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Advances on the separation of crocetane and phytane using GC–MS and GC×GC–TOFMS

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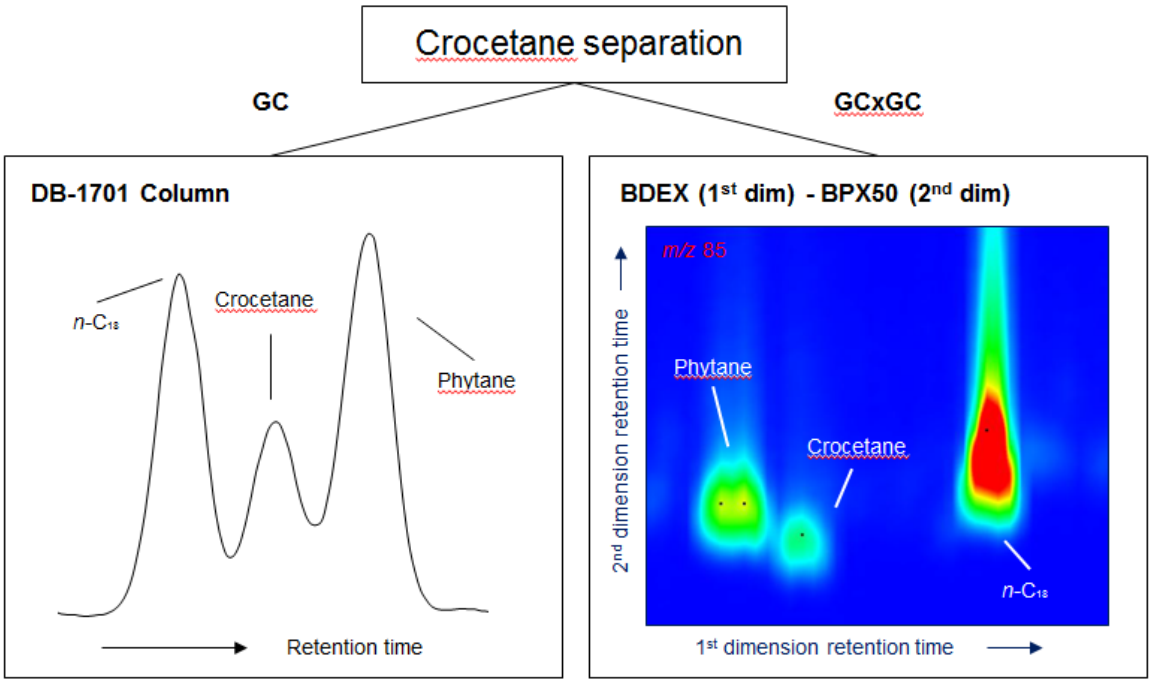
ABSTRACT

The C_{20} isoprenoid crocetane (2,6,11,15-tetramethylhexadecane) is an environmental diagnostic biomarker that can be used as a proxy for both anaerobic methane oxidation and photic zone euxinia. Crocetane co-elutes with its isomer phytane (2,6,10,14-tetramethylhexadecane) on many gas chromatographic capillary columns, making the detection of crocetane analytically challenging. The separation of crocetane and phytane is reported here using: (i) a one-dimensional gas chromatography (GC) method and (ii) a comprehensive two-dimensional gas chromatography (GC×GC) chiral method. The one-dimensional method employs a (14% cyanopropyl-phenyl)-methylpolysiloxane (DB-1701) capillary GC stationary phase where crocetane and phytane elute isothermally. This resulted in the highest resolution reported to date on a non-chiral column and allows for the analysis of both crocetane and conventional biomarkers in a single injection. The GC×GC chiral method reported here is capable of separating crocetane from phytane in whole oils without any pre-separation work, diminishing any effects and potential losses associated with sample preparation.

Keywords: Analytical separations, Biomarkers, Comprehensive two-dimensional gas chromatography, Crocetane, Crude oil, DB-1701, GC×GC, Phytane

Graphical Abstract



1. Introduction

The irregular tail-to-tail C_{20} isoprenoid crocetane (2,6,11,15-tetramethylhexadecane) has been detected in various cultured organisms and microbial communities (Schouten et al., 1997), as well as in sedimentary rocks and crude oils (e.g., Thiel et al., 1999; Barber et al., 2001b; Greenwood and Summons, 2003). Crocetane is commonly used as a proxy for anaerobic oxidation of methane (AOM), as it has been identified in sediments and microbial communities associated with methane seeps and mud volcanoes (e.g., Pancost et al., 2000; Thiel et al., 2001; Birgel et al., 2006; Bouloubassi et al., 2009) and in microbial communities comprising methanogenic archaea (e.g., Orphan et al., 2008). If crocetane is associated with AOM, the stable carbon isotopic signal is extremely depleted with $\delta^{13}C$ values as low as –142‰ (Bouloubassi et al., 2009). On the other hand, if crocetane originates from the C_{40} carotenoids characteristic for photic zone euxinic conditions (Summons and Powell, 1986; Grice et al., 2005), an estimated $\delta^{13}C$ value between –11 and –6‰ would be expected (Maslen et al., 2009). The ability to reliably detect crocetane without interference from co-eluting compounds is therefore of importance for a broad range of disciplines.

Detection of crocetane in complex hydrocarbon mixtures is difficult because it co-elutes with the regular head-to-tail C_{20} isoprenoid phytane (2,6,10,14-tetramethylhexadecane) on many conventional capillary GC columns (Robson and Rowland, 1993). Moreover, phytane has biological and thermal diastereomers (Dawson et al., 2007; Mcintyre et al., 2007), which can interfere with the separation if they don't elute at the same retention time. Phytane is one of the most common and abundantly used biomarkers in petroleum and environmental studies to evaluate anoxia, microbial productivity and organic matter maturity (Peters et al., 2005; Brocks and Summons, 2014; Grice and Eiserbeck, 2014). Additionally, the carbon isotopic composition of phytane relative to *n*-alkane isotopic composition provides

information on microbial diversity (Nabbefeld et al., 2010). The ability to distinguish phytane from crocetane is therefore highly desirable.

Baseline separation of phytane and crocetane is analytically challenging due to their high structural similarity. Previous studies have been able to partially separate the two compounds using non-polar methyl polysiloxane columns (Robson and Rowland, 1993; Thiel et al., 1999) and chiral (cyclodextrin) columns (Barber et al., 2001a) (Table 1). Selective ion monitoring (SIM) and multiple (metastable) reaction monitoring (MRM) gas chromatography mass spectrometry (GC–MS) analyses using a cyclodextrin GC stationary phase have also been performed to investigate the use of these techniques for the analysis of crocetane. Whilst providing enhanced specificity, the MRM of secondary transitions proved to be slightly less sensitive than SIM for the analysis of sediments and oils (Greenwood and Summons, 2003).

More recently, separation of crocetane and phytane was achieved using a chiral GC stationary phase (derivatised cyclodextrin) with the GC oven temperature at 135–140 °C (Huang and Armstrong, 2009). These temperatures are lower than those used by Barber et al. (2001a), where crocetane and phytane eluted above 200 °C (Table 1).

Even though a cyclodextrin (chiral) column is capable of baseline separation of phytane and crocetane, analysis of the full suite of biomarkers would be impossible on this column due to its upper temperature limit of 250 °C. Conventional biomarkers, such as hopanes and steranes, elute above 290 °C, making chiral columns unsuitable for routine biomarker analysis. Previous work (unpublished) has indicated that the (cyanopropyl-phenyl)-methylpolysiloxane DB-1701 column has potential to separate phytane and crocetane in aliphatic fractions and its upper temperature limits allow the detection of higher molecular weight biomarkers.

Recently, the use of comprehensive two-dimensional gas chromatography (GC×GC) has shown potential for enhanced resolution of co-eluting compounds whilst reducing sample

preparation (Ventura et al., 2008; Eiserbeck et al., 2011; Rowland et al., 2011; Gros et al., 2014; Naeher et al., 2016; Nelson et al., 2016). Particularly polar–non-polar (reverse) column configurations have shown enhanced separation of *n*-alkanes, branched alkanes and isoprenoids (Tran et al., 2006, 2011) in comparison to conventional GC–MS systems.

The overall aim of this study is to develop an analytical method that is capable of separating crocetane and phytane in a routine biomarker analysis. As most routine biomarker analyses are currently performed on GC–MS systems, the capability of a DB-1701 stationary phase to separate crocetane and phytane is investigated. With GC×GC becoming more widely used, environmental samples were additionally analysed on a GC×GC time of flight mass spectrometer (GC×GC–TOFMS) using various column configurations to investigate its separation capacity.

2. Experimental

2.1. Authentic standards and oil samples

Phytane was purchased from Fluka (Catalogue # 80165) and Ultra Scientific (Catalogue # FLHC-017, Lot NT0527718). Crocetane was donated by Dr Steve Rowland (Plymouth University). It was originally synthesised as described by Robson and Rowland (1993) with additional sample prepared from side products of the synthesis of branched alkyl tetralins and branched alkyl indanes (Booth et al., 2008).

