SPE-LC-MS/MS is also hampered because there are no isotopically labelled ICM standards, which complicates determination of recoveries. While a surrogate standard like desmethoxy-iopromide can be used instead, it is a metabolite of iopromide and therefore may also be present in wastewater, falsifying results. Furthermore, its own low recovery compared to other ICM lead to an over determination of ICM in sludges [18], while data for its performance in water and wastewater is ambiguous [8,19]. Alternatively, standard addition prior to SPE extraction can correct for recovery [6,11] but at least doubles the number of samples that require SPE pre-concentration, while using average recoveries to determined losses [20] requires the assumption that matrix effects are consistent over all samples.

Direct injection (DI) LC-MS/MS avoids the time consuming nature of SPE, the need to measure recovery with an internal standard, and should increase the overall robustness of the analysis. While achievable limits of detection (LOD) [21] are poorer than with SPE pre-concentration, they are still orders of magnitude lower than recommended guidelines in recycled drinking water [22]. In this work, we describe a rapid method to measure eight ICM (Table 1), including ioxaglic acid and iodipamide, which have not previously reported in the literature. The DI-LC-MS/MS method has been validated on both secondary and tertiary treated wastewater, and applied to samples from a water recycling facility using microfiltration (MF) and RO in Perth, Western Australia. Australian draft guidelines for maximum concentration of chemicals in recycled water for indirect potable reuse purposes are based on drinking water limits [22]. The draft guideline values for pharmaceuticals like ICM are based on human health considerations and are therefore relatively high (3-7 mg/L). The method developed in this study achieved LOD that were 10,000-fold lower than these guideline values, with sufficient sensitivity to quantitatively analyse ICM in secondary treated wastewater.

This study was part of a larger project investigating the effectiveness of advanced tertiary treatment processes, particularly MF/RO, to treat secondary wastewater for indirect potable reuse purposes. Samples were collected from the Kwinana Water Reclamation Plant (KWRP) in Perth, Western Australia. The plant incorporates an MF/RO unit that takes secondary treated effluent from the nearby Woodman Point wastewater treatment facility to produce a water supply, which is characterised by a very low content of dissolved organic carbon (DOC ~ 0.27 mg/L). The water produced by the KWRP plant (approx.16 ML/day) is currently used as general process water (e.g. for cooling, to generate high pressure steam) by neighbouring industrial facilities, reducing Perth's total demand for scheme water. However, similar technologies are being investigated at other Perth metropolitan wastewater treatment plants to produce high quality treated water for indirect potable reuse. As well as providing information of the efficacy of RO to remove specific ICM, these results also present the first published information on IDP iodipamide in secondary wastewater and specific ICM removal using an MF/RO process.

2. Experimental

2.1. Sampling

Composite and grab samples were collected on three days over a week-long period (30/05/2007 - 07/06/2007), pre and post-RO treatment. Composite samples were taken using an automated ISCO 4700 refrigerated sampler over 24 hours, while grab samples were collected from the corresponding sample stream on each of the three days. In addition, field and trip blanks were collected on each

day of sampling. Samples were preserved with 100 mg/L sodium azide, added as a solid to the amber glass sample bottles before sampling, and stored at 4 °C until analysis.

2.2. Standards and chemicals

Iopromide (IOP) was supplied by Bayer Schering Pharma AG, (Berlin, Germany), iomeprol (IOM) was supplied by Bracco s.p.a. (Milano, Italy), and iohexol (IOX), iopamidol (IOD), iothalamic acid (ITA), amidotrizoic acid (DTZ), ioxaglic acid (IXA) and iodipamide (IDP) were supplied by United States Pharmacopeia-USP, (Maryland, USA). Methanol and acetonitrile (ChromAR HPLC grade) were purchased from Mallinckrodt (New Jersey, USA); ammonium formate (purity 99.995%) was purchased from Sigma-Aldrich (NSW, Australia); formic acid (purity 99%) was purchased from Ajax FineChem (NSW, Australia). The MQ water used was purified using an IBIS Technology Ion Exchange System followed by Elga Purelab Ultra System. Disposable Ion Chromatography Acrodisc® Syringe Filters (0.45µm pore size, 25 mm diameter) were purchased from PALL Life Sciences (NY, USA). Single compound stock solutions were prepared in 5 mL volumetric flasks by dissolving c.a. 5 mg of each analytical standard in 2.5mL of MQ water. The solutions were placed in an ultrasonic bath for 5 minutes and then made up to volume with MQ water (nominal concentration 1µg/µL). Iodipamide was the only compound that was dissolved in MeOH due to its relatively low solubility in H_2O. A working solution containing all eight ICM (0.1 µg/µL) was prepared freshly for each analytical run, and calibration solutions ranging from 0.001 to 0.1 µg/µL were prepared by serial dilution of this working solution.

2.3. DI-LC-MS/MS method

All DI-LC-MS/MS measurements were performed using an Agilent 1100 HPLC system (Palo Alto, CA, USA) equipped with a solvent degasser unit, a quaternary pump and a 100 well-plate autosampler. Separation was achieved with a Phenomenex Gemini C18 column (125mm × 3mm I.D., 3 µm) with a flow rate of 200 µL/min. The mobile phase used in this work was modified from that reported by Seitz et al. [21] and consisted of eluent A, acetonitrile containing 0.01% of formic acid, and eluent B, MQ water containing 10 mM of ammonium formate, 0.5% (v/v) of formic acid and 1% (v/v) of acetonitrile. The chromatographic run began at 95% eluent B for 15 minutes, followed by a 5 minute linear gradient to 85% B, and a 1 minute linear gradient to 10% B. The mobile phase remained at 10% B for 29 minutes, until the end of the analytical run. Afterwards, eluent B was reduced to 0% B in 1 min, and the column washed with 100% eluent A for 4 min at an increased flow rate of 250 µL/min. The initial conditions were then re-established within 1 min and the column re-equilibrated at the normal flow rate of 200 µL/min for 19 min before injecting the next sample.

Prior to injection (100 µL), secondary treated wastewater was filtered through a 0.45 µm disposable syringe filter to remove suspended solids and particulate matter. The needle of the injector was also rinsed thoroughly in the injection port with a mixture of ACN:H_2O (50:50 v:v) before and after each injection to minimise potential carryover. Instrumental and laboratory contamination was also monitored by regular analysis of injector and procedural blanks every 10 injections, as well as field and trip blanks collected daily during field sampling. The analytical column was protected by a Phenomenex Gemini C18 security guard column (4mm × 3.0 mm I.D.). After every ~ 100 injections, the guard column was replaced, the analytical column back-flushed with ACN for 60 min, and the mass spectrometer thoroughly cleaned to ensure consistent system performance.

The LC was coupled to a Micromass Quattro Ultima Triple Quadrupole (Manchester, UK) system fitted with an electrospray interface (ESI) operated in positive ion mode. Prior to analysis, the triple quadrupole MS was turned on and left to stabilize for one hour, then calibration was checked across the range 85-1522 Da with a sodium iodide solution. Analyte identification was based on chromatographic retention time (RT) and compound-specific MRM transitions.

