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Short communication

Isolation of nine petroporphyrin biomarkers by reversed-phase high-performance liquid chromatography with coupled columns

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Abstract

It is difficult to directly study the petroporphyrin biomarkers in oil but is easier through the free porphyrins which are obtained after undergoing a lot of steps including extraction, purification, isolation and so on. In this work, nine free petroporphyrin compounds were successfully isolated by reversed-phase high-performance liquid chromatography. They are $C_{27}E$, $C_{28}E$, $C_{29}E$, $C_{30}E$, $C_{31}E$, $C_{29}D$, $C_{30}D$, $C_{31}D$ and $C_{32}D$. The isolation conditions were a C_{18} bonded column (5 μm, 150 mm×4.6 mm I.D.) in series with a –CN bonded column (5 μm, 150 mm×4.6 mm I.D.) used as stationary phase, a mobile phase of MeOH–water (97:3,v/v) at a flow-rate of 1.3 ml/min as elute and a detection wavelength of 400 nm. After isolation each fraction of petroporphyrin was collected according to the respective retention time and analyzed by mass spectrometry with direct sampler probe.

Keywords: Oil; Petroporphyrin biomarkers

1. Introduction

Biomarkers in crude oil play an important role when determining its genesis, delivery, maturity as well as the relationship of oil with rock. Studies in such fields, especially those on a molecular level, have great contribution to geochemistry. Petroporphyrin is one of the main biomarkers found in crude oil and sediment. The numerous type of petroporphyrin existing in crude oil [1,7,8], such as ETIO (etio-porphyrins), DPEP (deoxophylloerythroetio porphyrins), di-DPEP (dicyclic-deoxophylloerythroetio porphyrins), rhodo-DPEP (rhodo-deoxophylloerythroetio porphyrins), rhodo-ETIO (rhodoetio porphyrins) and rhodo-di-DPEP (rhodo-dicyclic-deoxophyll-oerythroetio porphyrins), have complex compositions and are present in low quantities, thus making it difficult to isolate and prepare individual porphyrin compounds as a standard sample for biomarker identification. Published research papers on studies of petroporphyrin are scarce. In this work, experiments are conducted on petroporphyrin isolation and preparation by reversed-phase high-performance liquid chromatography (RP-HPLC) with coupled columns. Nine free petroporphyrin compounds are isolated by mass spectrometry (MS) from crude oil. There are $C_{27}E$, $C_{28}E$, $C_{29}E$, $C_{30}E$, $C_{31}E$, $C_{29}D$, $C_{30}D$, $C_{31}D$ and $C_{32}D$.

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2. Experimental

2.1. Apparatus

The following apparatus was used: Varian Model 5060 HPLC system with variable-wavelength absorbency detector (190–750 nm), a Model UV-100; a Model VG7070 E-HF gas chromatography (GC)–MS system with direct sampler probe and a Shimadgu Model UV-3000 UV–Vis system.

2.2. Columns

The following columns were employed: a Micropak C_{18} banded column (5 μm, 150 mm×4.6 mm I.D.) and a Micropak –CN bonded column (5 μm, 150 mm×4.6 mm I.D.). A glass column (250 mm× 20 mm I.D.) packed with silica gel G (40~100 μm) was also used.

2.3. Chemicals

Methylene chloride, hexane, petroleum ether, 2-propanol and toluene (all analytical-reagent grade) were obtained from Beijing Chemical Plant (Beijing, China); HPLC-grade methanol was obtained from Siyou Chemical Plant (Tianjin, China); siliceous earth (200~500 μm) was obtained from Tianjin Second Chemical Plant (Tianjin, China); methyl sulfuric acid from Shanghai Chemical Plant (Shanghai, China) and silica gel G (40~100 μm) from Qingdao Hairang Chemical Plant (Shandong, China).