An ExxonMobil diesel fuel (purchased January 31st 2003 at a local gas station on Cape Cod, Falmouth, MA, USA), routinely used as a reference sample at the Woods Hole Oceanographic Institution, is employed in this study. This diesel fuel does not contain crocetane and was therefore spiked with crocetane for initial GC×GC analyses. Crude oil samples were provided by Geoscience Australia and include three crude oils from onshore and offshore Australian basins: Blina-1, Blina-4 and Cornea-1. Blina oils are known to

contain crocetane (Barber et al., 2001b; Greenwood and Summons, 2003), whereas crocetane has not been reported in Cornea-1 oil.

2.2. GC–MS and GC×GC–TOFMS analyses

For both GC and GC×GC analyses, initial measurements were performed on a flame ionization detector (FID). To identify crocetane and phytane using their mass spectrum, GC–MS and GC×GC–TOFMS was used. GC–FID and GC×GC–FID methods are provided in the supplementary information.

2.3. GC–MS

GC–MS analyses were performed using a HP-6890A gas chromatograph (Agilent, Santa Clara, CA, USA) interfaced to a HP-5973 mass selective detector (MSD) (Agilent). The method employed an Agilent J&W DB-1701 column (60 m × 0.25mm i.d., 0.25 µm film thickness). The GC oven was programmed from 40 °C (held 1 min) to 220 °C at 15 °C/min (held 5 min) and further heated to 295°C at 3 °C/min with a final hold time of 32 min. Ultra-high purity helium was used as carrier gas with a constant flow of 1ml/min. Sample injection was 1 µl pulsed splitless at 290 °C, HP-6890 series injector (Agilent). The MSD was operated at 70 eV with a source temperature of 230 °C. Mass spectra were acquired in both full scan mode and SIM mode. The resolution (R), a characteristic of the separation of two adjacent peaks, was calculated using the equation reported by McNaught and Wilkinson (2006).

2.4. GC×GC–TOFMS

A Leco Pegasus IV GC×GC–TOFMS system was used in this study. The instrument was equipped with an Agilent 6890 GC and configured with a split/splitless auto-injector

(7683B series) and a dual stage cryogenic modulator (Leco, Saint Joseph, MI, USA). Samples were injected in splitless mode. The thermal modulator operates with a cold and hot jet. The cold jet gas was dry N_2 chilled with liquid N_2. The hot jet temperature offset was 20 °C above the temperature of the main GC oven and the inlet temperature was isothermal at 225 °C.

2.5. Chiral GC×GC method

The chiral method employed a Restek Rt®-bDEXsm column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) in the first dimension and a 50% phenyl polysilphenylene-siloxane column (SGE BPX50, 1.2 m length, 0.10 mm i.d., 0.1 μm film thickness) in the second dimension. For crude oil analysis, the temperature program of the main oven was held isothermal at 50 °C (1 min) and was then ramped from 50 to 140 °C at 3 °C/min followed by a ramp from 140 to 170 °C at 0.5 °C/min and at final ramp from 170 to 230 °C at 5 °C/min. The hot jet pulse width was 0.75 sec and the modulation period was 6 sec with a 2.25 sec cooling period between stages. The second dimension oven was programmed from 55 to 145 °C at 3 °C/min followed by a ramp from 145 to 175 °C at 0.5 °C/min and at final ramp from 170 to 235 °C at 5 °C/min. A quicker temperature program was used for the ExxonMobil diesel fuel, which is listed in the supplementary information. To obviate the occurrence of ghost peaks (compounds from a previous run that remained on the column), the bDEX column was conditioned. Following each sample run a solvent injection was performed and the first dimension column temperature ramped rapidly to 230 °C (maximum T for the bDEX column) and held for 30 min.

2.6. TOFMS

TOFMS data was sampled at an acquisition rate of 100 spectra per second in the mass range of 40-400 Dalton. The transfer line from the second oven to the TOFMS was deactivated fused silica (0.5 m length, 0.18 mm i.d.), held at 235 °C. The TOF detector voltage was 1355 Volts and the source temperature 240 °C. The mass spectrometer employs 70 eV electron ionization and operates at a push pulse rate of 5 kHz allowing sufficient signal averaging time to ensure good signal-to-noise ratios while still operating at an adequate data acquisition rate to accurately process (signal average) spectra from the peaks eluting from the second dimension column in this high resolution separation technique (GC×GC–TOF second dimension peak widths range between 50 to 100 milliseconds). For GC×GC whole oil analysis, oil samples were brought up in *n*-hexane to a final concentration of 50 mg/ml of which 2 µl was injected. Asphaltenes were precipitated prior to injection.

3. Results and discussion

A GC–MS method was designed for the separation of crocetane and phytane suitable for the routine biomarker analysis of environmental organic extracts. Analyses were performed on aliphatic hydrocarbon fractions. The method used a standard DB-1701 column with a temperature program developed to maximise the separation of these compounds. Holding the temperature at 220 °C for 5 min, such that crocetane and phytane eluted during the isothermal stage, improved the separation. Previously, similar isothermal temperature programs were reported by Dawson et al. (2005, 2007) that used an isothermal stage of 60 min on an Rxi-5MS column for the determination of pristane and phytane diastereomer ratios. In this case, due to the separation capability of the DB-1701 phase, a shorter isothermal stage of 5 min is sufficient. Although beyond the scope of this work, it would be worthwhile to investigate the capability of a DB-1701 column to separate the diastereomers

of pristane and phytane. During preliminary studies, different phase thicknesses and column lengths were investigated and it was found that increasing column length and decreasing column thickness both improved the separation. With the reported DB-1701 methodology (60 m × 0.25 mm i.d., 0.25 µm film thickness), a maximum resolution of 1.0 was achieved using synthetic standards (Supplementary Fig. S1). An aliphatic fraction of Blina-4 crude oil (obtained using silica gel chromatography) spiked with crocetane was analysed under the same conditions resulting in similar resolution of crocetane, phytane and octadecane (R = 1.0 for crocetane–phytane, R = 1.1 for octadecane–crocetane) in the total ion current (TIC) (Fig. 1).

In contrast to chiral columns, the DB-1701 column can be used to analyse both crocetane and higher molecular weight biomarkers such as steranes and hopanes in a single run. In comparison to a standard 5%-phenyl-methylpolysiloxane (DB-5MS) capillary column, this method resulted in comparable sterane distributions with minor shifts in retention times (Supplementary Fig. S2). This demonstrates that a DB-1701 column is capable of partially separating crocetane without loss of resolution in the higher molecular weight region, and therefore can be used for routine biomarker analysis.

One major drawback of using traditional GC–MS is the need for sample preparation with possible inherent losses. Such preparative steps are not always required using GC×GC methodologies.

3.1. Crocetane-phytane separation by GC×GC

Several different GC×GC based approaches were used to evaluate its capability to separate: (i) crocetane and phytane, and (ii) higher molecular weight biomarkers (hopanes and steranes) in a single analysis. Non-polar–polar (normal phase) and chiral phase GC×GC methods were investigated.

3.1.1. Crocetane–phytane separation using a normal phase column configuration

The GC×GC-FID normal phase column method utilised in this study was not successful at separating crocetane and phytane (Fig. 2), despite the partial separation previously reported on an OV-1 column (Robson and Rowland, 1993). GC method details were not reported for the OV-1 separation. Although the separation in the first dimension could have been lost due to the modulation, Barber et al. (2001a) also stated that they could not replicate the OV-1 separation on a GC–MS system. The lack of separation could therefore be a result of different flow conditions (constant flow versus constant pressure), variations in bonding or cross-linking of the column phase, or column age.

3.1.2. Crocetane-phytane separation using a chiral column configuration

The GC×GC–TOFMS chiral method utilised here resulted in baseline separation of crocetane, phytane and *n*-C_{18}, including separation of two phytane epimers (Fig. 3 and Supplementary Fig. S3). The capability of separating phytane stereoisomers using a chiral capillary column has been reported previously (Huang and Armstrong, 2009). Blina-1, Blina-4 and Cornea-1, three crude oils known to vary in crocetane abundance, were analysed using the same method (Fig. 4). The advantage of this GC×GC method over the 1D Huang and Armstrong (2009) chiral chromatography method is that: (i) there is no need for silica gel fractionation or molecular sieving before gas chromatographic analysis, (ii) less organic solvents are used and (iii) as well as separating crocetane and phytane, it is also capable of separating the *n*-C_{18} alkane

3.2. Crocetane quantification

Crocetane was semi-quantified in Blina-1, Blina-4 and Cornea-1 based on the *m*/*z* 85 and *m*/*z* 169 ions using an external calibration curve on both GC–MS and GC×GC–TOFMS. Crocetane concentrations are listed in Table 2 and calibration curves are shown in Supplementary Fig. S4. Crocetane concentrations determined using GC–MS and GC×GC–TOFMS are not directly comparable due to the differences in methodologies. Apart from the chromatographic differences (GC vs GC×GC), GC analyses were performed on aliphatic hydrocarbon fractions with a quadrupole mass spectrometer. GC×GC analyses were conducted on the *n*-hexane soluble fraction of the whole oil with a TOFMS. Despite these methodological differences, the concentrations are in the same order of magnitude.