For optimum signal the ESI was operated with capillary and cone voltages optimised at 3200V and at 75V respectively. The molecular weight of the analytes targeted in this work ranged between 600 and 1300 Da. In order to efficiently transfer these high molecular weight ions to the first quadrupole, Hexapole1, Aperture and Hexapole2 required unusually high voltages (1 V, 0.8 V and 0.8 V respectively). Increasing these voltages dramatically increased the overall sensitivity of the analytical determination. However, increasing ion transfer to the first quadrupole meant that the ion block required cleaning more frequently than during normal operation to keep sensitivity at acceptable values. Desolvation temperature and source temperature were set to 345°C and 140°C, respectively. Nitrogen (cryogenic liquid) was used as both the desolvation and nebulizer gas; cone gas and desolvation gas flows were set to 110 L/h and to 550 L/h respectively. High purity Argon (99.997% purity) was used as collision gas ($P = 1.5 \times 10^{-2}$ Torr). Both quadrupoles (Q1 and Q3) were set at unit mass resolution; ion energy on Q1 was set to 1 while on Q3 it was set to 1.5.

To ensure method specificity, the two most intense transitions characteristic were identified for each analyte (Table 2). Peak identification was therefore based on the MRM ratio between these transitions and the chromatographic RT. To increase the sensitivity of the MS determination, the MRM transitions were grouped in three windows based on RT and the dwell time of each *m/z* monitored was set in proportion to the number of transitions in that window. Typically, a dwell time of 150 ms was used for the transitions that were selected for quantitation, while a slightly shorter dwell time (100ms) was used for the transitions selected for confirmation. Post-RO samples were quantified using external calibration curves built in MQ water. This was an appropriate quantitation

method because the DOC content of MQ water is very similar to that of post-RO water. Furthermore, the water produced by a MF/RO treatment plant is of consistent composition and DOC content on a day-to-day basis. It was therefore realistic to quantify a post-RO water with a calibration curve built in MQ because the matrix effects will be very similar and constant from sample to sample. As confirmation of this, minimal differences in the signal intensity (less than 5%) were observed when MQ water and Post RO water were spiked with the same amount of ICM (see Figure 5). Because it was quite possible for each wastewater sample to show a different matrix effect, quantitation in pre-RO samples was performed using the standard addition method [23]. In these cases, a second replicate sample was spiked with a known concentration of ICM (usually in the range 1-5ug/L) and the area of the peaks in the sample and peaks in the spiked sample were compared to calculate the ICM concentration. Data processing was carried out using MassLynx NT 4.0 SP4 software, while data quantitation was performed using QuanLynx 4.0.

3. Results and discussion

3.1. MRM Transitions

Direct infusion experiments were used to optimise general MS and MS/MS tuning parameters. Single compound standard solutions (10 ng/μL) prepared in 50:50 (v:v) mixture of eluent A and eluent B were introduced into the mass spectrometer at a flow rate of 5 μL/min using a Harvard Apparatus syringe pump (NSW, Australia).

In the presence of ammonium formate, the most intense precursor ion observed for all ICM was the ammonium adduct $[M+NH_4]^+$; other characteristic ESI precursor ions such as the sodium adduct $[M+Na]^+$or the proton adduct $[M+H]^+$ were substantially weaker. Although the loss of NH_3 ($[M+NH_4]^+ \rightarrow [M+H]^+$) is a "soft" transition and not characteristic of a real MS/MS phenomenon (the compound is simply exchanging NH_4^+ with a H^+ in the collision cell), it was normally the most intense transition in the MS/MS fragmentation spectra and occurred at low collision energies. As an example, MS/MS spectra showing the fragmentation pathway of IDP under optimised collision energy values are shown in Figure 1. Other MRM transitions were observed with increasing collision energy, although these were generally non-specific. Common fragmentation pathways of ICM included the loss of NH_3, HI, I, I_2, H_2O and loss of the hydrophilic chain. The latter process was characterised by either breaking of the amide bond (O=C-NHR) and elimination of an amine (NH_2R) or, in some cases (e.g. IOP, IOD, ITA, IOM), breaking the bond between the aromatic ring and the amide group (Φ-CONHR) and loss of –CONHR (see Table 2). Most of the fragments lost were identified, except for a few minor transitions where the change in *m/z* could not be reconciled with the ICM structure, (i.e. IDP). Some of the fragments shown in Table 2 are in agreement with

those previously reported in literature [4] but it has to be noted that erroneous fragmentation assignments (i.e. IOM, IOD) have been also reported [4,19]. The other fragments have not been reported before.

Generally the transition corresponding to the loss of ammonia was about an order of magnitude higher in intensity than the second mass transition chosen. Thus, the transition corresponding to the loss of ammonia was selected for quantitative purposes, while the secondary transition was used for confirmation purposes.

3.2. Development of the chromatographic separation

Three analytical columns from Phenomenex (Torrance, CA, USA) were tested for the chromatographic separation. Initially a Synergi Polar RP (125 × 4.6mm I.D., 4µm particle size) column was trialled, using a flow rate of 300 µL/min. This column was chosen because its I.D. allowed relatively large sample volumes to be injected (~100 µL), similar to the column used by Seitz et al. [21]. However, the high flow rates required to achieve optimum performance (typically in the range 0.5-2 mL/min) were incompatible with the ESI fitted onto the mass spectrometer. Even at a flow rate of 300 µL/min, the ESI was unable to completely convert the liquid mobile phase into an ionised vapour. Water droplets were observed on the sample cone fitted on the ion source, particularly at the beginning of the chromatographic run when the mobile phase was ~ 95% H_2O. Attempts were made to reduce the flow rate to the ESI source by fitting a zero-dead volume tee-piece with a split ratio of 70:30 between the column and the mass spectrometer. While this reduced the formation of water droplets on the ion source, the signal was unstable and the signal intensity decreased to ~ 30-40% of the intensity prior to the addition of the tee-piece. The best results that were achievable using the Synergi Polar-RP with a flow rate of 300 µL/min and the tee-piece are shown in Figure 2. Baseline separation was achieved for only three analytes, IOD, IOX and IOM. Two of the other analytes, DTZ and ITA, have the same molecular weight and similar transitions, and these practically co-eluted, which made it difficult to use the transition corresponding to the loss of ammonia for quantitation. Finally, IOP, IXA and IDP showed broad peaks and undesirable fronting and tailing, probably partly due to the excessively low flow rate.

The second column tested was the Phenomenex Gemini C18 (150mm × 2mm I.D., 3 µm particle size) at a flow rate of 200 µL/min. As this column is specifically designed for LC-MS, it can be operated at flow rates of 100-200 µL/min and lower. However, the injection volume recommended by the manufacturer (5-20 µL) is also lower because of its reduced I.D. Despite these specifications, a volume up to 100 µL was injected to test the sensitivity of the mass spectrometer under the desired DI conditions. As demonstrated in Figure 3, this column produced satisfactory resolution, selectivity and peak shape for all eight ICM. However, the limited retention of more polar ICM (i.e.