2.4. Extraction of crude petroporphyrins

A 200-g amount of crude oil from Liaohe (China) was weighed. The viscous crude oil sample was diluted with 200 ml hexane and 200~500 μm of siliceous earth was added to the diluted oil sample.

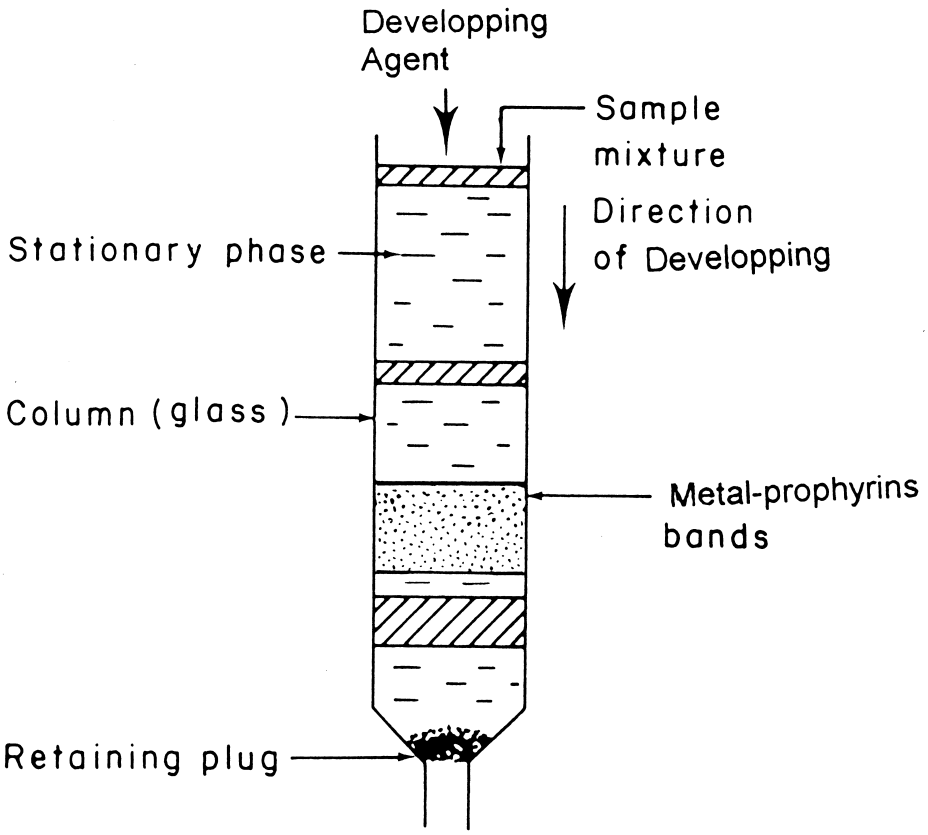

Fig. 1. Purification of petroprophyrins by dry LC.

The ratio of siliceous earth and diluted oil is (7.5:10, v/v). The oil was dispersed on particles of siliceous earth for effective extraction. With evaporation of hexane, crude oil sample was uniformly loaded onto particles of siliceous earth. These particle carriers were mixed with 300 ml of extraction solvent (two times) which was a combination of methanol–methylene chloride (1:2, v/v). Three hours of sample mixing with application of an ultrasonator vibrator was carried out before the upper clear solution portion was taken out. The extraction solvents from the upper clear solution were eliminated in a rotating vacuum drier to obtain the crude extract of petroporphyrins [2,9].

3. Results and discussion

3.1. Purification by dry LC and thin-layer chromatography (TLC)

Because the crude extract was very complex mixture (including a lot of alkyl hydrocarbons, aromatic hydrocarbons, high-molecular-mass organic compounds, a small amount petroporphyrins and others), it was necessary to further purify the extract by dry LC and TLC. The purpose of using dry LC was to save developing solvent and increase the treatment volume.