Using GC–MS, the calculated crocetane concentrations in Cornea-1 were at the limit of detection (LoD) of 0.25 µg/ml for the *m*/*z* 169 ion and 0.26 µg/ml for the *m*/*z* 85 ion (determined using method described by Armbruster and Pry, 2008). Using GC×GC–TOFMS crocetane was below the detection limit in Cornea-1. As Cornea-1 had no detectable crocetane as measured by GC×GC–TOFMS, it was used for a spike-and-recovery assessment. The recovery of crocetane ranged between 97.5% using the *m*/*z* 113 ion and 87.5% using the *m*/*z* 169 ion, indicating that despite the complexity of the crude oil, the sample matrix had minimal effects on crocetane detection.

The reliability of the calculated concentrations using the *m*/*z* 85 ion versus the *m*/*z* 169 ion depends on the GC method. For GC–MS, peak area integrations performed on the *m*/*z* 169 ion are more reliable because of more Gaussian peak shapes. Moreover, the potential interference from branched alkanes is minimized as *m*/*z* 169 is not a discriminant ion in these compounds. The downside of using *m*/*z* 169 for peak area integration is that this is not a major ion in the mass spectrum of crocetane. Therefore, slight differences in peak area integration with the *m*/*z* 169 ion translate into more noise in the concentration values.

3.3. Future applications

Crocetane can be used as a proxy for both anaerobic methane oxidation and photic zone euxinia. The isotopic composition of crocetane can discriminate between these two sources. To complement the separation methods described here and obtain the isotopic signal of crocetane, preparative GC and preparative GC×GC could be investigated (Kim and Marriott, 2012).

4. Conclusions and outlook

Moderate separation of crocetane and phytane was achieved using a (14% cyanopropyl-phenyl)-methylpolysiloxane GC stationary phase (R = 1). This is the best separation of crocetane and phytane reported to date using a non-chiral column. Comprehensive GC×GC–TOFMS was capable of baseline separation of crocetane and phytane using a chiral – polar column configuration. This work demonstrates the capability of comprehensive GC×GC–TOFMS to perform complete separation on the hexane soluble fraction of whole oils, eliminating the need for molecular sieving and silica gel chromatography.

For routine biomarker analyses on GC–MS systems a DB-1701 column can be used for the analysis of crocetane without chromatographic losses of other biomarker compounds. For rapid analyses without any time consuming pre-separation work GC×GC–TOFMS can be used.

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Table Captions

Table 1

Previously reported separations of phytane and crocetane using various GC stationary phases.

Table 2

Crocetane concentrations determined using external calibration curves on both GC–MS and GC×GC, resulting in comparable concentrations despite methodological differences.

Figure Captions

Fig. 1. Gas chromatogram of (A) aliphatic fraction of Blina-4 oil showing elution region of crocetane (B) magnified with corresponding mass spectrum of crocetane (C); (D) magnified region of Blina-4 oil spiked with additional crocetane standard and (E) gas chromatogram of overlaid individual standards.

Fig. 2. GC×GC chromatogram of Blina-4 crude oil analysed on the normal phase column configuration (Rxi-1 (Restek), 60 m × 0.25 mm i.d., 0.25 µm film thickness) first dimension and BPX50 (SGE; 1 m × 0.1 mm i.d., 0.1 µm film thickness, second dimension) showing co-elution of phytane and crocetane.

Fig. 3. Mass fragmentograms for *m/z* 85, 113 and 169 of crocetane and phytane standards analyzed using the GC×GC chiral column configuration.

Fig. 4. Mass fragmentogram for *m/z* 169 showing separation of phytane and crocetane in: (a) Blina-1, (b) Blina-4 and (c) Cornea-1.

Tables

Table 1

GC stationary phase	Separation	Resolution	Separation temperature (°C)	Reference
OV-5	no separation	NR	NR	Robson and Rowland, 1993
OV-1	partial separation	NR	NR	Robson and Rowland, 1993
DB5-HT	no separation	NR	NR	Thiel et al., 1999
HP-1	no separation	NR	NR	Thiel et al., 1999
OV-101	no separation	NR	NR	Thiel et al., 1999
Combined HP-1 and OV-101	partial separation	0.7	NR	Thiel et al., 1999
CPSil 2 CB	partial separation	0.5	NR	Thiel et al., 1999
Permethyl-β-cyclodextrin (β-CYDEX)	partial separation	0.8	200-220	Barber et al., 2001a
Permethyl-γ-cyclodextrin	partial separation	0.6	200-220	Barber et al., 2001a
Permethyl-α-cyclodextrin	no separation	NR	NR	Barber et al., 2001a
6-tributyldimethyl-β-cyclodextrin	no separation	NR	NR	Barber et al., 2001a
BP-1	no separation	NR	NR	Barber et al., 2001a
BP-5	no separation	NR	NR	Barber et al., 2001a
BP-20	no separation	NR	NR	Barber et al., 2001a
Chiraldex B-PH	baseline separation	1.6	140	Huang and Armstrong, 2009
Chiraldex B-DM	baseline separation	1.5	135	Huang and Armstrong, 2009
Chiraldex G-DM	no separation	NR	NR	Huang and Armstrong, 2009
Chiraldex B-DA	partial separation	0.4	120	Huang and Armstrong, 2009
Chiraldex G-TA	no separation	NR	NR	Huang and Armstrong, 2009
Chiraldex B-TA	baseline separation	1.5	140	Huang and Armstrong, 2009
Chiraldex G-PN	no separation	NR	NR	Huang and Armstrong, 2009
Chiraldex G-BP	no separation	NR	NR	Huang and Armstrong, 2009

NR: Not Reported. Resolution: a characteristic of the separation of two adjacent peaks (McNaught and Wilkinson, 2014).

Table 2.

Oil sample		Crocetane concentration (µg/mg aliphatics) (DB-1701 method) [a]	Approximate crocetane concentration (µg/mg oil) (DB-1701 method)	Crocetane concentration (µg/mg oil) (GC×GC chiral method)
Blina-1	*m/z* 169	0.49	0.30	0.27
	m/z 85	0.71	0.43	0.37
Blina-4	*m/z* 169	0.85	0.51	0.29
	m/z 85	1.59	0.95	0.45
Cornea-1	*m/z* 169	0.25 [b]	-	bdl
	m/z 85	0.26 [b]	-	bdl

bdl: below detection limit

[a] Crocetane was quantified in the aliphatic fraction on GC–MS systems. For comparison with quantification on GC×GC crocetane concentrations were converted from µg/mg aliphatics to µg/mg oil using the silica gel fractionation mass balance.

[b] Crocetane concentrations in Cornea-1 were at the limit of detection (LoD) of 0.25 µg/ml for the *m/z* 169 ion and LoD of 0.26 µg/ml for the *m/z* 85 ion.

