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An effective pre-treatment method for the determination of short-chain fatty acids in a complex matrix by derivatization coupled with headspace single-drop microextraction

Yuan Chen, Yun Li*, Yongqiang Xiong, Chenchen Fang, Xiaotao Wang

State Key Laboratory of Organic Geochemistry (SKLOG), Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

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ABSTRACT

We have developed a sample preparation method involving derivatization combined with headspace single-drop microextraction (HS-SDME) for the determination of short-chain fatty acids (SCFAs) in complex matrices. The derivatization of SCFAs was conducted using the BF3/ethanol method prior to HS-SDME. The HS-SDME extraction conditions for the derivatization products (ethyl esters) of SCFAs were optimized using 1.0 μL of dibutylphthalate (DBP), 1000 rpm stirring speed, 30% (w/v) NaCl, 20 min extraction time, and 7 mL of sample solution in a 12 mL vial. Quantitative determination of ethyl esters was performed using gas chromatography (GC). Linear calibration curves and excellent reproducibility were obtained using these optimized extraction conditions. Compared with our previous work, the significantly lower detection limits (0.11, 0.017, 0.0060, and 0.0024 μg/mL for C_2 to C_5 SCFAs, respectively) indicate that this new method is suitable for quantitative analysis of SCFAs in complex matrices, such as the RuO4 oxidation products of kerogen or asphaltene.

1. Introduction

Kerogen and asphaltene are insoluble macromolecular organic matter (OM), which is common in hydrocarbon source rocks and crude oils. The structures of kerogen and asphaltene are mainly composed of polyaromatic nuclei, with aliphatic rings, alkyl side chains, and heteroatoms (e.g., oxygen, nitrogen, and sulfur). Developing a better understanding of the molecular structures of kerogen and asphaltene will assist in evaluating their sources and origin. A number of studies have revealed that the abundance of aliphatic carbon in kerogen gradually decreases and that carbon chains shorten with increasing maturity, whereas the content of aromatic carbon increases [1]. The composition and distribution of alkyl side chains on the aromatic structures of macromolecular OM are largely related to the nature of the source rocks [2]. Moreover, the structural characteristics of asphaltenes in petroleum may provide unique insights into the history of crude oils [3–5].

Ruthenium-ion-catalyzed oxidation (RICO) is a common approach for releasing alkyl side chains from macromolecular OM. The method has high selectivity that can quantitatively oxidize aromatic carbons to carbon dioxide, whilst maintaining the structural integrity of aliphatic and naphthenic units [4,6]. Aromatic-attached aliphatic appendages are converted to their corresponding carboxylic acids, with the aromatic carbon at the site of attachment becoming a carboxylic carbon on the carboxylic acid [2,4,7,8]. Therefore, the amount and distribution of the carboxylic acids produced by the RICO reaction can be used to estimate the proportion and chain length distribution of alkyl groups attached to aromatic carbons, as well as that of the methylene bridges connecting two aromatic units [4,5,9–12]. In addition, the carboxylic acids liberated from the RICO reaction of petroleum asphaltenes can be used for oil–source and oil–oil correlations [2,5,8,9].

Most previous studies have focused on long-chain fatty acid products (C_{6+}) of the RICO process, and less work has been conducted on short-chain fatty acids (SCFAs), due to their volatile, hydrophilic, and highly polar nature. The RICO reaction system includes both organic and aqueous phases. The fatty acids in the organic phase are typically collected by extraction with organic solvent. However, SCFAs are prone to also being in the aqueous phase, and thus organic solvent extraction may not work for SCFAs. Moreover, conventional sample pre-treatment processes, including extraction, concentration, and derivatization will inevitably cause a loss of volatile SCFAs, resulting in incomplete information on the SCFAs.

The SCFAs examined in this study represent low molecular weight organic acids, mainly including acetic acid (C_2), propionic acid (C_3), butyric acid (C_4), and valeric acid (C_5). The primary problem is how these SCFAs can be extracted from complex matrices by

* Corresponding author. Tel.: +86 20 85290744; fax: +86 20 85290706.
E-mail address: yunli1002@163.com (Y. Li).