For the dry LC method, the silica gel G was put into a glass column to a final bed height of 18 cm. Then the extracted sample was added to the top of bed and developed with a mix solvent of petroleum ether–toluene–methylene chloride (3:1:1, v/v/v). While the sample was developing the rosy color ring metal-porphyrins appeared in the column [2]. Developing was stopped after the mix solvent reached the bottom of the glass column. With evaporation of solvent in column the silica gel bed became desiccated. The bed was then removed from the column by applying pressure from the end of the column. The rosy color ring was cut and re-filled into a small column. This column was eluted with methylene chloride to obtain metal-porphyrin biomarkers as shown in Fig. 1. If necessary further purification can be completed by TLC with the same silica gel G and developing agent as shown in Fig. 2.

The metal-porphyrin sample was demetallized under return flow with methyl sulfuric acid, 2-propanol and toluene at 110°C for 1.5 h. Neutralization was conducted for the return solution with solid $NaHCO_3$ and extraction with methylene chloride to obtain the free petroporphyrins. Free petroporphyrin biomarkers have five characteristic peaks on UV–Vis

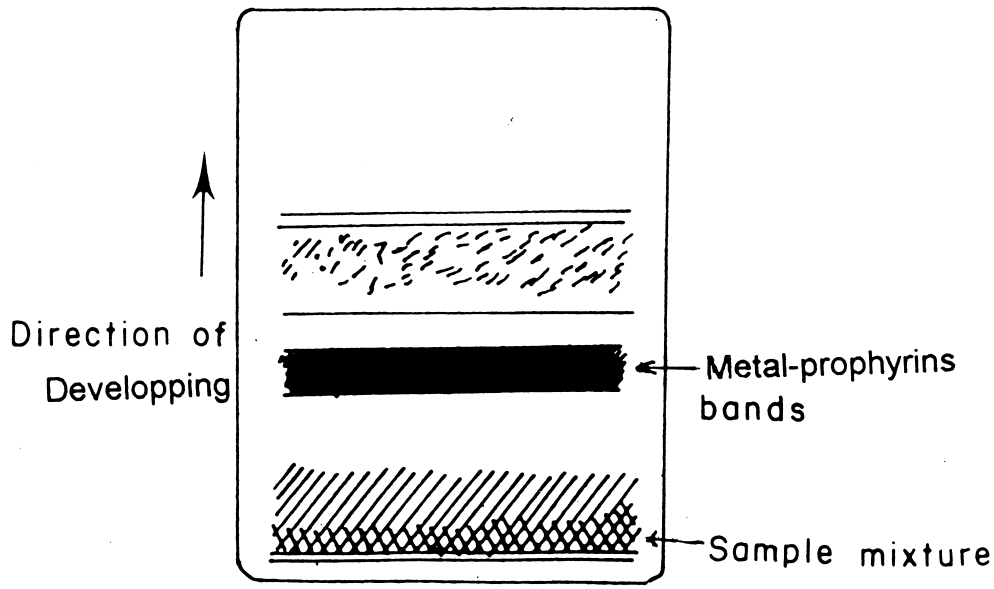

Fig. 2. Purification of petroprophyrins by TLC.

spectrum at wavelengths of 398, 496, 528, 564 and 616 nm.

3.2. Isolation of porphyrins by HPLC

Compared with most of the published papers on the separation of petroporphyrins, which are normally conducted with normal-phase or reversed-phase columns, this work is done by means of reversed-phase HPLC with coupled columns which features a simple solvent system, a high efficiency, good repeatability and stability [3,10]. The experimental results from this study indicate that a successful separation can be obtained with the application of a C_{18} bonded column in series with a –CN bonded column as stationary phase and MeOH–water as mobile phase [4]. Separation procedure and conditions are listed in Table 1. The possible separation mechanism is believed to be a selective separation according to the functional group which takes place in the –CN column, where certain types of petroporphyrin were segregated at first. The C_{18} bonded column provides further separation.