IOD RT = 3.96 min, IOX RT = 6.06min and IOM RT = 6.71 min) could be a disadvantage if matrix components in highly polluted samples (e.g. wastewater) co-elute with the analytes of interest. Such co-elution may result in ion suppression/enhancement effects that cannot be easily predicted or controlled without appropriate internal standards. Furthermore, RT of IOD, IOX and IOM were also highly irreproducible, making analyte identification difficult. The poor reproducibility of these RT was attributed to the large sample volume injected. Thus, a third column, the Phenomenex Gemini C18 column, with the larger I.D. (125mm × 3mm I.D., 3 µm particle size) was tested, also with a flow rate of 200 µL/min (Figure 4). This column showed increased retention for more polar ICM (IOD RT = 9.86 min, IOX RT = 16.50min and IOM RT = 17.09 min), improved selectivity and base-line resolution for DTZ and ITA, and much improved chromatographic reproducibility. We note that there are peaks with two maxima for IOX, IOP and IOM. Each of these compounds contains two chiral carbon atoms, which in turn leads to the presence of two diastereoisomers with slightly different physico-chemical properties. Other ICM (e.g. ioversol) also demonstrate stereoisomerism as it helps achieve the solubility required for a useful contrast agent [24] and similar observations in LC-MS/MS have been previously reported [19,21]. The area under both peaks was used in the quantitation process in this work.

3.3. Method linearity and limits of detection

The DI-LC-MS/MS method was validated using both MQ water and secondary treated wastewater spiked with varying amounts of the combined ICM working solution. A nine-point calibration curve was prepared in MQ water between 0.1-100 µg/L to test the LC-MS/MS response to very high ICM concentrations. A six-point calibration curve, spanning the concentrations expected in real samples, was prepared in secondary treated wastewater between 0.5 and 10 µg/L. Calibration curves for all ICM were linear over both concentration ranges tested; correlation coefficients for each ICM are presented in Table 3 and were typically better than 0.996 in MQ water and 0.967 in wastewater.

Limits of detection were calculated from the concentration equivalent to a signal to noise ratio (S/N) of three [25], either using the software MassLynX 4.0 software or, in some cases, manual S/N calculation using a peak of a known concentration. This method is able to calculate LOD for each analytical determination and therefore both average and LOD ranges are presented (Table 3). Average LOD in MQ water ranged between 0.10 and 0.58 µg/L depending on the characteristic absolute response of each compound. Average LOD in wastewater were comparable to those in MQ water, ranging between 0.11 and 0.97 µg/L. Noise was comparatively low at the relatively high MW of the ICM, which aided detection at the ng/L-µg/L level. For comparison to LOD determined in post-RO water, LOD in MQ water were also determined by replicate analysis (n = 10) of low concentration standards (1 µg/L, 3 to 4 times higher than the estimated LOD) and calculating a 1-

tailed 95% confidence interval (Table 3). While the LODs calculated with the statistical method are higher than using the S/N method (8-35% higher than the average values, except for ITA which resulted 17% lower) the agreement between the two methods is very good. Only in the case of IDP does the statistical LOD (0.15) lie outside the range calculated using S/N=3 (0.09-0.14). The same statistical approach could not be used to estimate LOD in wastewater because of the inherent elevated ICM concentrations already present.

Proposed drinking water limit guidelines were (Table 3) calculated by Western Australian Department of Health using the equation used to formulate the draft Australian guidelines for water recycling [22]. The values used the lowest therapeutic dose from the pharmacopeia, with a safety factor of 100 for an adult of 70 kg of body weight and assuming 2 litres of water consumption per day. For both MQ water and secondary treated effluent, the LODs achieved by the DI-LC-MS/MS method were 3 to 4 orders of magnitude lower than the health target LOD values, set at 10% of the drinking water guidelines

3.4. Accuracy, precision and peak identification.

Accuracy and precision of the analytical method in MQ water and wastewater, were determined by measuring ten replicate samples spiked with known amounts of ICM (10 µg/L). The average percent accuracy of these spikes was excellent, ranging from 100 to 108% in MQ water and 93 to 102% in wastewater (Table 4). In comparison, other methods utilising SPE pre-concentration have reported recoveries lower than 50% [11,17,19,20]. The precision of the ten replicates was also excellent, with relative standard deviation values ranging between 2 and 9% in MQ water and 3 and 10% in wastewater.

Analysis of these spiked replicates also provided data on the reproducibility of the RT and MRM ratios in both MQ water and wastewater (Table 4). As discussed before, the RT of the earlier eluting ICM (in particular IOD, IOM and IOX) were more variable than those that eluted later. The standard deviations of MRM ratio, as defined by a Commission Decision of the EU on the performance of analytical methods [26], were well within permitted tolerances.

3.5. Reproducibility

The reproducibility of the analytical method was determined by repeating measurements of three different secondary treated wastewater samples on three different days (Table 5). Generally reproducibility was better than 10%, very similar to the in-run precision reported from spiked matrices. It should be noted that IOM, IXA and ITA were always below detection and therefore reproducibility data is not yet available for these compounds.

3.6. *Matrix effects*

Matrix effects can compromise quantitative analysis by LC-ESI-MS. Co-eluting residual matrix components present in the sample can affect the ESI source, resulting in signal suppression or enhancement that leads to erroneous results. Several different approaches have been proposed in the literature to account for matrix effects including use of surrogate standards, standard addition method, and dilution of the SPE extracts [27]. In our study we have overcome the problem of matrix effects by the standard addition method rather than by using surrogate standards mainly because appropriate deuterated standards were not available, while dilution of the samples would decrease analyte concentrations. To investigate suppression/enhancement effects for ICM analysis by DI-LC-MS/MS, post-RO water, MQ water, tap water, and secondary effluent were spiked with the same nominal amount of ICM (20 μg/L) and each sample was injected three times. Unspiked samples were also analysed in order to account for any ICM inherently present in the samples (only secondary effluent showed appreciable amounts of ICMs). The suppression/enhancement effect of each matrix was determined by comparison to MQ water. A positive value indicates "ion enhancement" and a negative value indicates "ion suppression". Results are presented in Figure 5. The general trend in signal suppression followed the order: MQ water ~ post-RO water < tap water < secondary effluent. As expected, signals in spiked MQ water (DOC = 0.05mg/L) and in Post RO water (DOC=0.25 mg/L) were almost identical and signal intensity variations can be attributed to random experimental errors rather than a real matrix effect. Tap water (DOC = 1.33mg/L) also showed only a small degree of ion suppression (+2 to -10%) compared to MQ water. In contrast, secondary effluent showed between -3.5% up to -45% signal suppression, with highest ion suppression were IOP, IXA and IDP in wastewater. Similar results have been previously reported [21] and it is also interesting to note that matrix effects in secondary effluent do not appear to be as significant as those that can be seen when injecting SPE extracts [27]. This is probably because SPE cartridges concentrate both the analytes as well as all the matrix components that show affinity for the stationary phase. These matrix components become more concentrated in the SPE extract than in the original sample, and could suppress the ESI signal to a higher degree. In the future, we intend to conduct a more rigorous study of the matrix effect for ICMs in a range of different samples (i.e. surface water, groundwater and different wastewaters) to better understand the phenomena.