Figures

Fig. 1

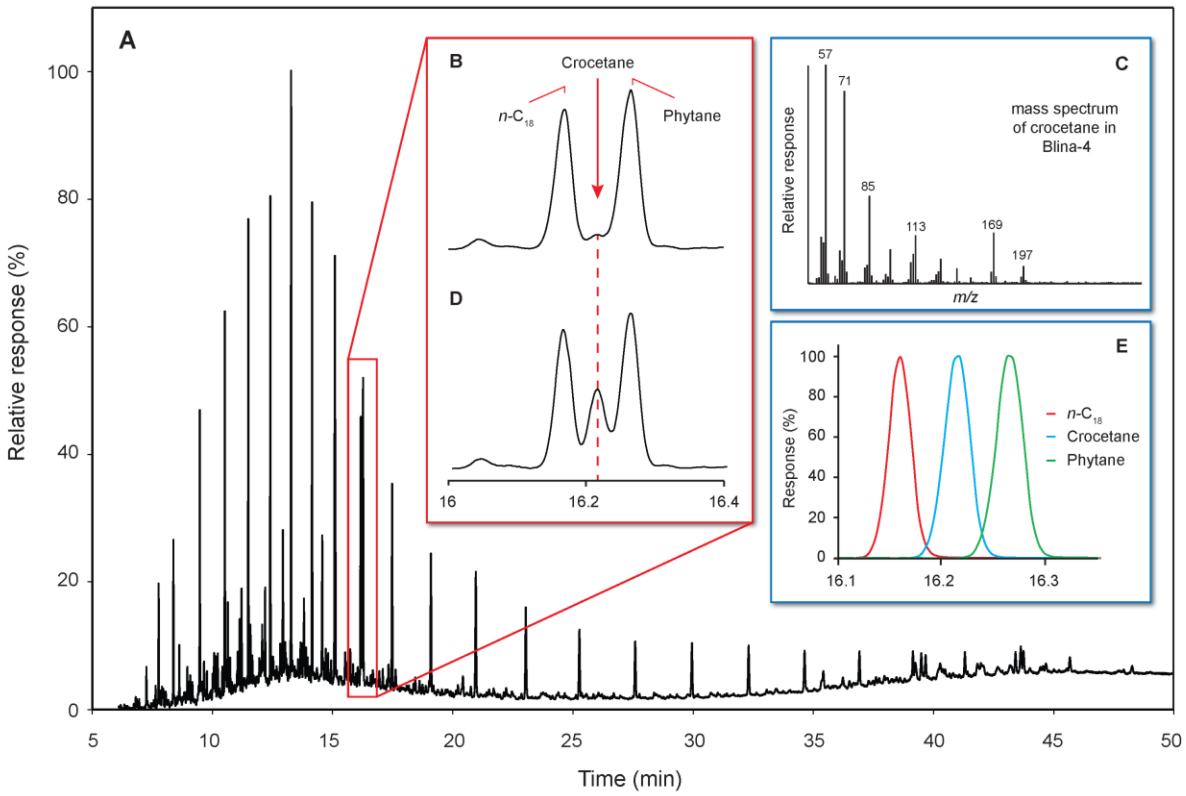


Fig. 2

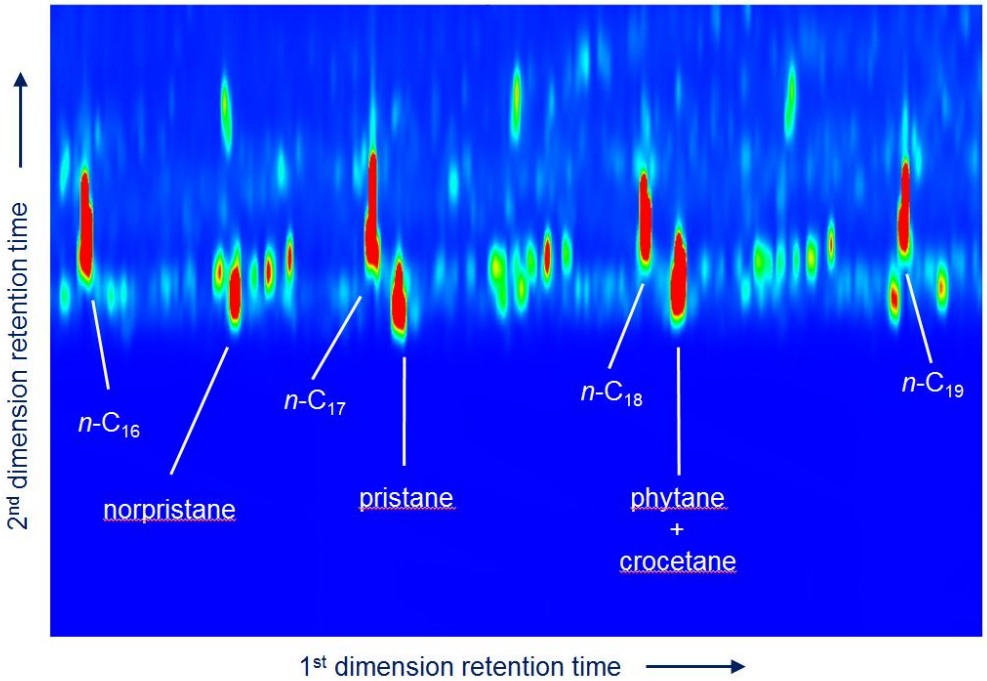


Fig. 3

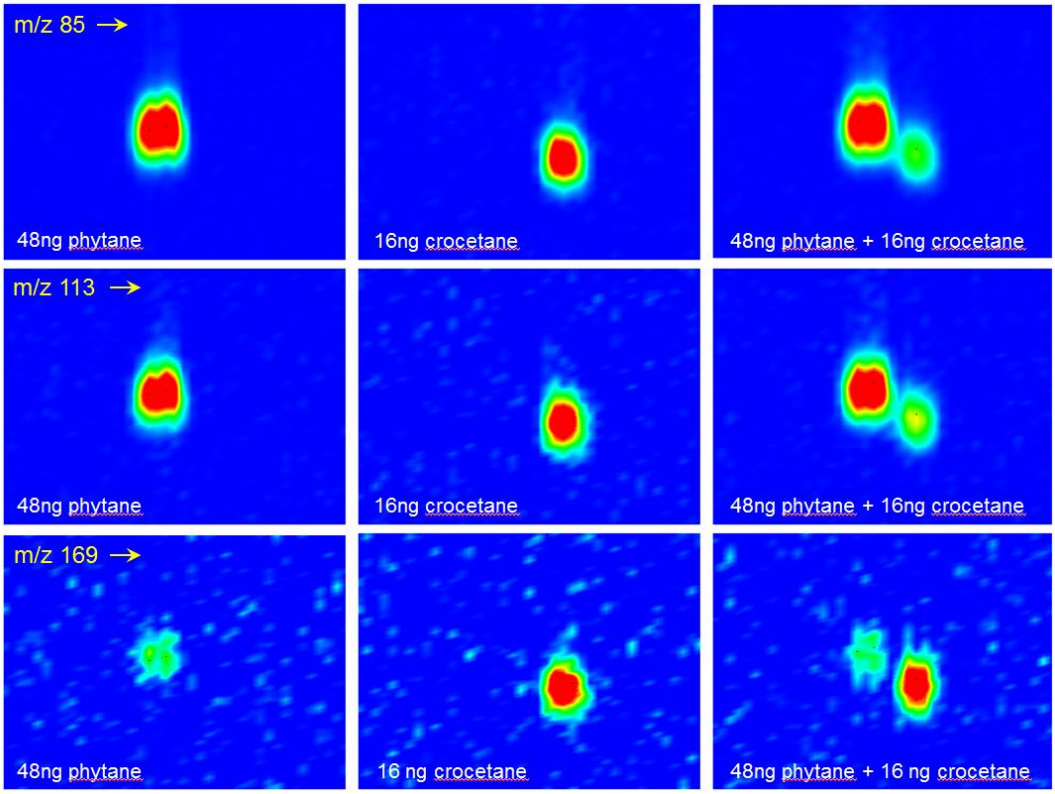
