Journal of Chromatography A 1612 (2020) 460621

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Separation of crocetane and phytane and measurement of their compound-specific carbon isotopic compositions

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ARTICLE INFO

Article history:
Received 22 August 2019
Revised 9 October 2019
Accepted 12 October 2019
Available online 13 October 2019

Keywords:
Crocetane
Phytane
GC separation
Compound-specific isotopic composition
DB-17MS column

ABSTRACT

Crocetane and phytane are two isoprenoids isomers with similar molecular structures and often present together in methane-seep sediments and some Palaeozoic crude oils. Their commonly co-elution on gas chromatography is challenging for quality and quantity analysis, making it impossible to determine their compound-specific isotopic composition, and thus, insight their geological and geochemical significance. A new gas chromatography method is reported here using a DB-17MS column (50%-phenylmethyl polysiloxane as the stationary phase) that successfully achieved baseline separation of crocetane and phytane and can be used to accurately identify and quantify them on gas chromatography and gas chromatography–mass spectrometry. Routine steroids and terpenoids biomarkers can also be analysed simultaneously. Additionally, their compound-specific carbon isotopic compositions were also measured without matrix influence using this method. This is the first time that a simple chromatographic method for direct determination of compound-specific carbon isotopic composition of crocetane has been reported publicly.

1. Introduction

Crocetane (2,6,11,15-tetramethylhexadecane; Cr) is an irregular tail-to-tail C_{20} isoprenoid hydrocarbon (Fig. 1). Its occurrence is usually connected to methane-oxidizing archaea and sulphate-reducing bacteria and can be used as one of the diagnostic biomarkers of anaerobic oxidation of methane (AOM) [1–4]. Cr was also inferred to have an origin from Chlorobi carotenoids and can be seen as a potential biomarker for photic zone euxinia in high maturity samples [5,6].

Phytane (2,6,10,14-tetramethylhexadecane; Ph) is the most discussed head-to-tail isoprenoid biomarker in crude oils and sediment organic matter (Fig. 1). It can be used with pristane (Pr) together as Pr/Ph to indicate depositional redox conditions [7]. However, the proxy is often perturbed by thermal maturity and variable source input as well as co-elution with other isoprenoid hydrocarbons such as Cr with Ph [8]. Although Ph mostly originates from oxidation of the phytol side chain in chlorophylls during the diagenetic process [9], it can also derive from archaeal isopranyl lipids [10], with a plausible biological source of methane-oxidizing archaea [4].

The mass spectra of Cr and Ph are almost identical in the electron impact ionization (EI) mode (Fig. 1), but there are still some diagnostic differences [8]. For example, Cr yields a relatively high intensity of *m/z* 169 and nearly no *m/z* 183 ions (Fig. 2). They can be identified by gas chromatography–mass spectrometry (GC–MS). Improved measurements can be obtained using the diagnostic mass transitions of 197 → 127/126 and 169 → 126 for Cr and 183 → 127 for Ph by the gas chromatography–tandem quadrupole mass spectrometry (GC/MS/MS) [11].

Cr and Ph often occurred in microbial carbonate deposits [1] and some Palaeozoic crude oils together [5,12]. Because of their similar chemical structure, Ph and Cr often co-elute on most capillary columns, which interferes with their quantitation and isotope determination [5,8]. Their co-elution highlights the need for very careful identification and quantification of the trace amount of one isomer in the presence of higher amounts of another, even when using the GC/MS/MS. Baseline separation of Ph and Cr on gas chromatography using different columns has been repeatedly tested without an effective solution [13,14]. Chiraldex columns with cyclodextrin-based stationary phases provide a feasible method for baseline separation of Ph and Cr [15]. Recently, Spaak et al. [16] successfully separated Ph and Cr with a resolution (R) of 1.0 by a one–dimensional gas chromatography (GC) method using a DB-1701 column. They also provided a comprehensive two-dimensional gas chromatography chiral method, which is capable of separation of Cr and Ph in whole oils without any pre-separation work.