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the RICO process, particularly from the aqueous phase, with as little loss as possible. In addition, the high polarity of SCFAs limits their direct analysis by gas chromatography (GC) or gas chromatography mass spectrometry (GC–MS). As such, derivatization is often necessary prior to the GC or GC–MS analyses. For example, esterification with phenacyl bromide has been applied to convert SCFAs to higher molecular weight esters to reduce the loss of SCFAs and increase the derivatization efficiency [7,9]. Peng et al. [4] used octadecylation for the determination of relatively low molecular weight fatty acids ($C_{<12}$). It is notable that common derivatization reagents are usually moisture-sensitive, which requires there be no traces of water in the reaction system. However, during esterification, the removal of water is laborious and time-consuming, and the longer reaction times and higher temperatures required may cause more acetic acids to be generated by acetonitrile (CH_3CN) hydrolysis [5,7]. Recently, water-phase derivatization methods for fatty acids in water have been reported, with some performed prior to extraction [13] and some performed simultaneously during extraction in a solvent micro-drop [14]. However, these studies have shown that the water-phase derivatization is of low reaction yield, which may be due to reagent hydrolysis or catalyst dissolution in water. During derivatization in a solvent micro-drop it is also difficult to control the reaction temperature, which can potentially compromise the linearity of the reaction yield. In comparison, methanol coupled with sulfuric acid has been successfully applied to the derivatization of formic acid in aqueous samples and obtains acceptable recoveries [15], which may give inspiration to the derivatization of SCFAs in the oxydate of RICO reaction.

Headspace single-drop microextraction (HS-SDME) is a rapid, simple, inexpensive, and environmentally friendly sample preparation technique, in which a single liquid collecting drop is suspended from the tip of a microsyringe needle and exposed to the headspace of a stirred sample solution [16]. And there are several factors that could influence the efficiency of HS-SDME, such as drop solvent type[17,18], microdrop volume [19,20], extraction time [21] and so on. In order to avoid the loss of SCFAs during the pre-treatment processes, we have previously tested various extraction technologies, and the HS-SDME extraction method coupled with GC–flame ionization detection (FID) analysis has been successfully used for the analysis of SCFAs in RICO products [21]. 1-Butanol was used as an extraction solvent, and the SCFAs in the aqueous phase extracted by HS-SDME were directly analyzed by GC coupled to a HP-FFAP fused silica capillary column (30 m × 0.25 mm × 0.25 μm) without derivatization. Although this method is a promising tool for the determination of volatile SCFAs in RICO products from complex matrices, the chosen extraction solvent (1-butanol) has no enrichment effect for the SCFAs, and this may hamper its application to low concentration analysis of SCFAs, particularly as is the case for highly mature kerogen or asphaltene. Moreover, the complicated matrix injected directly without any sample preparation is damaging to the chromatographic column, even in the case of a polar column (e.g., HP-FFAP). Based on our experience, after analysis of 30–50 samples, the column efficiency degrades to the point where it can no longer be used.

Here we report a modified method that couples derivatization with HS-SDME to determine SCFAs in the RICO products of kerogen and asphaltene. Prior to HS-SDME, SCFAs in the RICO products were subjected to derivatization using ethanol and BF_3–diethyletherate catalysis. After derivatization, HS-SDME was used to extract the corresponding ethyl esters of the SCFAs. The objective of this study was to develop an improved method for analysis of SCFAs in RICO products in terms of detectability (selectivity and sensitivity) of the target analytes, and of reducing potential degradation to the column or analysis instrument. To do this, we explored options for optimizing the derivatization and extraction conditions.

2. Experimental

2.1. Chemical reagents

Ethyl acetate (99%), ethyl propionate (99%), ethyl butyrate (99%), ethyl valerate (98%), methyl valerate (99%), ethylbenzene (99%), boron (tri)fluoride diethyl etherate (98%), formic acid (97%), and ruthenium(III) chloride hydrate (PGM basis; 99.9%) were all purchased from Alfar Aesar China (Tianjin, China). Sodium periodate, potassium hydroxide, sodium chloride, and ethanol were obtained from Qianhui Chemicals and Glassware (Guangzhou, China). Carbon tetrachloride (HPLC; ≥99.8%) was purchased from Merck (Shanghai, China). Acetonitrile (HPLC; 99.9%) was purchased from CNW Technologies GmbH. All the water used in the experiments was ultrapure water from a Milli-Q Integral Water Purification System.

One mixed stock solution was prepared with 51.1, 6.54, 6.85, and 7.16 mg/mL concentrations of short-chain fatty acid ethyl esters (SC-FAEEs), respectively, by dissolving the appropriate amounts of each SC-FAEE in ethanol. The stock solution was stored at 4 °C and used to prepare working solutions by dilution with ethanol.

Toluene that was used as a surrogate standard for the volume calibration of the sample solutions was prepared in ethanol at a concentration of 5.59 mg/mL. Methyl valerate in ethanol at a concentration of 1.10 mg/mL was used as an internal standard for the quantification of SC-FAEEs.