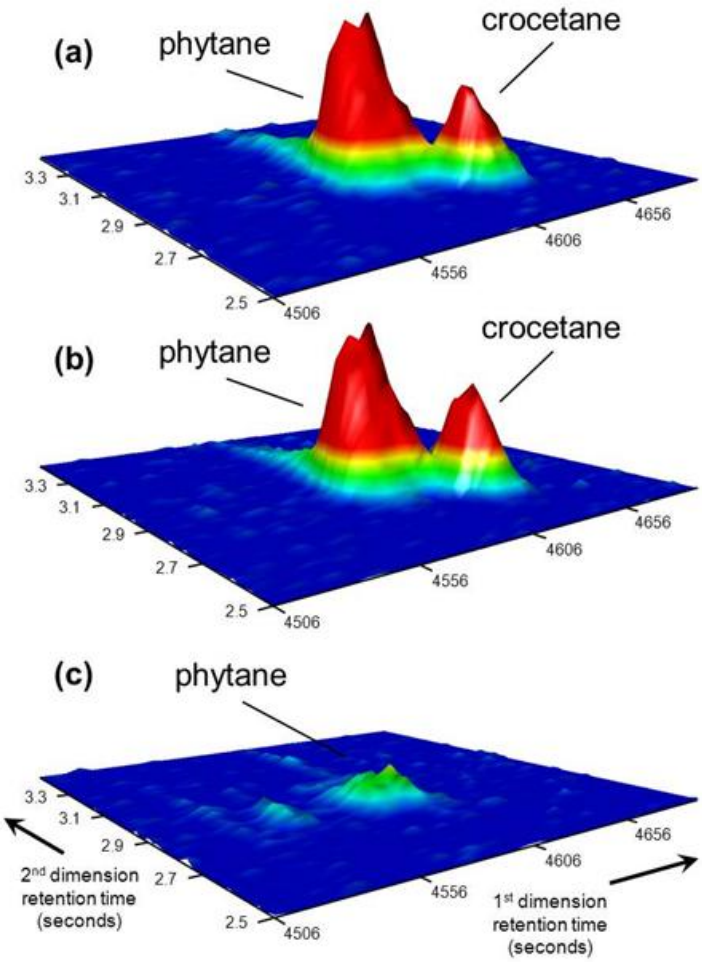
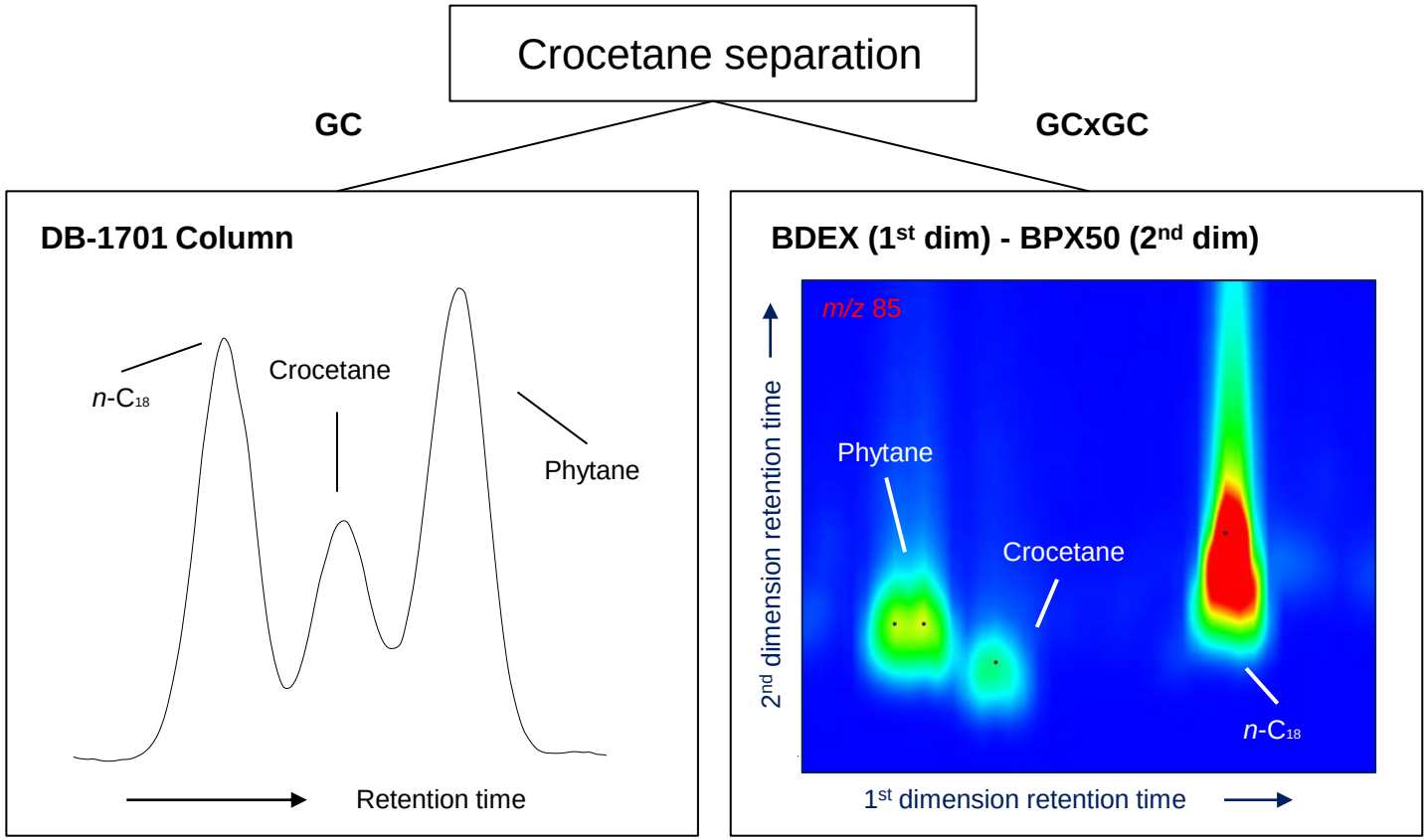
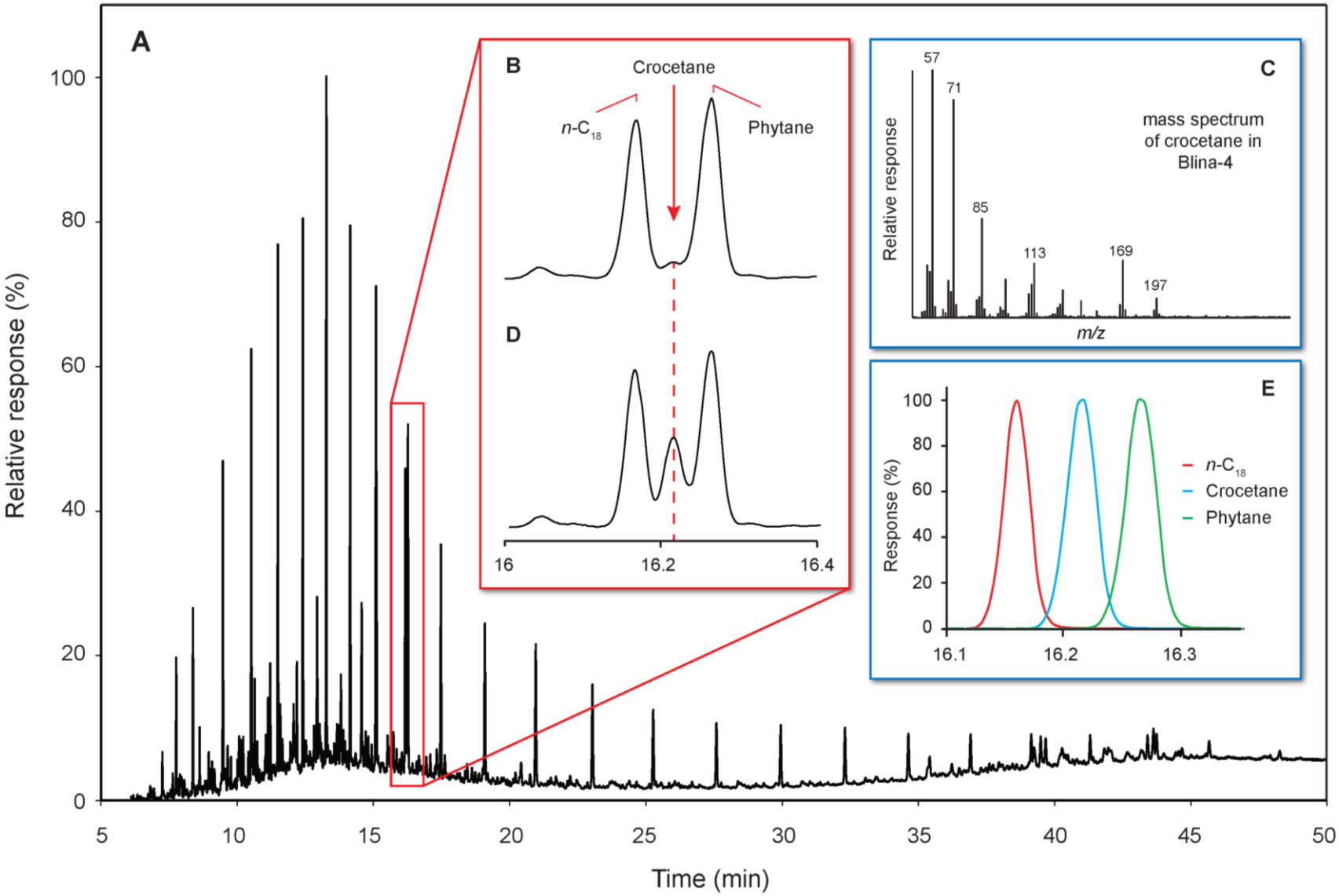