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https://doi.org/10.1016/j.chroma.2019.460621
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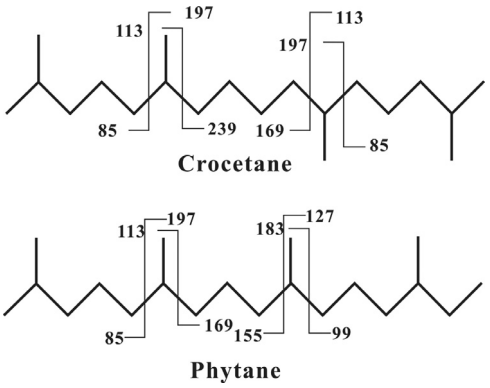

Fig. 1. Chemical structures and major mass fragments (electron impact ionization) of crocetane and phytane [17].

Stable carbon isotope compositions ($\delta^{13}C$) of Cr or Ph provide insights into their biological sources and can be used to trace back into the geological record [17,18]. For example, the extremely ^{13}C depleted of Cr can be used as a specific marker for the process of anaerobic oxidation of methane [19]. The $\delta^{13}C$ of Ph is also helpful to study microbial diversity [18]. However, commonly co-elution of Cr and Ph makes it impossible to directly measure their individual carbon isotope ratios [17]. So far, few effective chromatographic methods have been reported for the direct determination of $\delta^{13}C$ of Cr and Ph in a sample [15,16]. The $\delta^{13}C$ values can only be inferred indirectly by measuring the $\delta^{13}C$ of Pr and the combined Ph/Cr peaks, assuming that the $\delta^{13}C$ of Pr and Ph are equal, and calculating the $\delta^{13}C$ of Cr by difference [17]. Otherwise, only the stable carbon isotope composition of combined Ph/Cr is discussed [1,13].

Here, we report a one-dimensional GC method for the separation of Cr and Ph using a conventional non-chiral column with a flame ionization detector (FID) and mass spectrometry (MS) detector. This method provides an ideal and simple way to directly determine the compound-specific carbon isotope ratios of Cr and Ph avoiding their mutual interference and without too much pretreatment.

2. Materials and methods

2.1. Materials

Some authentic standards and artificial mixtures were used in this work (Table 1). The analyte E is the saturated fraction of a