Eighteen samples of petroprophyrins in oil and rock from the Xinjiang, Liaohe, Yumen areas were analyzed by means of the established HPLC method and their characteristic fingerprints obtained. Fig. 3 illustrates the fingerprint of petroporphyrins biomarkers in oil from Liaohe.

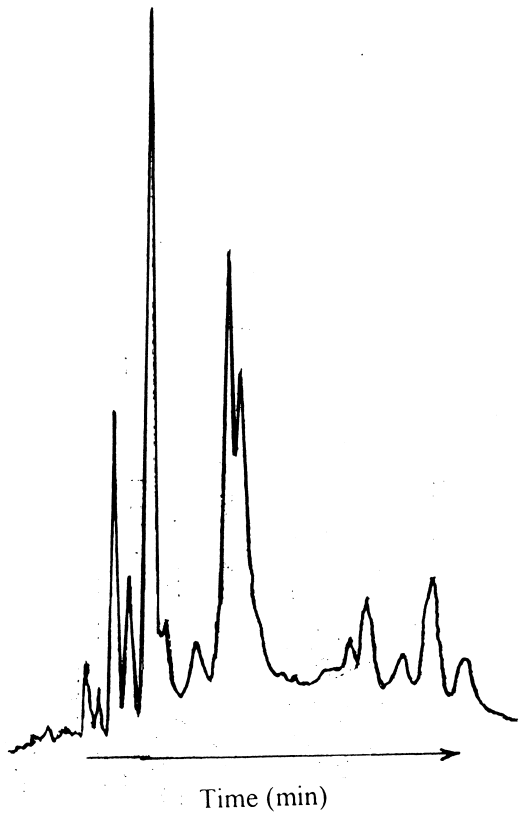

Fig. 3. Chromatograms for separation of petroporphyrins by HPLC. Chromatographic conditions: C_{18} in series with a –CN bonded column, MeOH–water (95:5), flow-rate 1.2 ml/min (see Table 1), injection amount 20 μl, detection wavelength 400 nm.

3.3. Collection and determination of free porphyrin fractions

The purpose of collection of free porphyrin fractions is the preparation of a single porphyrin compound as the standard material for use in the identification of porphyrin fingerprints.

A preparation method with good repeatability is needed for collecting porphyrin compounds. Modification of the above mentioned HPLC separation conditions were as follows: (1) use of an isocratic elution to replace the gradient elution to ensure the reproducibility of retention time; (2) keeping the mobile phase (MeOH–water; 97:3,v/v) at a constant flow-rate, 1.3 ml/min; and (3) increasing the sample size from 20 μl to 200 μl. Fig. 4 shows the HPLC isolation graph under the modified conditions. The shaded portion represents an overlapping peak area that is mainly caused by the increased sample size and the changed elution process. Fractions from this area should be avoided [5]. All other fractions collected need to be dried under a vacuum evaporator to eliminate the elute. The residual resulting from the drying process are the isolated petroporphylin compounds. The identities of the

Table 1
Procedure and conditions of coupled column HPLC

No.	Time (min)	Event	Value
1	0.0	Flow-rate	1.2 (ml/min)
2	0.0	MeOH	95%
3	17.0	Flow-rate	1.5 (ml/min)
4	20.0	Flow-rate	1.7 (ml/min)
5	30.0	MeOH	100%
6	50.0	MeOH	95%