m/z 85 →
48ng phytane
16ng crocetane
48ng phytane + 16ng crocetane
m/z 113 →
48ng phytane
16ng crocetane
48ng phytane + 16ng crocetane
m/z 169 →
48ng phytane
16 ng crocetane
48ng phytane + 16 ng crocetane

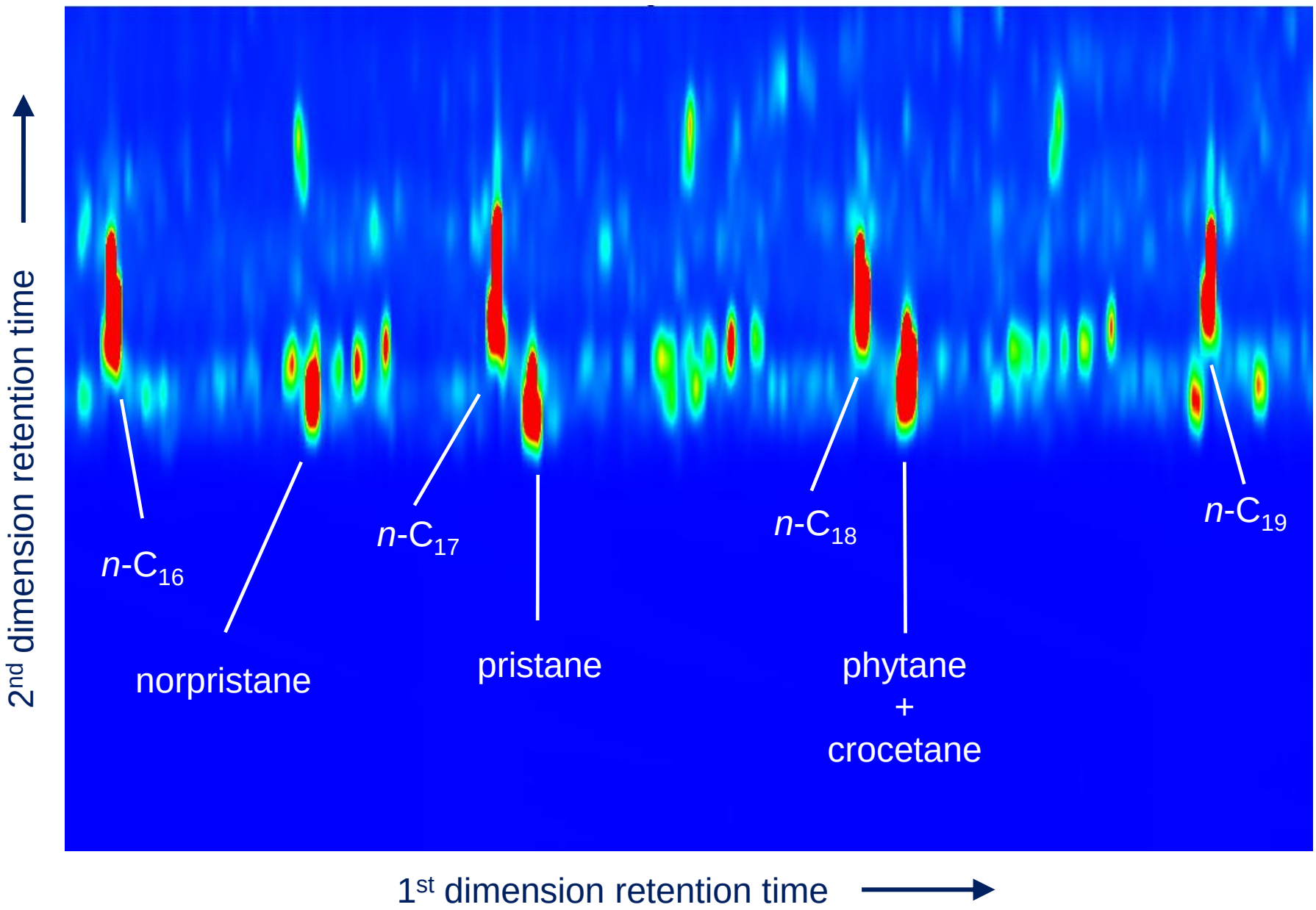
Fig. 4

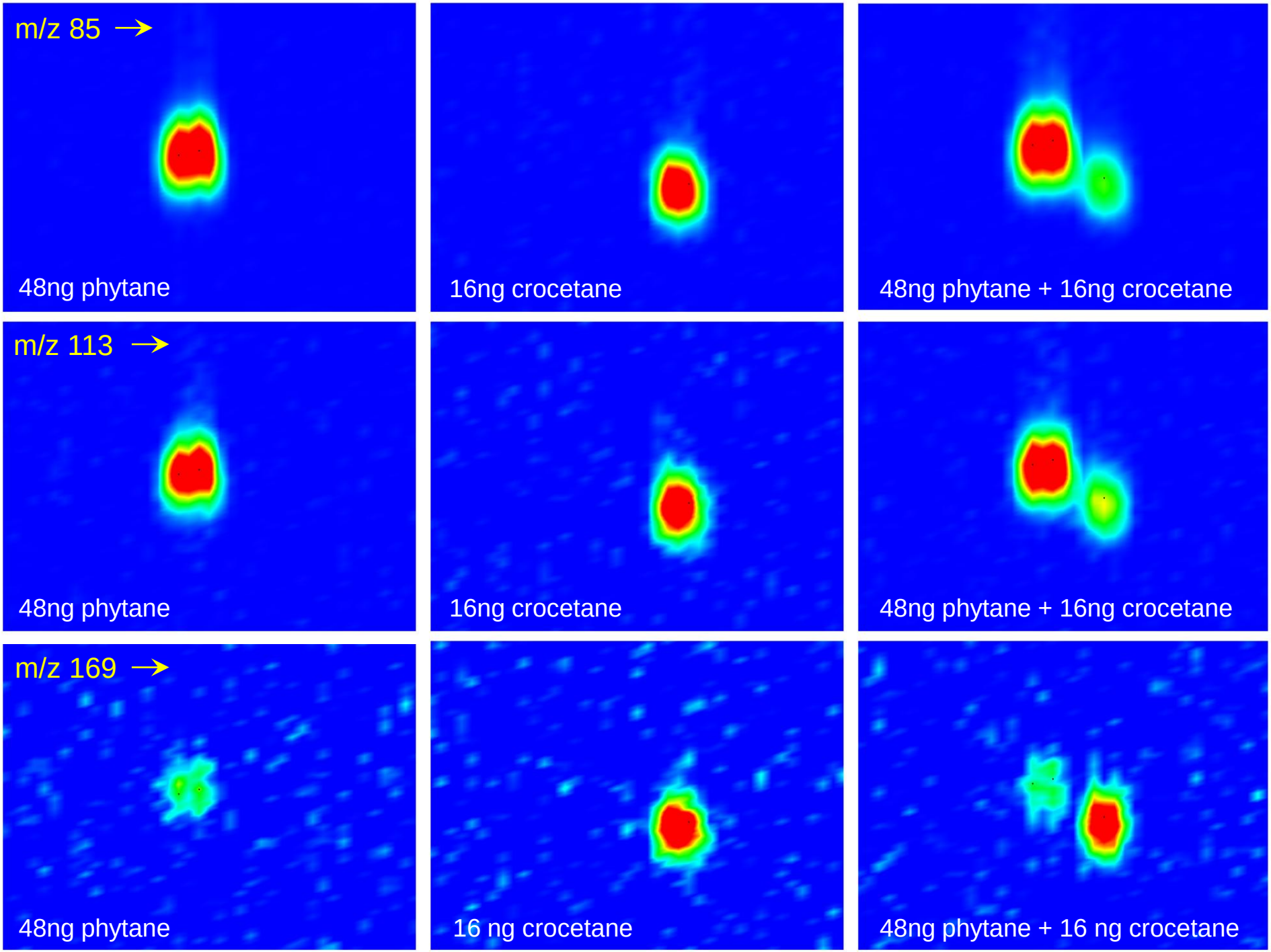

(a)
phytane
crocetane
3.3
3.1
2.9
2.7
2.5
4506
4556
4606
4656
(b)
phytane
crocetane
3.3
3.1
2.9
2.7
2.5
4506
4556
4606
4656
(c)
phytane
3.3
3.1
2.9
2.7
2.5
4506
4556
4606
4656
2nd dimension retention time (seconds)
1st dimension retention time (seconds)