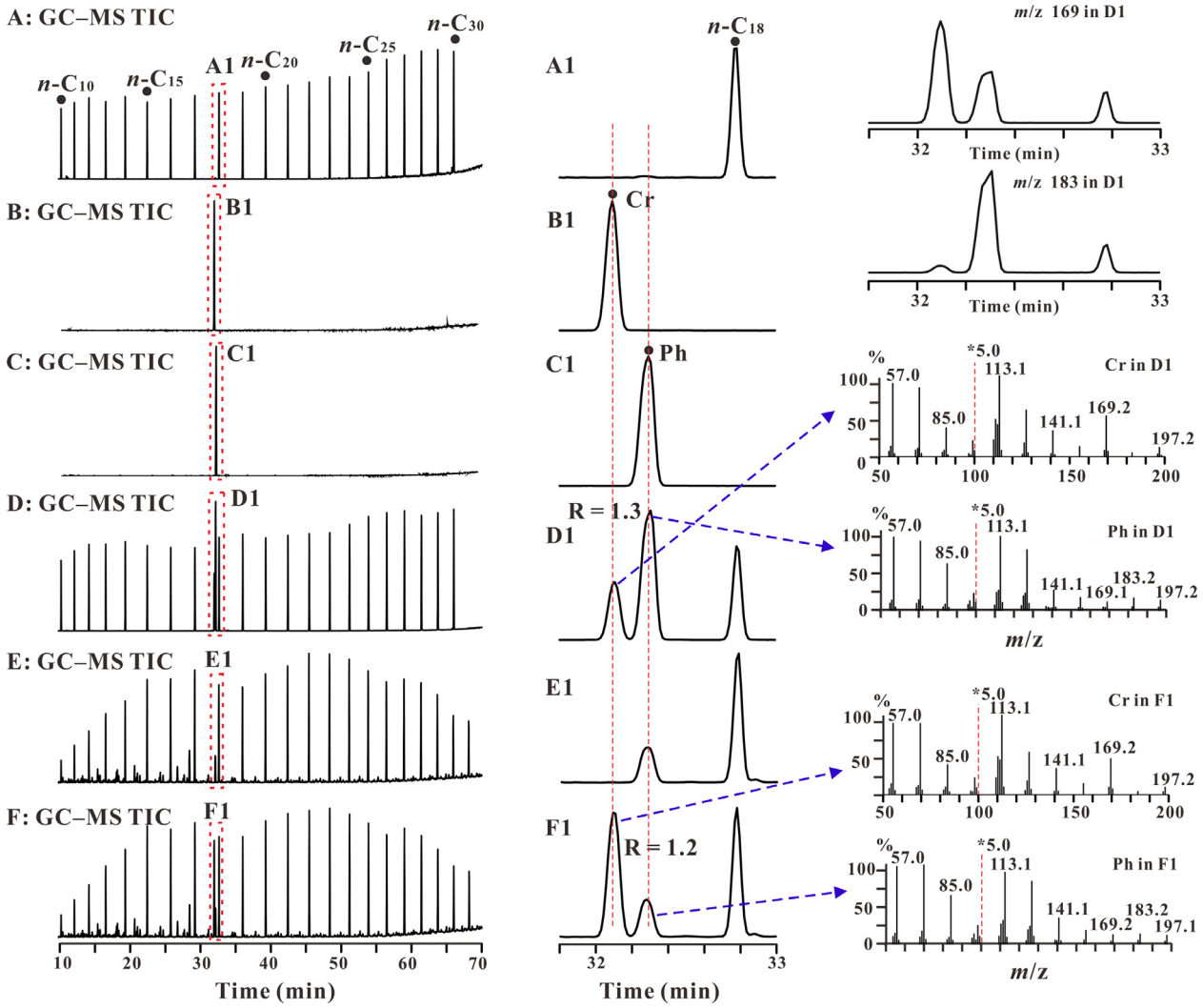

Fig. 2. Total ion chromatogram (TIC) of GC–MS for the different analytes (left), magnified partial chromatograms for the Cr, Ph, and n-C_{18} (middle), and partial m/z 169 and 183 mass chromatogram of analyte D and mass spectrum of Cr and Ph (right). A to F are the different analytes (Table 1). Cr = crocetane, Ph = phytane. R is resolution calculated based on [22].

Table 1
Details of the analytes.

Code	Analyte composition	Producer or references
A	n-C_7 to n-C_{30}	SUPELCO (Bellefonte, PA, USA)
B	Cr (Crocetane, 99.5%, CAS NO. 504-44-9)	Chiron AS (Trondheim, Norway)
C	Ph (Phytane, CAS NO. 638-36-8)	TRC Inc.(Toronto, ON, Canada)
D	n-C_7 to n-C_{30}, Cr and Ph	Artificial mixing of A, B and C in this work
E	Saturated fraction from a crude oil	Sample ID Gao898 [20]
F	Cr and Saturated fraction from crude oil	Artificial mixing of B and E in this work

crude oil originating from a lacustrine source rock [20], which lacks Cr but contains a certain amount of Ph. The analyte F is a mixture of the saturated fraction (E) and the authentic standard Cr (B). Analytes D and F were used to verify the accuracy of isotope measurements of Cr and Ph at different concentrations with mutual interference.