2.2. Ruthenium-ion-catalyzed oxidation reaction

Ethylbenzene was used as a model compound to examine the efficiency of our whole procedure, including RuO_4 oxidation and derivatization. Approximately 3.48 mg of ethylbenzene, 2 mL of acetonitrile, 2 mL of carbon tetrachloride, 1 mg of ruthenium trichloride trihydrate, 3 mL of water, and 320 mg of sodium periodate were added to a 12 mL vial, and the vial was then sealed with a rubber septum and an aluminum cap to prevent loss of volatile compounds. The mixture was shaken for 24 h at 35 °C in a water bath, and then adjusted to pH > 9 by addition of 1 M KOH solution. After centrifugation, the supernatant was transferred to a 25 mL glass vial, and then dried in an oven at 110 °C for 2 h.

2.3. Derivatization procedure

BF_3–methanol esterification is one of the most commonly used methods for the derivatization of fatty acids. In our study, ethanol was used as the derivatization reagent to convert the SCFAs to their corresponding ethyl esters by boron (tri) fluoridediethyl etherate catalysis. Formic acid was used to adjust the pH of the reaction solution. A reaction solution of 0.5 mL boron (tri) fluoridediethyl etherate, 0.5 mL ethanol, and 0.2 mL formic acid was added to the vial with the dried degradation products and sealed immediately. The sealed vial was then placed in a water bath (85 °C) for 50 min.

2.4. Headspace single-drop microextraction process

After derivatization, 100 μL of toluene solution (5.59 mg/mL) was added as a spike to the vial for sample volume calibration during SC-FAEE quantification. Following this, 60 μL of the product solution and 30 μL of methyl valerate were sequentially added as spikes to a 12 mL glass vial containing a certain volume of NaCl solution, along with a magnetic stir bar. The vial had been previously closed with a rubber septum and sealed with an aluminum cap to prevent sample loss. A 10 μL microsyringe was used as both the extraction and injection syringe. First, a volume of extraction solvent was quantitatively drawn into the microsyringe. The syringe needle was then inserted through the rubber septum of the

sample vial until its tip was 0.5 cm above the surface of the working or sample solution. The syringe plunger was depressed slowly and a solvent droplet was suspended from the needle tip. During the extraction, the microsyringe was fixed above the extraction vial using a metal clamp. After extraction, the droplet was retracted into the needle and injected immediately into the GC system for analysis.

2.5. Gas chromatography

GC analyses were conducted using an Agilent 7890A GC system fitted with a flame ionization detector and an HP-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm). The injection and detection temperatures were both 300 °C and the injection was operated at a 5:1 split mode. Nitrogen (≥99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The GC oven temperature was held for 2 min at 50 °C, and then programmed to sequentially rise from 50 °C to 150 °C at 5 °C/min and at 15 °C/min to 280 °C, at which point this temperature was maintained for 10 min. Quantification of SC-FAEEs was achieved by integration of the peak areas. The response factors of SC-FAEEs relative to the internal standard were determined based on the peak area ratios of each C_2–C_5 ethyl ester as compared with the internal standard (A_{Cn}/A_{IS}). Blank samples were processed through the above procedures along with samples.

3. Results and discussion

3.1. Derivatization of short-chain fatty acids

To enhance the sensitivity of SCFAs analysis by GC and decrease the potential damage to the column and/or instrument, derivatization of SCFAs was carried out before extraction by HS-SDME. Lee et al. [15] successfully applied a method using methanol and sulfuric acid to the derivatization of formic acid in body fluids, such as blood and urine. As is the case for body fluids, SCFAs are present in the water phase of the RICO reaction system and, as such, the method of Lee et al. [15] was adopted in our study. However, preliminary experiments indicate that the presence of water as the by-product of neutralization reactions will compromise the derivatization efficiency of fatty acids, even though sulfuric acid should absorb most of the water. In addition, the strong oxidizing nature of sulfuric acid can give rise to some unexpected side reactions with the complex mixture of the RICO reaction system, which can compromise analysis of SC-FAEEs. Therefore, direct derivatization in the water phase may not be appropriate for SCFAs in our study, necessitating the development of a process for water removal. To avoid loss of SCFAs after the RICO reaction, the mixture was adjusted to pH > 9 by addition of 1 M KOH solution to convert the SCFAs to their corresponding salts. After centrifugation, the supernatant was transferred to a 25 mL glass vial, and then dried in an oven at 110 °C for 2 h. Both water and some other volatile components are evaporated by this step, resulting in the elimination of a variety of interferences. Subsequently, 0.5 mL of BF_3–diethyletherate, 0.5 mL of ethanol, and 0.2 mL of pure formic acid were added to the vial with the dried degradation products and sealed immediately. The sealed vials were then placed in a water bath for derivatization at 85 °C for 50 min.