3.7. *Concentration of ICM in KWRP samples and RO membrane rejection*

The analytical method developed and validated in various aqueous matrices was applied to the determination of ICM in pre and post-RO treated water from KWRP. Results from the 3 days of sampling are presented in Table 6. Data from field and trip blanks were not included as all results for these samples were below LOD. Five of the eight ICM were regularly found in KWRP pre-RO

water, specifically IOD, DTZ, IDP, IOX and IOP at concentration levels ranging from 0.14 µg/L up to 9.2 µg/L. Despite being detected, concentrations measured in this secondary treated wastewater were still two to three orders of magnitude lower than the suggested guidelines for drinking water. In contrast to pre-RO water, ICM concentrations in all post-RO samples were below detection, demonstrating that RO is capable of removing the ICM measured from secondary treated wastewater at these concentrations. For pre-RO waters, the concentrations measured in composite and grab samples were generally similar and differences may indicate that ICM concentrations can vary within wastewater treatment facilities, related to diurnal variations in discharge from within the catchment.

These preliminary results demonstrated that RO was an effective treatment for removal of IOD, DTZ, IDP, IOX, and IOP. The process of rejection by an RO membrane can be influenced by many factors including compound–specific physico–chemical properties (e.g. molecular size, solubility, diffusivity, polarity, hydrophobicity, and charge), specific membrane properties (e.g., permeability, pore size, hydrophobicity, and charge), as well as membrane operating conditions (e.g., flux, transmembrane pressure, and regeneration). Several studies have reported that the molecular size of the molecule is the most important structural property for membrane rejection [28,29]. The MW range (600-1300Da) of the compounds considered in this work is high compared to the nominal MW cut-off (MWCO) of the RO membrane (approx. 100-150 Da). The ionic ITA, DTZ, IDP and IXA, are all characterised by one or more free carboxylic groups with a pKa estimated to be approx. 3.5 (e.g. DTZ, pKa = 3.4 [30]). For pH > pKa, these ionic ICM are negatively charged, so the main mechanism of RO rejection is most likely to be size/steric exclusion. Furthermore, repulsion between the charged ICM and the RO membrane will mean that adsorption on membranes can be excluded as mechanism of rejection [29,31]. In contrast, the non-ionic ICM (IOP, IOX, IOD, IOM) are triiodinated benzene derivatives containing amide and hydroxyl functionalities. At neutral pH they are uncharged [4] and have relatively high solubility in water. For these compounds a moderate to high rejection is expected because MW > MWCO, molecular width > RO membrane pore size, pH < pKa, and Log Kow can be assumed to be < 2 [29]. These results agree with other studies investigating the efficacy of RO for contaminant removal [5,32]. In one study in which organic iodine was used as surrogate for the triiodinated benzene derivative ICM [5] the decrease in AOI between feed water (10.3 µg I/l) and post-RO water (<0.4 µg I/l) implied organic iodine rejection exceeded 97%. Drewes et al. [32] have estimated that IOP rejection would exceed 90% in RO systems based the compound's high MW. The importance of size exclusion for larger molecules was also highlighted in a study of ultrafiltration (UF, MWCO=8000 Da) and nanofiltration (NF, MWCO=600) in which retention of IOP by NF membranes exceeded 58%, while UF membranes showed retention of less than 25% [33].

4. Conclusion

A DI-LC-MS/MS method was developed for the analysis of ICM in MQ water and wastewater samples. The DI-LC-MS/MS method is faster and considerably cheaper than comparable SPE-LC-MS/MS methods, with superior accuracy and precision. By avoiding SPE, the procedure is far less labour intensive, contamination due to sample handling is minimised and expensive SPE cartridges and hazardous solvents are not required. The LODs achieved easily detected concentrations of ICM at levels found in secondary wastewater and proved suitable for studies of the efficacy of advanced tertiary treatment processes (e.g. MF/RO) for further removal of these compounds. While several ICM were measured in secondary treated wastewater, all concentrations were orders of magnitude lower than drinking water limits. The non-detection of any ICM in post-RO treated water samples was attributed to the high molecular weight of the ICM, promoting RO membrane rejection of the compounds.

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6. Figures and Tables

Figure 1: Three spectra showing the fragmentation pathway of IDP with collision energy values of 0, 12 and 25. With collision energy = 0, the spectra is dominated by *m/z* 1158, attributed to $[M+NH_4]^+$, the chosen precursor ion. A smaller peak at *m/z* 1141 is attributed to $[M+H]^+$. When collision energy = 12, the spectra is dominated by *m/z* 1141, indicating the 'soft' transition of $[M+NH_4]^+ \Rightarrow [M+H]^+$. When collision energy is increased to 25, the fragmentation is also increased with the major ion (*m/z* 626) equal to the precursor ion minus NH_3 and $C_7H_4NO_2I_3$. Other ions identified (m/z 598, 498 and 481) were less sensitive.