2.2. Apparatus and methods

GC–MS analysis was performed on a Shimadzu GC–MS QP2010 Ultra, equipped with a DB-17MS column (60 m × 0.25 mm i.d., 0.25 μm film thickness, 50%–phenyl-methyl polysiloxane as the stationary phase). The GC oven was held isothermally at 40 °C for 2 min, then ramped at 10 °C/min to 120 °C, and finally ramped to 300 °C at 3 °C /min holding for 30 isothermal. Helium was used as the carrier gas with a constant flow of 1.0 ml/min. The inlet temperature was 290 °C. The ion source was operated in EI mode (70 eV) with a source temperature of 230 °C. The mass spectrometer measurements were acquired in both selective ion monitoring (SIM, m/z 113, 127, 169, 183, 197, 191, and 217) and full scan modes in the scanning range from 50 to 550 m/z. The GC–FID analysis was performed on a Shimadzu GC QP 2010 Ultra. The GC conditions were the same as for the GC–MS analysis.

The compound-specific carbon isotopic composition ($\delta^{13}C$) analysis was performed on an Isoprime IRMS instrument interfaced to a HP6890 GC via a combustion interface (GC–IRMS). A mixture of standards of n-alkanes (C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{25}, C_{28}, C_{30} and C_{32}, from A. Schimmelmann of Indiana University) was measured one or two times daily to monitor the accuracy of the GC–IRMS system. The instrument requires that the $\delta^{13}C$ of each compound must be within ± 0.5‰ of the standard value. Except for using a 90 m DB-17MS column, the GC conditions were the same as for the GC–MS analysis. Every sample was analysed at least twice, and the deviation for the two runs was no greater than 0.5‰ for $\delta^{13}C$. The average for the two runs of each sample was reported relative to the VPDB standard.

3. Results and discussion

3.1. Separation of Cr and Ph

Three standard analytes A, B, and C were analysed by GC–MS respectively to confirm their elution sequence, in the order of Cr, Ph, and n-C_{18} on the DB-17MS column (Fig. 2). The elute sequence is clearly different from HP-5MS [21], DB-1701 [16], and chiral columns [11]. This is mainly attributed to the polarity difference and the mutual interactivity between the column and the target analytes.

Ph and Cr have common diagnostic fragment ions such as m/z 99, 113, 127, 141, 155, and 197 (Fig. 2). Additionally, Ph yields significant m/z 183 ions, while Cr yields nearly no m/z 183 ions. Despite of the different relative intensity of m/z 169 to 197, the accuracy of quality and quantity of Ph and Cr will be discounted a lot using the common diagnostic m/z 169 ion. Here, baseline separation of Ph and Cr was achieved on a DB-17MS column (Fig. 2). The resolution (R) for Ph and Cr in the mixed standards D reached to 1.3 using the calculating equation reported by McNaught and Winkinson [22]. The resolution reported here is obviously comparable with results using varieties of cyclodextrin stationary phase [15] and DB-1701MS columns [16], and is much better than conventional columns [21].

Cr was not detected in analyte E. Baseline separation of Ph and Cr was achieved in analyte F (R = 1.2, Fig. 2). Meanwhile, the resolution for Ph and n-C_{18} reached 3.9. The mass fragment ions and retention time of Cr and Ph are consistent in mixed standard D and analyte F (Fig. 2), indicating that no other compounds, such as straight-chain or branched-chain alkanes, cycloalkanes etc. in the saturate fraction of crude oil, might co-elute with Cr and Ph on this column. The excellent separation performance allows for accurate quality and quantity analysis of Ph and Cr. Furthermore, routine biomarkers of high molecular weight or boiling point can be eluted on the same column with one injection because the maximum temperature of DB-17MS reaches 325 °C and is equal to the common columns (e.g., HP-1MS and HP-5MS columns). This facilitates quality and quantity analysis of routine biomarkers, Ph, and Cr in one GC–MS analysis. The eluotropic sequence of hopanes and steranes is slightly different from that on the commonly used columns because of the different phase polarity. The detailed differences in eluotropic sequence require further research.