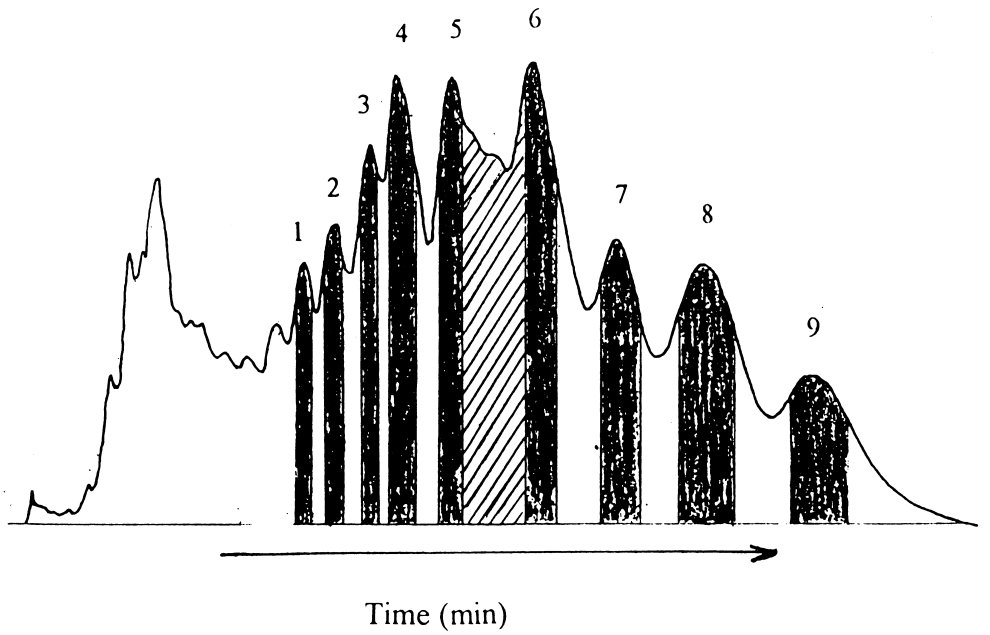

Fig. 4. Chromatograms for isolation and preparation of nine compounds of petroporphyrin. Chromatographic conditions: C_{18} in series with a –CN bonded column, MeOH–water (97:3), flow-rate 1.3 ml/min, injection amount 200 μl, detection wavelength 400 nm.

porphyrins in the nine residuals were established using MS.

The sample was directly injected into the ion source by a probe (electron impact; EI type). The electron energy was 18 eV and the source temperature was 270°C. The MS spectrum is shown in Fig. 5 and other results are listed in Table 2. Because the structure of petroporphyrins is cyclical and stable so the molecular ion peak is very strong and the fragment peaks are a few [6]. In this case, there is a preponderant molecular ion peak and a few non-homologous fragment peaks for every isolated porphyrin fraction in this work. It is clear that the isolated porphyrin compound was more pure and the isolation method successful. By way of example there is a $m/e=436$ major peak for $C_{29}E$, $m/e=450$ for $C_{30}E$, $m/e=462$ for $C_{31}D$ and $m/e=476$ for $C_{32}D$ in Fig.5.

4. Conclusions

In this paper, an available method for isolation and preparation of petroporphyrins was established. The method has good repeatability and stability. Nine single petroporphyrin components were obtained by RP-HPLC with coupled columns. The MS analysis results illustrate that collected petroporphyrin fractions are more pure. The results of this work will help to determine the chromatography fingerprint of petroporphyrins in an oil and thereby aid quantitation. It is important that an oil could be fingerprinted for these porphyrin components and quickly characterized. This procedure has used to successfully study petroporphyrins in oil from Yumen oil.

Of course, it also provides important information, at a molecule level, such as a better understanding of petroporphyrin biomarkers, the relationship of crude oil with the sedimentary environment, biodegradability, maturation and other geochemical aspects.