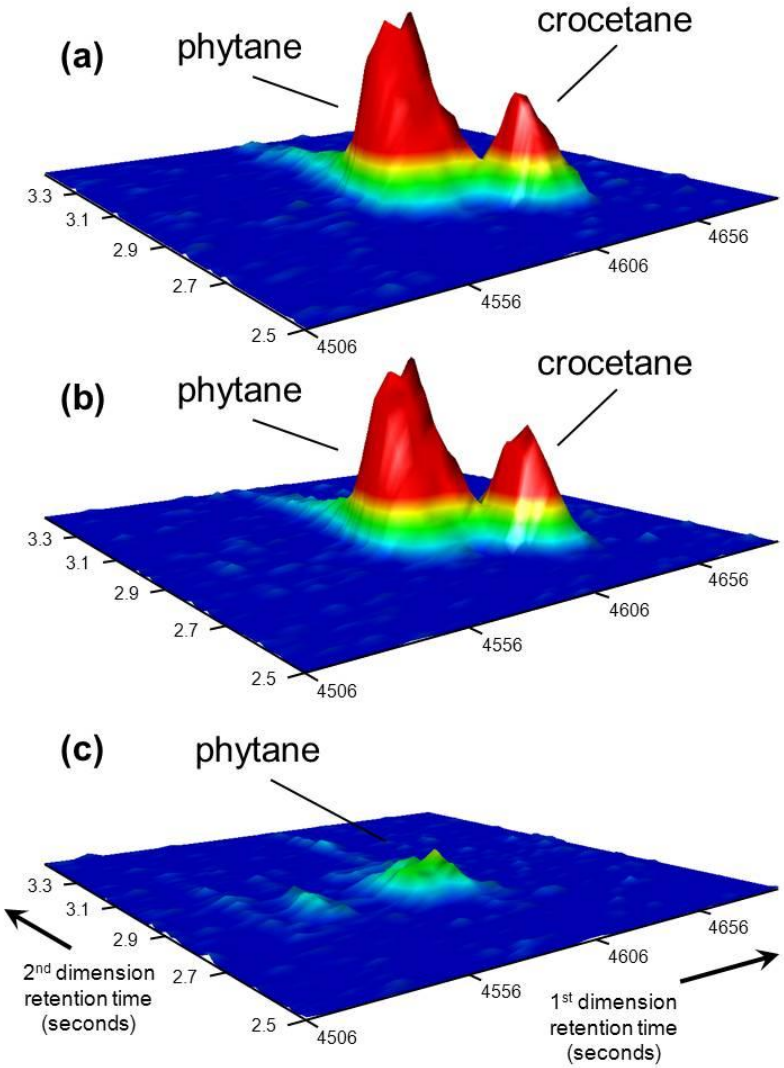
Figure

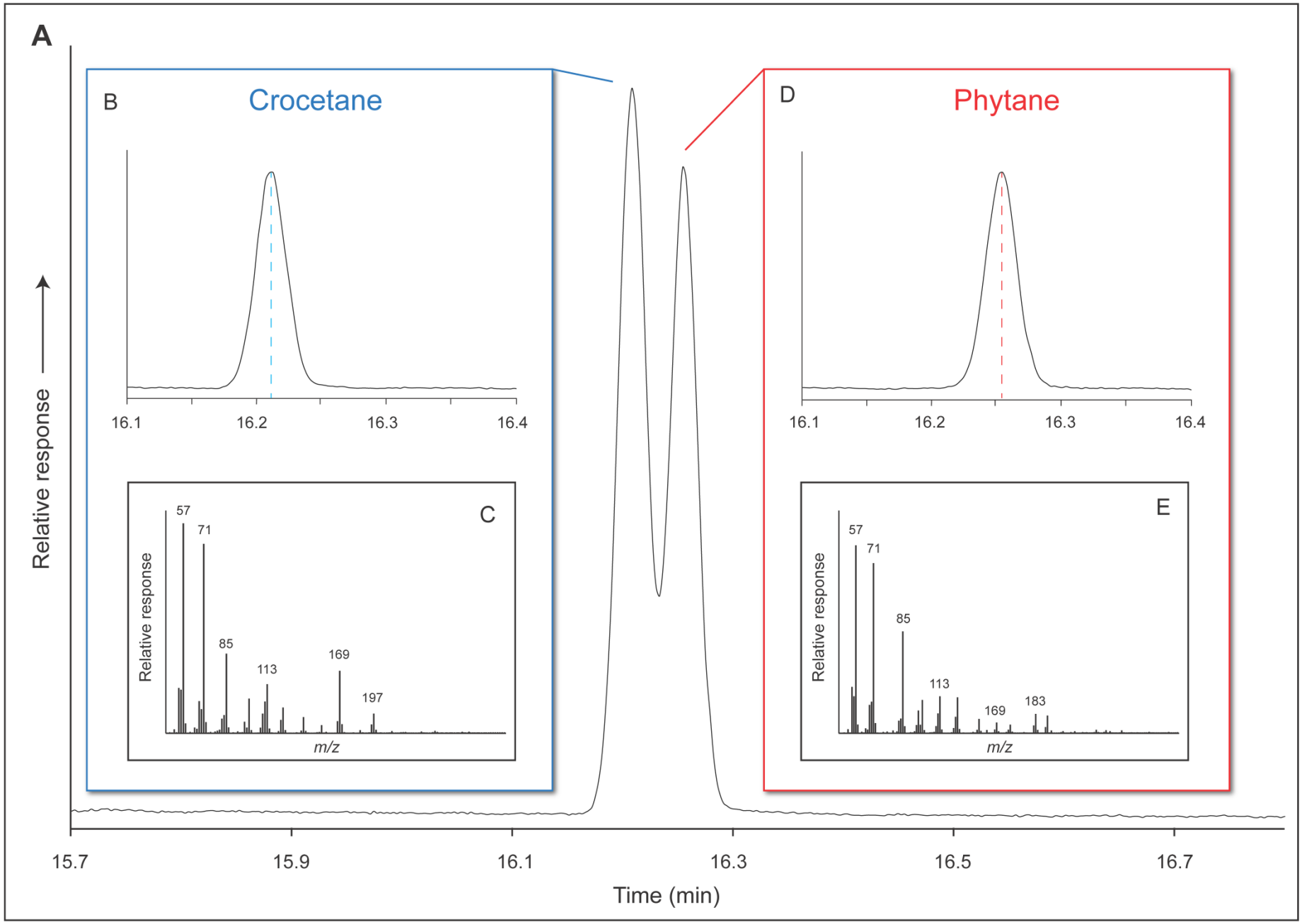

Crocetane separation
GC
GCxGC
DB-1701 Column
n-C18
Crocetane
Phytane
Retention time
BDEX (1st dim) - BPX50 (2nd dim)
m/z 85
Phytane
Crocetane
n-C18
2nd dimension retention time
1st dimension retention time


A
Relative response (%)
Time (min)
B
Crocetane
n-C18
Phytane
D
16
16.2
16.4
C
mass spectrum
of crocetane in
Blina-4
Relative response
m/z
57
71
85
113
169
197
E
Response (%)
n-C18
Crocetane
Phytane
16.1
16.2
16.3

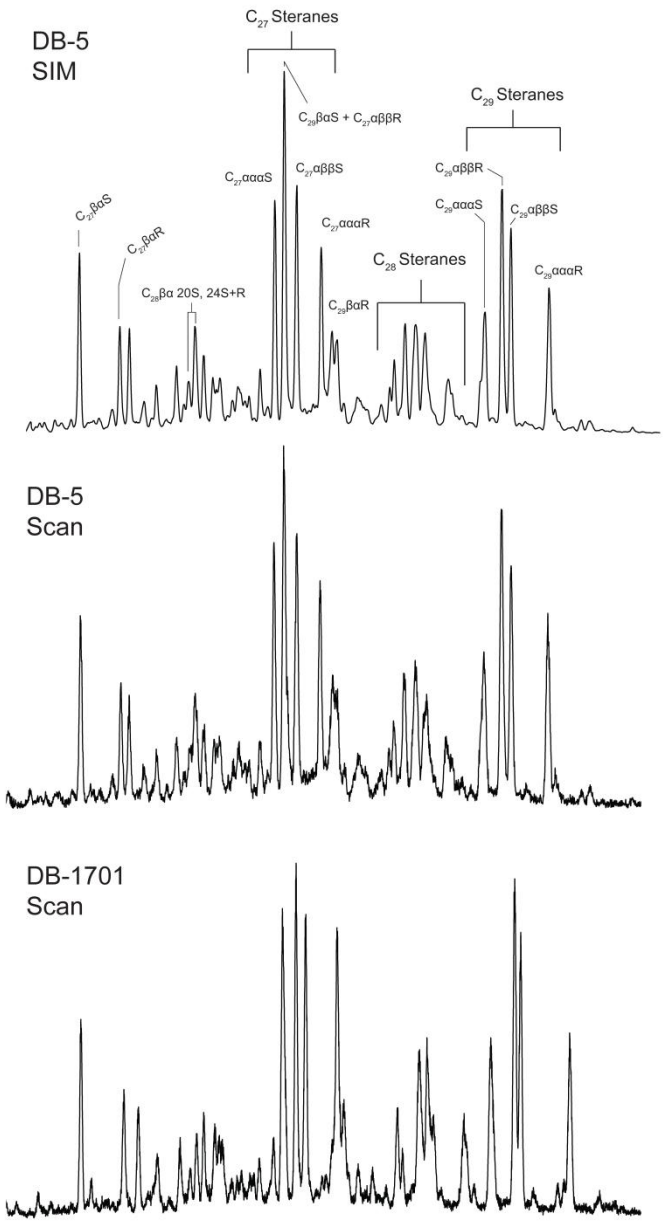

2nd dimension retention time
n-C16
norpristane
n-C17
pristane
n-C18
phytane
+
crocetane
n-C19
1st dimension retention time

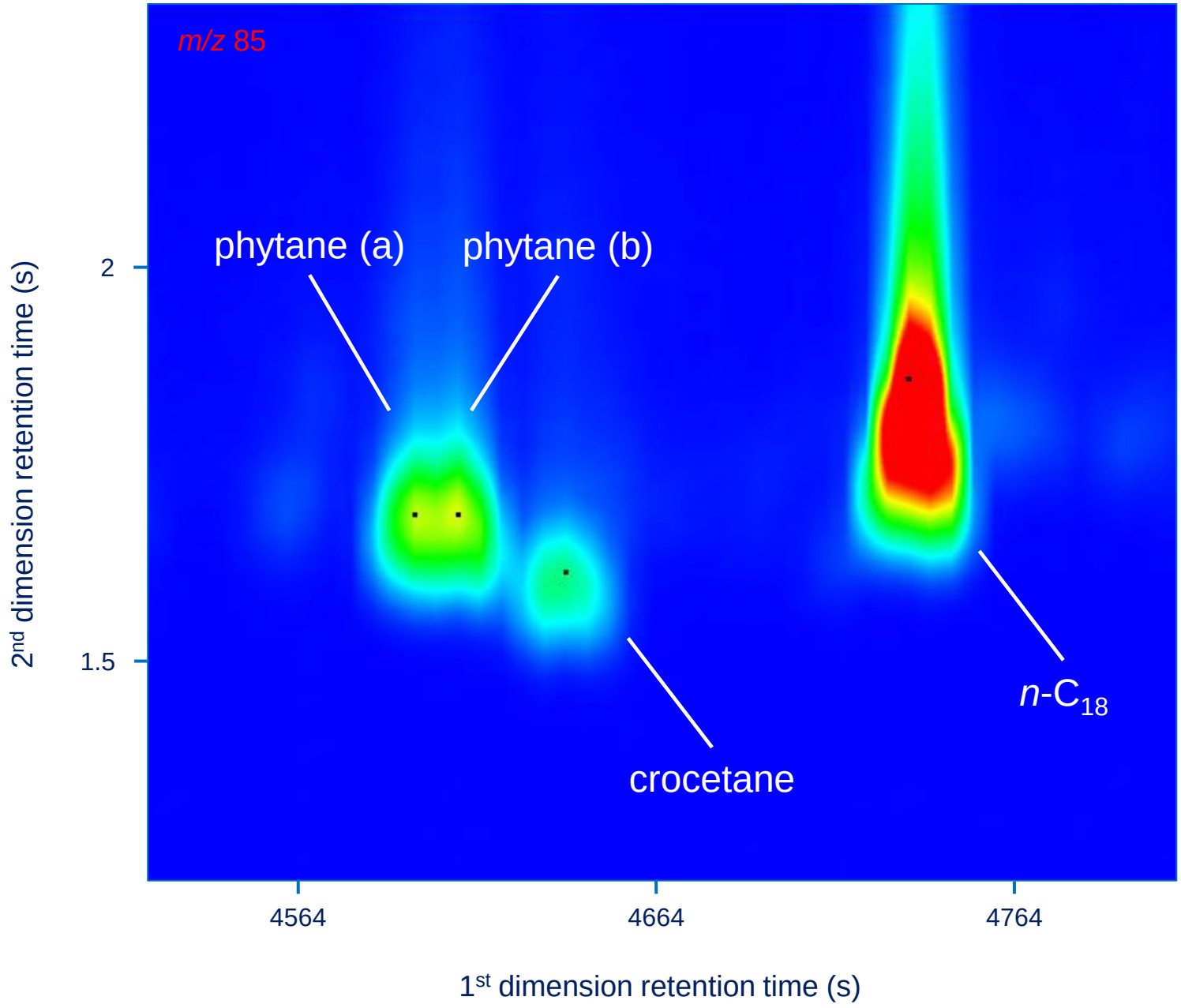

m/z 85 →
48ng phytane
16ng crocetane
48ng phytane + 16ng crocetane
m/z 113 →
48ng phytane
16ng crocetane
48ng phytane + 16ng crocetane
m/z 169 →
48ng phytane
16 ng crocetane
48ng phytane + 16 ng crocetane