Cr, Ph, and n–C_{18} in analytes D and F can also be effectively separated by GC–FID with the DB-17MS column (Fig. 3). The perfect normal chromatographic peak shape and consistence retention time of Cr and Ph in analyte F compared with the standard samples (B, C and D) indicate that they have little possibility of co-elution with other compounds in the saturate fraction by the chro-

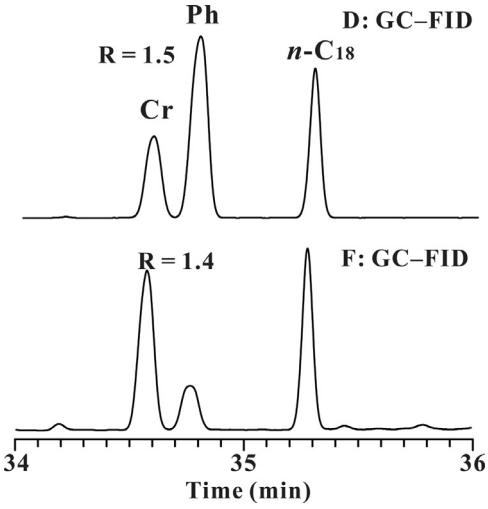

Fig. 3. Partial gas chromatogram (GC–FID) of the mixture of Cr, Ph, and n-alkane standards (analyte D) and the saturated fraction of a crude oil spiked with additional Cr standard (analyte F). Cr = crocetane, Ph = phytane. R is resolution calculated based on [22].

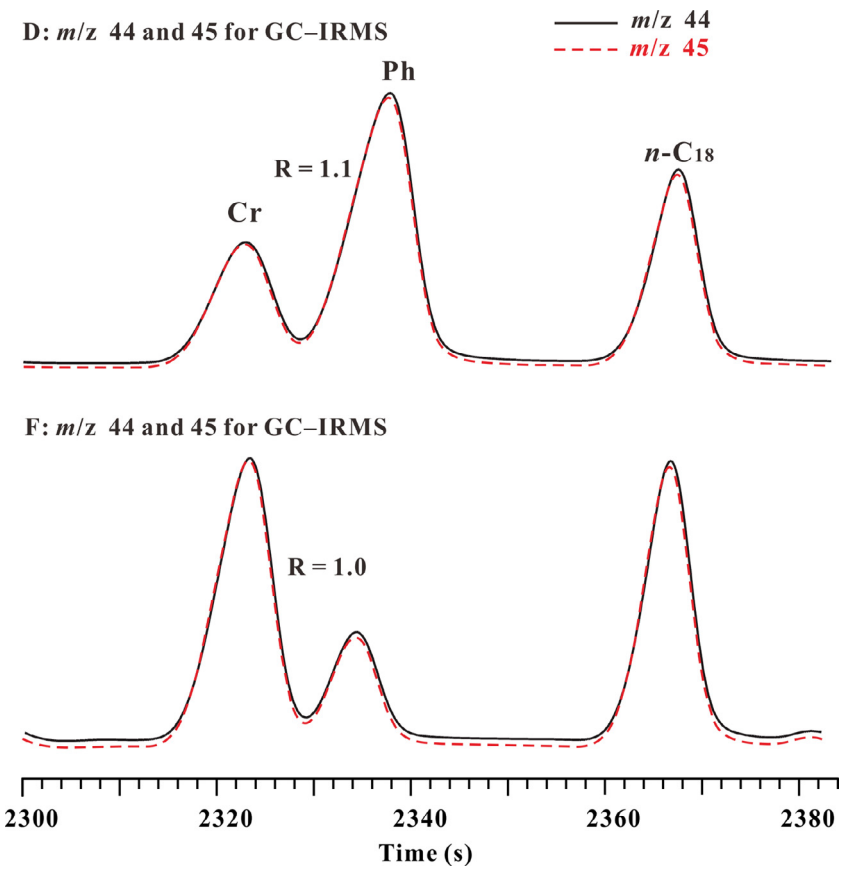

Fig. 4. Partial *m*/*z* 44 and 45 mass chromatograms of gas chromatography–isotope ratio mass spectrometry (GC–IRMS) for the analytes D and F. Cr = crocetane, Ph = phytane. R is resolution calculated based on *m*/*z* 45.