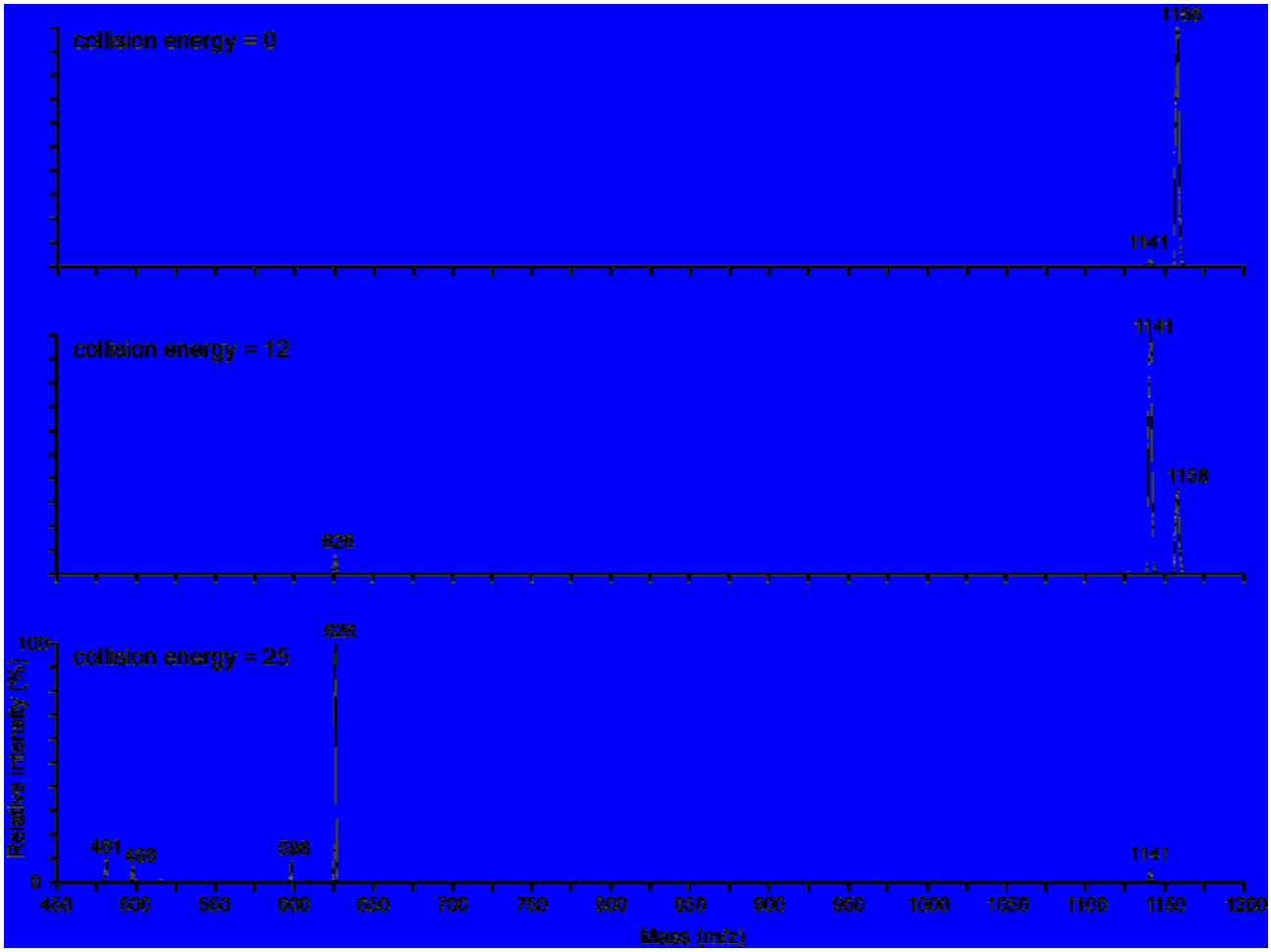


Figure 2: Chromatogram of eight ICM obtained using the Synergi Polar RP (125 × 4.6mm I.D., 4µm particle size) column, using a flow rate of 300 µL/min flow rate and a zero dead volume tee-intersection to reduce flow into the mass spectrometer to ~ 210 µL/min. This column proved unsatisfactory because IOP, IXA and IDP showed broad peaks and undesirable fronting and tailing, probably in part because the flow rate was inappropriate for the column. In addition, DTZ and ITA, which have the same molecular weight and similar transitions, co-eluted, making it difficult to use the transition corresponding to the loss of ammonia for quantitation.

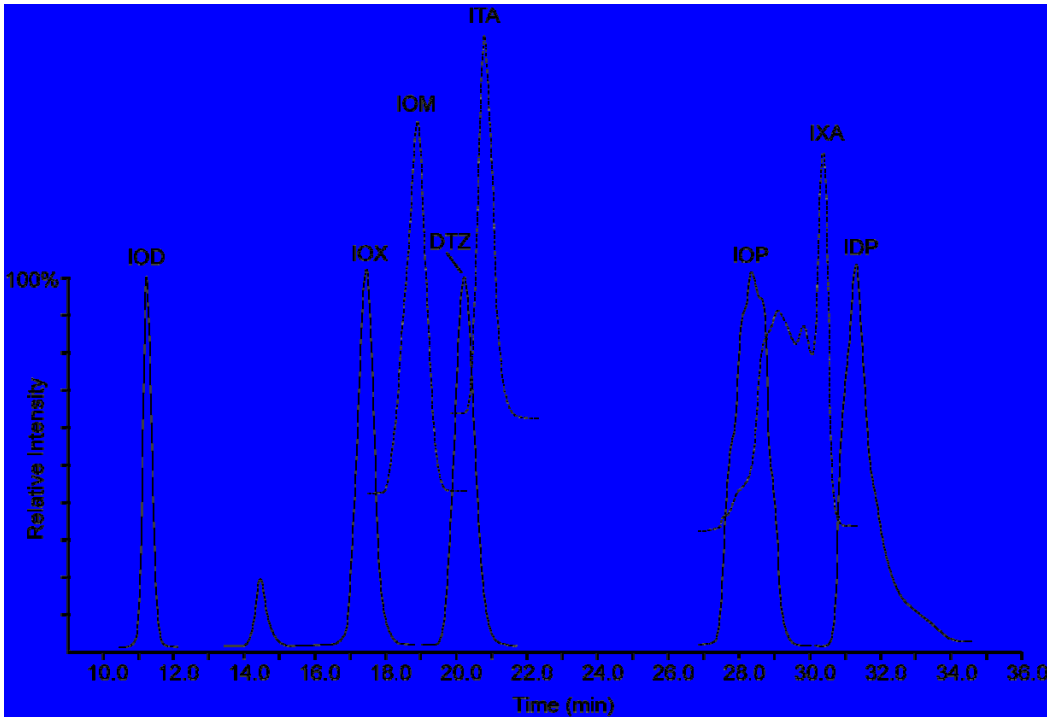


Figure 3: A typical chromatogram of eight ICM obtained using the Phenomenex Gemini C18 (150mm × 2mm I.D., 3 µm particle size) at a flow rate of 200 µL/min. This column produced satisfactory resolution, selectivity and peak shape for all eight ICM, however the short and irreproducible RT of more polar ICM (i.e. IOD, IOX and IOM) meant that this column was ultimately inappropriate for DI of large volume samples.

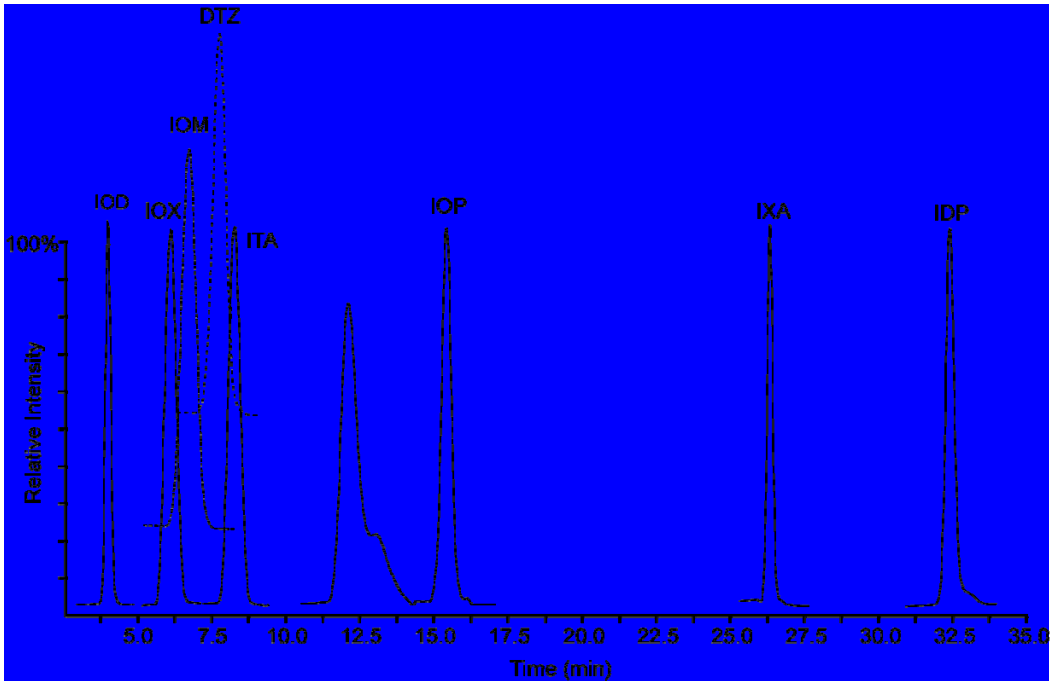


Figure 4: A typical chromatogram of eight ICM obtained a Phenomenex Gemini C18 column characterised by a slightly larger internal diameter (125mm × 3mm I.D., 3 µm particle size) at a flow rate of 200 µL/min. Compared to the 2mm I.D. column, this column showed increased retention for more polar ICM, improved selectivity and base-line resolution for DTZ and ITA, and much improved chromatographic reproducibility. Multiple maxima for IOX, IOP and IOM are the result of diastereomers with slightly different physico-chemical properties. The area under both peaks was used for quantitation for these ICM.