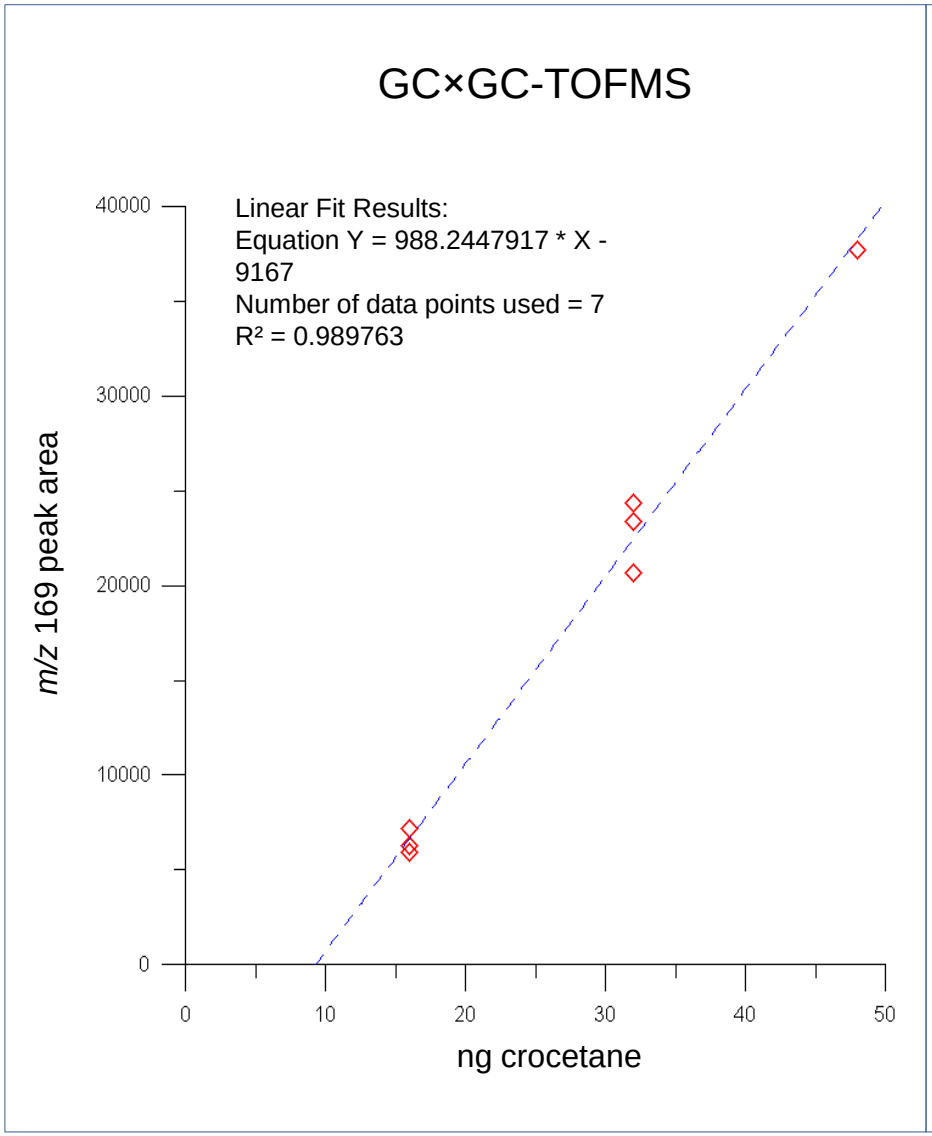

(a)
phytane
crocetane
3.3
3.1
2.9
2.7
2.5
4506
4556
4606
4656
(b)
phytane
crocetane
3.3
3.1
2.9
2.7
2.5
4506
4556
4606
4656
(c)
phytane
3.3
3.1
2.9
2.7
2.5
4506
4556
4606
4656
2nd dimension retention time (seconds)
1st dimension retention time (seconds)

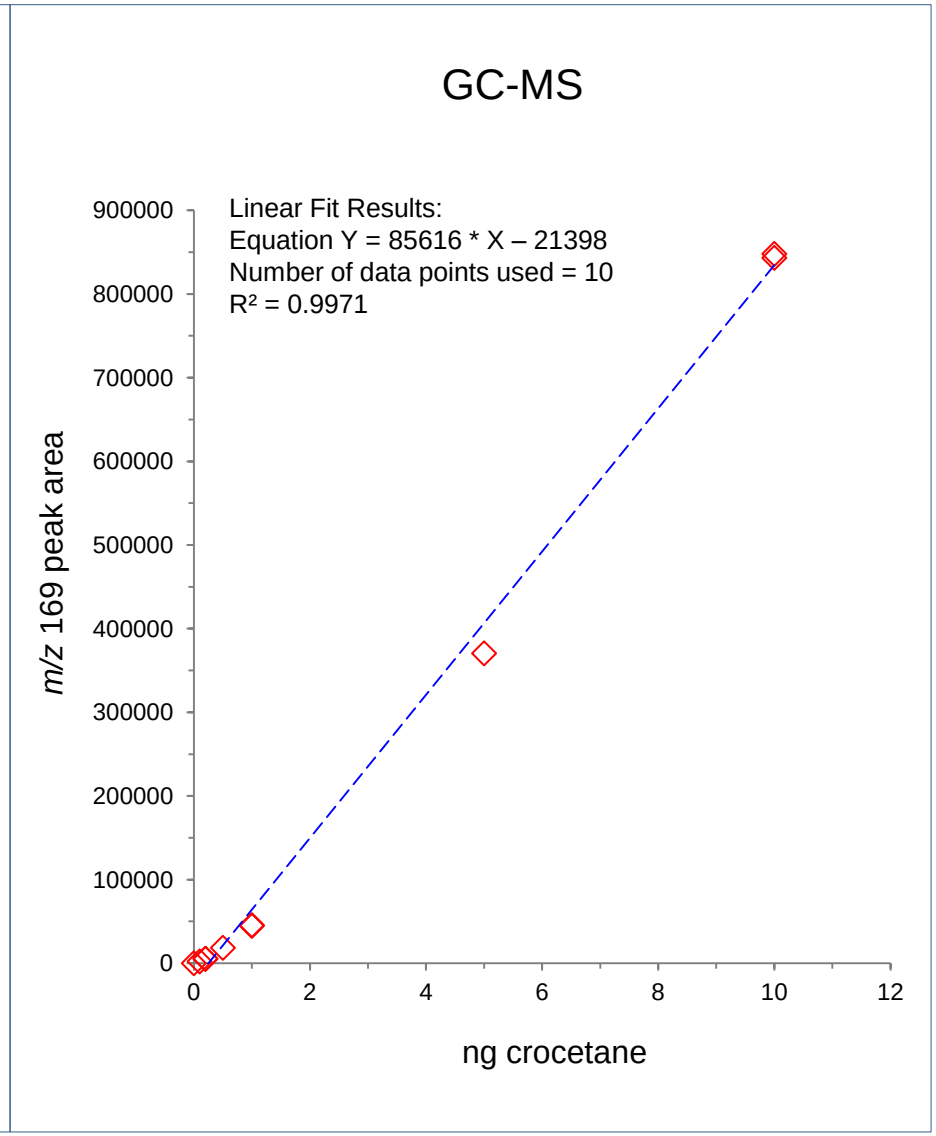

A
B
Crocetane
16.1
16.2
16.3
16.4
C
57
71
85
113
169
197
Relative response
m/z
D
Phytane
16.1
16.2
16.3
16.4
E
57
71
85
113
169
183
Relative response
m/z
Relative response
15.7
15.9
16.1
16.3
16.5
16.7
Time (min)

Blina-4 crude oil aliphatic fraction m/z 217




m/z 85
phytane (a)
phytane (b)
crocetane
n-C18
2
1.5
2nd dimension retention time (s)
4564
4664
4764
1st dimension retention time (s)


GC×GC-TOFMS
Linear Fit Results:
Equation Y = 988.2447917 * X - 9167
Number of data points used = 7
R² = 0.989763
m/z 169 peak area
ng crocetane
0
10000
20000
30000
40000
0
10
20
30
40
50


GC-MS
Linear Fit Results:
Equation Y = 85616 * X – 21398
Number of data points used = 10
R² = 0.9971
m/z 169 peak area
ng crocetane
0
100000
200000
300000
400000
500000
600000
700000
800000
900000
0
2
4
6
8
10
12