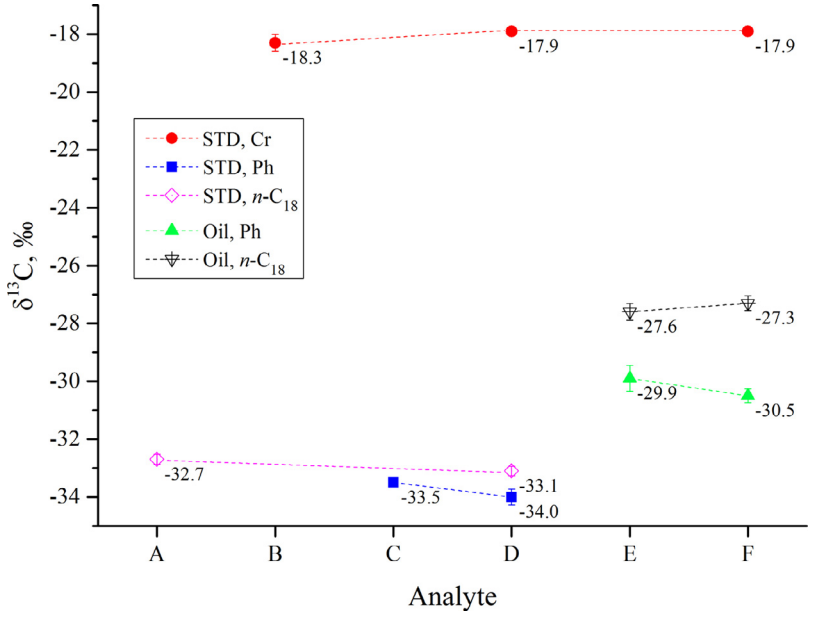

Fig. 5. Carbon isotope composition ($\delta^{13}C$) of Cr and Ph in different analytes (A to F). Cr = crocetane, Ph = phytane, STD = standard.

matographic method (Fig. 3). The resolution of Cr and Ph reached 1.5, and for Ph and *n*-C_{18} reached 4.4 in analyte D, and 1.4 for Cr and Ph and 4.4 for Ph and *n*-C_{18} in the artificial mixing analyte F. Regardless of the relative contents of Ph and Cr (Ph > Cr in analyte D, while Cr > Ph in analyte F) and the interference from other compounds such as high abundance isomer alkanes and biomarkers, the resolutions for Cr and Ph are very close in analytes D and F (R = 1.5 and 1.4, respectively). This indicates that the relative abundance of Cr and Ph, and the matrices have little influence on the resolution of Cr and Ph. Therefore, the GC–FID method reported

here can be used to quantitatively analyse Cr and Ph in other complex samples.

3.2. Compound-specific carbon isotopic composition of Cr and Ph

Nearly baseline separation of Cr and Ph was also achieved on DB-17MS column for GC–IRMS. The resolution of Cr and Ph in analytes D and F reached 1.0 and 1.1, respectively (Fig. 4). The separation of them by this chromatographic method of GC–IRMS allows for measurement of their compound-specific isotopic composition ($\delta^{13}C$, Fig. 5). The $\delta^{13}C$ of Cr in analytes B, D, and F are –18.3‰, –17.9‰, and –17.9‰, respectively, with relative standard deviations (RSD) of 0.23%, indicating that the relative abundance of Ph will not interfere with the isotope results of Cr. The $\delta^{13}C$ of Ph in analytes C and D are –33.5‰ and –34.0‰, with RSD of 0.35%. The $\delta^{13}C$ of Ph in the saturated fraction of the crude oil is –29.9‰, and after mixing with the standard sample of Cr, the $\delta^{13}C$ of Ph is –30.5‰, with RSD of 0.45%. The consistency of the $\delta^{13}C$ of Ph indicates the addition of Cr or the relative abundance of Cr does not influence the measurement of the $\delta^{13}C$ of Ph.