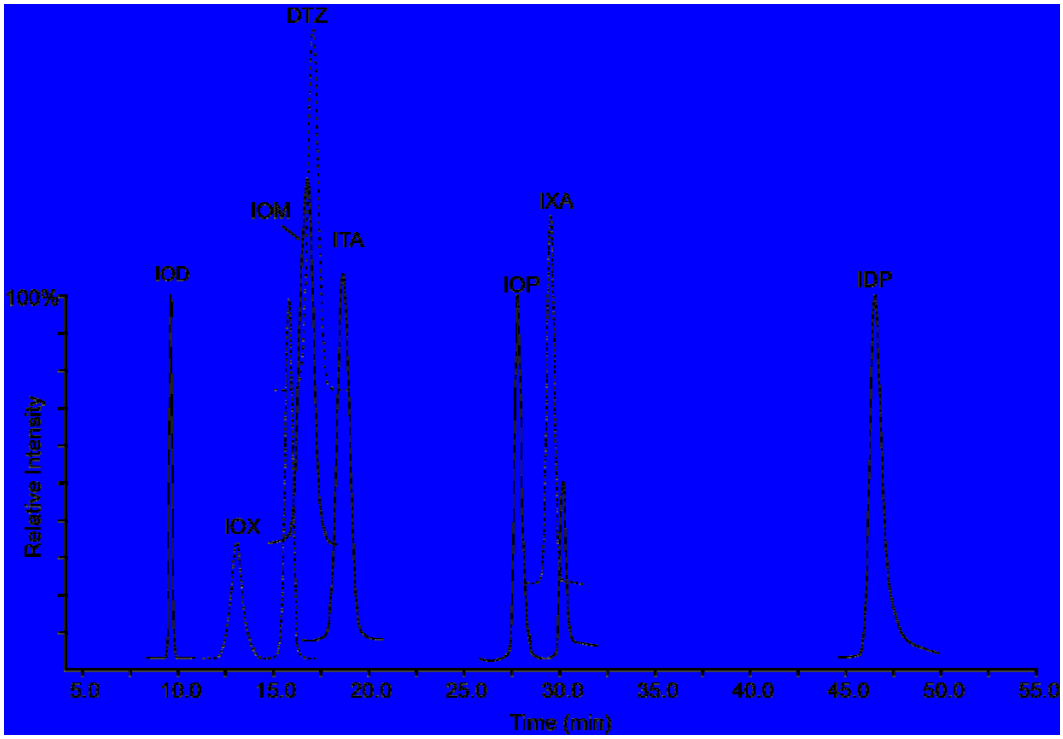


Figure 5: Matrix effects in post-RO water, tap water and secondary effluent. The suppression/enhancement effect of each matrix was determined by comparison to MQ water. A positive value indicates "ion enhancement" and a negative value indicates "ion suppression". The general trend in signal suppression followed the order: MQ water ~ post-RO water < tap water < secondary effluent.

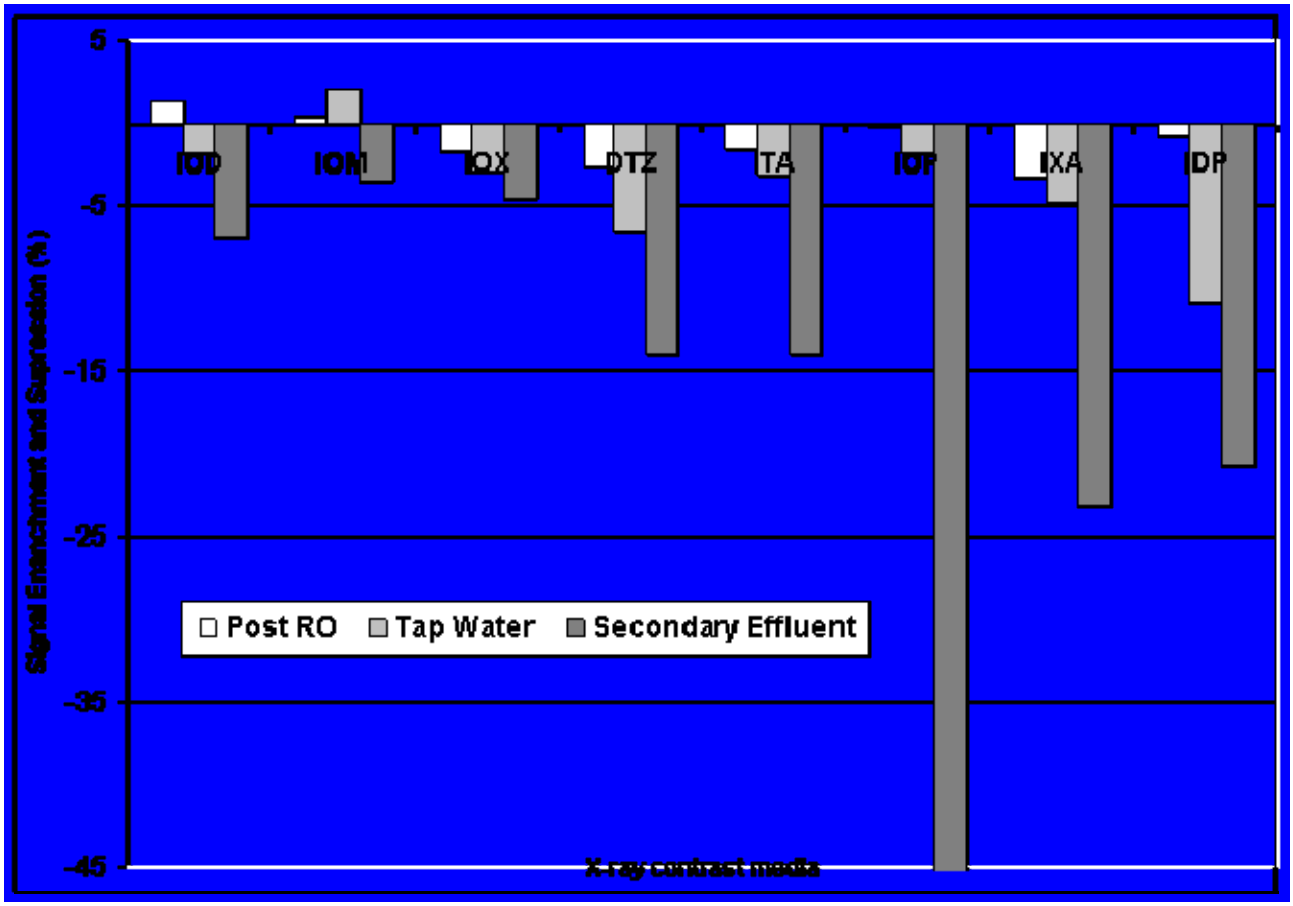


Table 1: Structure, molecular weight, and CAS number of the iodinated contrast media (ICM) under investigation. All ICM are stable in aqueous solutions, partly due to their hydrophilic side chains.