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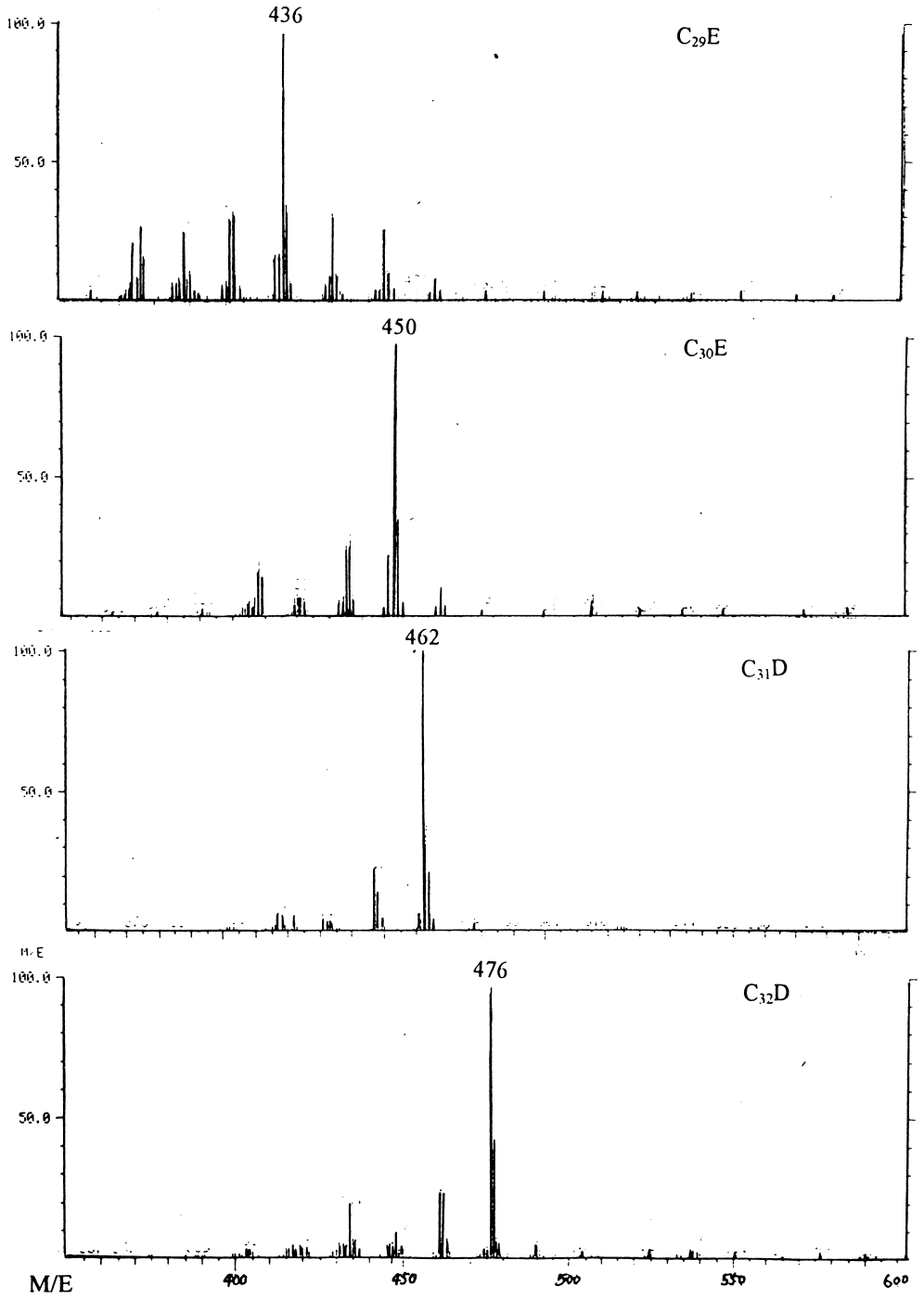

Fig. 5. Mass spectrum of collected petroporphyrin fraction.

Table 2
HPLC–MS analysis results of fractions

Fraction No.	Retention time (min)	*m/e*	Porphyrin name
1	10.23	408	$C_{27}E$
2	11.06	436	$C_{29}E$
3	12.14	422	$C_{28}E$
4	13.23	464	$C_{31}E$
5	15.88	450	$C_{30}E$
6	18.52	448	$C_{30}D$
7	21.30	434	$C_{29}D$
8	24.32	476	$C_{32}D$
9	27.89	462	$C_{31}D$

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