Previous measurement of $\delta^{13}C$ of Ph and *n*-alkanes often need further urea adduction or molecular sieve to separate *n*-alkanes and iso- and cyclic-alkanes after the group fractions, and then the $\delta^{13}C$ of *n*-alkanes and isoprenoids will be tested separately by GC–IRMS [23,24]. Here, the $\delta^{13}C$ of the paraffin compound (*n*-C_{10} to *n*-C_{30+}) can also be analyzed in a single run. For example, in the analysis of E and F, the $\delta^{13}C$ of *n*-C_{18} alkanes are –27.6‰ and –27.8‰, respectively (Fig. 4). This method will facilitate the analysis of compound-specific isotopic compositions of these compounds simultaneously.

4. Conclusions

Baseline separation of Ph and Cr was achieved on a DB-17MS column by GC–FID or GC–MS. It solved the difficulties in quality and quantity of Ph and Cr caused by their co-elution. It supplies a possible method to analyse routine biomarkers with Cr at the same time. Furthermore, this method can also accurately measure the compound-specific carbon isotopic composition of Ph and Cr. Meanwhile, the $\delta^{13}C$ of normal alkanes can be measured together, avoiding time-consuming sample preparation and losses of low boiling-point compounds. The $\delta^{13}C$ of Cr and Ph will be conducive to explain their origins and further unearth their geochemical implications.

Declaration of Competing Interest

We declared that we have no conflicts of interest to this work.

Acknowledgements

We appreciate Dr. Simon George and an anonymous reviewer for their critical comments that significantly improved the quality of the manuscript. We thank Drs. Shutao Gao and Hongxiang Guan for their help to this study. This work was supported by the State Key Laboratory of Organic Geochemistry Project (Grant No. SKLOGA2016-A08), the Natural Science Foundation of Guangdong Province (Grant No. 2017A030313236), and the National Natural Science Foundation of China (Grants Nos. 41502121 and 41973067). This is contribution No. IS–2760 from GIGCAS.