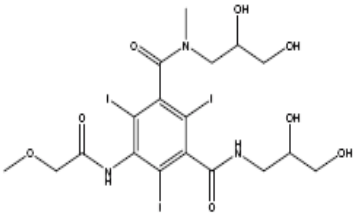
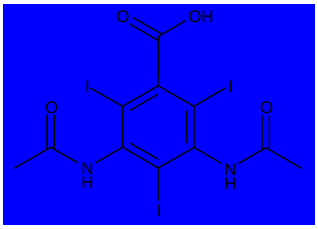
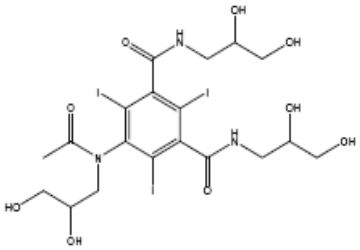
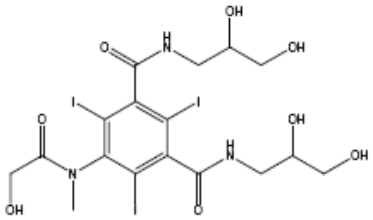
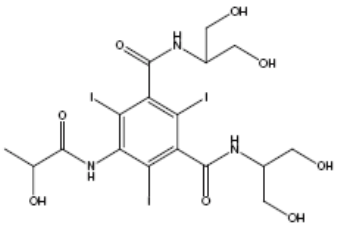
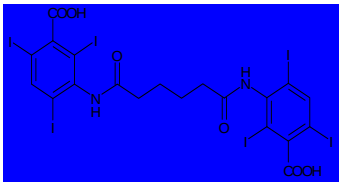
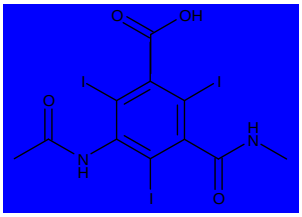
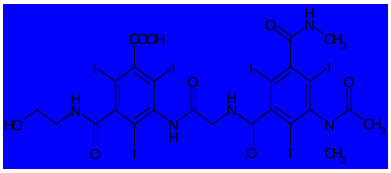
Compound	Structure	Compound	Structure
Iopromide (IOP) ($C_{18}H_{24}I_3N_3O_8$) MW: 791.8 CAS: 73334-07-3 Non-ionic		Amidotrizoic acid (DTZ) ($C_{11}H_9I_3N_2O_4$) MW: 613.9 CAS: 117-96-4 Ionic	
Iohexol (IOX) ($C_{19}H_{26}I_3N_3O_9$) MW: 821.1 CAS: 66108-95-0 Non-ionic		Iomeprol (IOM) ($C_{17}H_{22}I_3N_3O_8$) MW: 777.1 CAS: 78649-41-9 Non-ionic	
Iopamidol (IOD) ($C_{17}H_{22}I_3N_3O_8$) MW: 777.1 CAS: 62883-00-5 Non-ionic		Iodipamide (IDP) ($C_{20}H_{14}I_6N_2O_6$) MW: 1139.8 CAS: 606-17-7 Ionic	
Iothalamic acid (ITA) ($C_{11}H_9I_3N_2O_4$) MW: 613.9 CAS: 2276-90-6 Ionic		Ioxaglic acid (IXA) ($C_{24}H_{21}I_6N_5O_8$) MW: 1268.9 CAS: 59017-64-0 Ionic	

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Table 2: Precursor and product ions identified from ICM spectra, plus collision energy and dwell times for the transitions monitored during analysis. The collision energy to produce other fragments was variable. Most of the fragments lost were identified, except for a few minor transitions where the change in *m/z* could not be reconciled with the ICM structure, or where more than fragment structure was possible (see IXA). The product ions from the monitored transitions are underlined while an asterisk denotes the transition used for quantification.

Compound	Precursor Ion (*m/z*)	Product Ions (*m/z*)	Fragment Lost	Dwell Time (ms)	Collision Energy
IOP	809.3	792.3*	NH_3	150	8
		774.3	NH_3, H_2O	100	25
		701.2	NH_3, H_2O, $C_3H_5O_2$		
		687.2	NH_3, H_2O, $C_3H_6NO_2$		
		573.3	NH_3, HI, $C_3H_9NO_2$		
		559.2	NH_3, HI, $C_4H_{11}NO_2$		
		532.3	NH_3, HI, $C_5H_{10}NO_3$		
IOX	839.4	822.3*	NH_3	150	10
		804.1	NH_3, H_2O	100	22
		731.2	NH_3, $C_3H_9O_2N$		
		653.2	NH_3, HI, C_2H_3O		
		640.1	NH_3, $2C_3H_9O_2N$		
		603.2	NH_3, HI, $C_3H_9O_2N$		
IOD	795.3	778.3*	NH_3	200	10
		760.4	NH_3, H_2O	200	25
		705.4	NH_3, $C_3H_5O_2$		
		686.9	NH_3, $C_3H_9NO_2$		
		559.4	NH_3,HI, $C_3H_9NO_2$		
		541.6	NH_3, H_2O, HI, $C_3H_9NO_2$		
		531.2	NH_3, HI, $C_4H_9NO_3$		
ITA	632.3	615.2*	NH_3	150	8
		487.3	NH_3,HI		
		469.2	NH_3,H_2O, HI		
		361.3	NH_3, 2I		
		177.3	NH_3, 3I, C_2H_4NO	100	40
DTZ	632.2	615.0*	NH_3	150	12
		487.2	NH_3, HI		
		361.3	NH_3, 2I		
		233.3	NH_3, HI, 2I	100	30
IOM	795.3	778.1*	NH_3	150	25
		687.1	NH_3, $C_3H_9NO_2$	100	40
		559.3	NH_3, HI, $C_3H_9NO_2$		
		532.0	NH_3, HI, $C_4H_8NO_3$		
		405.2	NH_3, HI, I, $C_4H_8NO_3$		
IXA	1287.3	1270.1*	NH_3	150	10
		1252.2	NH_3,H_2O	100	35
		1195.1	NH_3,H_2O, C_2H_4NO		
		1123.8	NH_3, C_2H_4NO, $C_3H_6NO_6$		
		668.0	NH_3,$C_{10}H_9N_2O_4I_3$		
		641.2	NH_3,$C_{11}H_9N_2O_5I_3$		
		611.0	NH_3,$C_{12}H_{12}N_3O_5I_3$		

		542.1	NH_3,$C_{13}H_{12}N_3O_6I_3$, C_2H_3O		
		512.0	NH_3,$C_{13}H_{12}N_3O_6I_3$, C_2H_3O, CH_4N		
IDP	1158.1	1141.2*	NH_3	150	12
		626.1	NH_3, $C_7H_4NO_2I_3$	100	25
		598.1	NH_3, $C_8H_4NO_3I_3$		
		498.2	NH_3, HI, $C_7H_4NO_2I_3$		
		481.2	not identified		

Table 3: Linear regression data and LODs [A]calculated using the concentration equivalent to S/N = 3 in MQ water and wastewater and [B]calculated using the standard deviation of the peak area resulting from ten injections of 1ug/L of ICMs in MQ water.