References

[1] D. Birgel, V. Thiel, K.-U. Hinrichs, M. Elvert, K.A. Campbell, J. Reitner, J.D. Farmer, J. Peckmann, Lipid biomarker patterns of methane-seep microbialites from the Mesozoic convergent margin of California, Org. Geochem. 37 (2006) 1289–1302.
[2] H. Niemann, M. Elvert, Diagnostic lipid biomarker and stable carbon isotope signatures of microbial communities mediating the anaerobic oxidation of methane with sulphate, Org. Geochem. 39 (2008) 1668–1677.
[3] T. Himmler, D. Birgel, G. Bayon, T. Pape, L. Ge, G. Bohrmann, J. Peckmann, Formation of seep carbonates along the Makran convergent margin, northern Arabian Sea and a molecular and isotopic approach to constrain the carbon isotopic composition of parent methane, Chem. Geol. 415 (2015) 102–117.
[4] J. Peckmann, V. Thiel, Carbon cycling at ancient methane–seeps, Chem. Geol. 205 (2004) 443–467.
[5] E. Maslen, K. Grice, J.D. Gale, C. Hallmann, B. Horsfield, Crocetane: a potential marker of photic zone euxinia in thermally mature sediments and crude oils of Devonian age, Org. Geochem. 40 (2009) 1–11.
[6] L.E. Hays, K. Grice, C.B. Foster, R.E. Summons, Biomarker and isotopic trends in a Permian-Triassic sedimentary section at Kap Stosch, Greenland, Org. Geochem. 43 (2012) 67–82.
[7] B.M. Didyk, B.R.T. Simoneit, S.C. Brassell, G. Eglinton, Organic geochemical indicators of palaeoenvironmental conditions of sedimentation, Nature 272 (1978) 216–222.
[8] J.N. Robson, S.J. Rowland, Synthesis, chromatographic and spectral characterisation of 2,6,11,15-tetramethylhexadecane (crocetane) and 2,6,9,13-tetramethyltetradecane: reference acyclic isoprenoids for geochemical studies, Org. Geochem. 20 (1993) 1093–1098.
[9] T.G. Powell, D.M. McKirdy, Relationship between ratio of pristane to phytane, crude oil composition and geological environment in Australia, Nat. Phys. Sci. 243 (1973) 37.
[10] S.J. Rowland, S.J. Hird, J.N. Robson, M.I. Venkatesan, Hydrogenation behaviour of two highly branched C 25 dienes from Antarctic marine sediments, Org. Geochem. 15 (1990) 4.
[11] P.F. Greenwood, R.E. Summons, GC–MS detection and significance of crocetane and pentamethylicosane in sediments and crude oils, Org. Geochem. 34 (2003) 1211–1222.
[12] C.J. Barber, K. Grice, T.P. Bastow, R. Alexander, R.I. Kagi, The identification of crocetane in Australian crude oils, Org. Geochem. 32 (2001) 943–947.
[13] C.J. Barber, T.P. Bastow, K. Grice, R. Alexander, R.I. Kagi, Analysis of crocetane in crude oils and sediments: novel stationary phases for use in GC–MS, Org. Geochem. 32 (2001) 765–769.
[14] V. Thiel, J. Peckmann, R. Seifert, P. Wehrung, J. Reitner, W. Michaelis, Highly isotopically depleted isoprenoids: molecular markers for ancient methane venting, Geochim. Cosmochim. Ac 63 (1999) 3959–3966.
[15] K. Huang, D.W. Armstrong, GC–MS analysis of crocetane, phytane and some of their stereoisomers using cyclodextrin-based stationary phases, Org. Geochem. 40 (2009) 283–286.
[16] G. Spaak, R.K. Nelson, C.M. Reddy, A.G. Scarlett, G.E. Chidlow, K. Grice, Advances on the separation of crocetane and phytane using GC–MS and GC×GC–TOFMS, Org. Geochem. 98 (2016) 176–182.
[17] K.E. Peters, C.C. Walters, J.M. Moldowan, in: The Biomarker Guide, second ed, Cambridge University Press, Cambridge, UK, 2005, p. 1155.
[18] B. Nabbefeld, K. Grice, R.J. Twitchett, R.E. Summons, L. Hays, M.E. Bottcher, M. Asif, An integrated biomarker, isotopic and palaeoenvironmental study through the Late Permian event at Lusitaniadalen, Spitsbergen, Earth Planet. Sci. Lett. 291 (2010) 84–96.
[19] V. Thiel, J. Peckmann, H.H. Richnow, U. Luth, J. Reitner, W. Michaelis, Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat, Mar. Chem. 73 (2001) 97–112.
[20] Z.W. Zhan, X.H. Lin, Y.R. Zou, Z. Li, D.Y. Wang, C. Liu, P.A. Peng, Chemometric differentiation of crude oil families in the southern Dongying Depression, Bohai Bay Basin, China, Org. Geochem. 127 (2019) 37–49.
[21] Y. Miyajima, Y. Watanabe, Y. Yanagisawa, K. Amano, T. Hasegawa, N. Shimobayashi, A late Miocene methane-seep deposit bearing methane-trapping silica minerals at Joetsu, central Japan, Palaeogeogr., Palaeoclimatol., Palaeoecol. 455 (2016) 1–15.
[22] G. Book, Compendium of chemical terminology, Int. Union Pure Appl. Chem. 528 (2014).
[23] K. Grice, R.d. Mesmay, A. Glucina, S. Wang, An improved and rapid 5A molecular sieve method for gas chromatography isotope ratio mass spectrometry of n-alkanes (C8–C30+), Org. Geochem. 39 (2008) 284–288.
[24] S. Yu, X. Wang, B. Xiang, J. Ren, E. Li, J. Wang, P. Huang, G. Wang, H. Xu, C. Pan, Molecular and carbon isotopic geochemistry of crude oils and extracts from Permian source rocks in the northwestern and central Junggar Basin, China, Org. Geochem. 113 (2017) 27–42.