Compound	MQ water			Wastewater		Proposed guidelines[¥]	
	R^2	LOD^A (µg/L) Average (Range)	LOD^B (µg/L)	R^2	LOD^A (µg/L) Average (Range)	Drinking water limit (µg/L)	LOD (µg/L)
IOD	0.9997	0.19 (0.15-0.28)	0.23	0.9974	0.22 (0.14-0.28)	4000	400
IOM	0.9992	0.22 (0.038-0.59)	0.24	0.9989	0.73 (0.23-1.25)	9000	900
IOX	0.9986	0.22 (0.27-0.59)	0.27	0.9887	0.80 (0.37-0.98)	7200	720
DTZ	0.9988	0.38 (0.25-0.49)	0.39	0.9675	0.83 (0.41-1.12)	11000	1100
ITA	0.9993	0.58 (0.42-0.73)	0.49	0.9893	0.97 (0.15-1.89)	9000	900
IOP	0.9965	0.20 (0.09-0.32)*	0.24	0.9883	0.20 (0.11-0.31)	7500	750
IXA	0.9996	0.10 (0.076-0.13)*	0.12	0.9986	0.11 (0.09-0.15)	n/a	n/a
IDP	0.9984	0.11 (0.09-0.14)*	0.15	0.9980	0.11 (0.10-0.16)	n/a	n/a

¥: ICM guideline limits as calculated in the Draft Australian Guidelines for Water Recycling [22].

*= LOD manually calculated in unsmoothed chromatograms.

Table 4: Retention time (RT), MRM ratio, accuracy and precision measured in MQ and wastewater samples. The numbers reported are averages of ten replicate samples and error is reported as standard deviation.

Compound	MQ water				Wastewater			
	RT (min)	MRM ratio	Conc (µg/L)	Accuracy and Precision (%)	RT (min)	MRM ratio	Conc (µg/L)	Accuracy and Precision (%)
IOD	9.3 ± 1.06	3.3 ± 0.11	10.5 ± 0.15	105 ± 1.5	9.2 ± 1.12	3.0 ± 0.21	10.0 ± 0.90	101 ± 9.0
IOM	17.4 ± 2.06	10.7 ± 0.96	10.2 ± 0.42	102 ± 4.2	16.9 ± 1.42	11.2 ± 0.11	9.8 ± 0.38	98 ± 3.8
IOX	14.7 ± 1.79	1.25 ± 0.16	10.2 ± 1.21	102 ± 12.1	13.8 ± 1.81	1.32 ± 0.12	10.2 ± 0.65	102 ± 6.5
DTZ	17.6 ± 1.50	6.6 ± 0.6	10.8 ± 0.71	108 ± 7.1	18.0 ± 1.11	10.1 ± 0.6	9.2 ± 0.96	92 ± 9.6
ITA	18.9 ± 1.37	6.9 ± 0.4	10.9 ± 0.63	108 ± 6.3	19.3 ± 1.04	6.8 ± 0.4	10.2 ± 0.81	102 ± 8.1
IOP	27.9 ± 0.34	10.2 ± 0.48	10.7 ± 0.86	107 ± 8.6	27.5 ± 0.40	12.7 ± 1.67	9.7 ± 1.02	97 ± 10.2
IXA	29.7 ± 0.07	11.5 ± 0.23	10.0 ± 0.23	100 ± 2.3	30.5 ± 0.04	13.5 ± 0.42	9.3 ± 0.31	93 ± 3.1
IDP	46.0 ± 0.51	1.6 ± 0.05	10.5 ± 0.58	105 ± 5.8	52.7 ± 0.20	1.8 ± 0.08	9.8 ± 0.32	98 ± 3.2

Table 5: Reproducibility data determined by repeating measurements of three different secondary treated wastewater samples on three different days. It should be noted that IOM and ITA were always below detection and therefore data is not available for these compounds.

		Wastewater 1 (µg/L)	Wastewater 2 (µg/L)	Wastewater 3 (µg/L)
IOP	Day 1	1.56	2.20	0.96
	Day 2	1.12	2.48	1.19
	Day 3	1.41	2.34	1.02
	Average (± %RSD)	**1.35 (± 14.8%)**	**2.34 (± 6.0%)**	**1.06 (± 11.0%)**
IDP	Day 1	0.43	0.79	0.94
	Day 2	0.37	0.78	1.19
	Day 3	0.38	0.93	1.34
	Average (± %RSD)	**0.39 (± 8.4%)**	**0.83 (± 9.9%)**	**1.15 (± 17.4%)**
IOD	Day 1	0.95	<LOD	0.73
	Day 2	0.89	<LOD	0.69
	Day 3	1.02	<LOD	0.79
	Average (± %RSD)	**0.95 (± 6.8%)**	**…**	**0.74 (± 6.4%)**
IOX	Day 1	1.59	<LOD	12.8
	Day 2	1.30	<LOD	10.3
	Day 3	1.81	<LOD	8.8
	Average (± %RSD)	**1.57(± 16.2%)**	**…**	**10.7 (± 19.1%)**
DTA	Day 1	1.99	2.11	2.55
	Day 2	1.98	1.99	2.52
	Day 3	2.24	1.94	3.13
	Average (± %RSD)	**2.10 (± 7.0%)**	**2.01 (± 4.3%)**	**2.73 (± 12.6%)**

Table 6: ICM concentration in pre and post-RO samples collected from the Kwinana Water Reclamation Plant (KWRP), Perth WA.

Compound	Sampling Date: 30/05/07				Sampling Date: 04/06/07				Sampling Date: 07/06/07			
	Pre-RO		Post-RO		Pre-RO		Post-RO		Pre-RO		Post-RO	
	Comp	Grab	Comp	Grab	Comp	Grab	Comp	Grab	Comp	Grab	Comp	Grab
IOD (µg/L)	0.40	0.61	<LOD	<LOD	0.62	0.24	<LOD	<LOD	0.45	0.40	<LOD	<LOD
ITA (µg/L)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DTZ (µg/L)	4.90	2.91	<LOD	<LOD	3.36	0.98	<LOD	<LOD	0.9	1.1	<LOD	<LOD
IXA (µg/L)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IDP (µg/L)	0.14	0.14	<LOD	<LOD	0.24	<LOD	<LOD	<LOD	0.23	<LOD	<LOD	<LOD
IOX (µg/L)	2.86	9.20	<LOD	<LOD	2.80	0.79	<LOD	<LOD	4.76	2.17	<LOD	<LOD
IOM (µg/L)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IOP (µg/L)	0.54	0.67	<LOD	<LOD	1.35	<LOD	<LOD	<LOD	0.43	0.28	<LOD	<LOD

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