3.2. Headspace single-drop microextraction optimization

After ethanol derivatization, the corresponding SC-FAEEs were extracted by the HS-SDME method. According to our previous HS-SDME work, factors affecting the extraction efficiency include drop solvent type, extraction time, sample ionic strength, and sample volume (when the bottle volume is fixed). An appropriate drop solvent, which is a prerequisite to obtaining good results, must possess three key characteristics. First, the solvent must have low volatility in order to avoid major evaporative losses during the extraction procedure. Second, as shown by this study, the solvent should have a good affinity for the SC-FAEEs. Finally, the solvent should have excellent gas chromatographic behavior. In this study, *n*-butyl alcohol, *n*-undecane, and dibutylphthalate were tested as drop solvents. The volatility of *n*-butyl alcohol and low extraction efficiency of *n*-undecane for SC-FAEEs limited their application in the extraction of ethyl esters. Our results show that dibutylphthalate was a suitable extraction solvent due to its relatively low volatility, better GC behavior, and extraction efficiency for the target analytes. Fig. 1 shows a typical chromatogram after HS-SDME extraction of the ethyl esters using dibutylphthalate as the extraction solvent

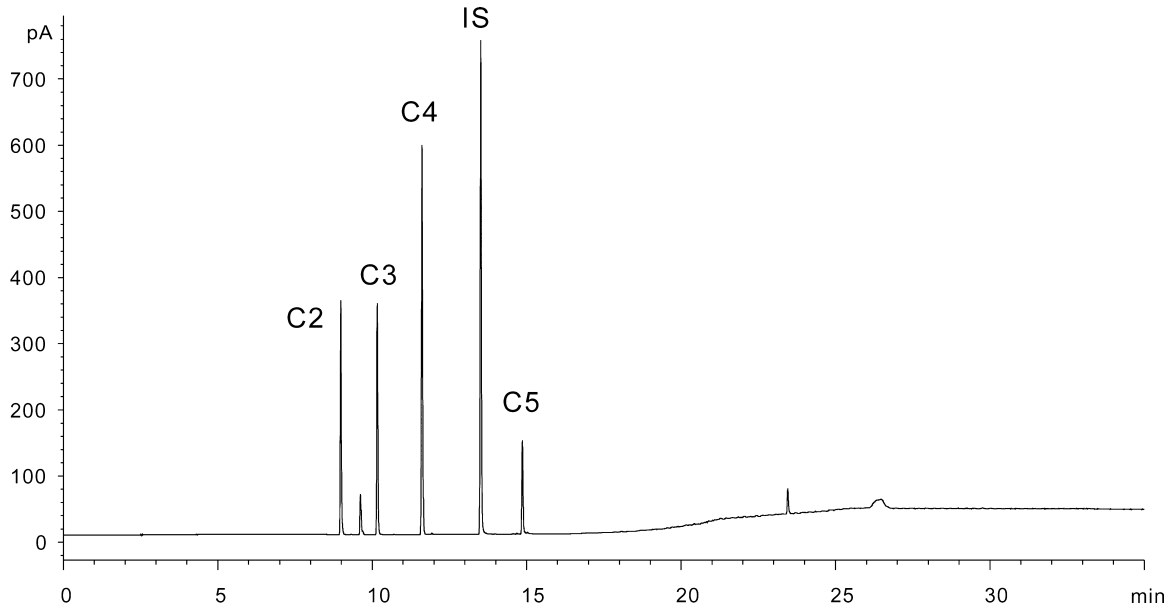

Fig. 1. A typical chromatogram after HS-SDME extraction of the SC-FAEEs using dibutylphthalate as the extraction solvent. C_2: ethyl acetate, C_3: ethyl propionate, C_4: ethyl butyrate, IS: methyl valerate, C_5: ethyl valerate.

Table 1
Different extract conditions of the HS-SDME and the corresponding ratio between peak areas of each target and the internal standard (A_i/A_{IS}).

Number	NaCl (W/V) (%)	Extraction time (min)	Solution volume (mL)	Drop volume (μL)	A_i/A_{IS}			
					C_2	C_3	C_4	C_5
1	30	10	6	1	3.19	3.30	5.40	6.40
2	30	20	6	1	3.37	3.55	6.19	8.37
3	30	30	6	1	3.43	3.51	5.75	7.37
4	0	20	6	1	0.41	0.30	0.48	0.84
5	15	20	6	1	0.58	0.52	0.92	1.74
6	30	20	6	1	3.37	3.55	6.19	8.37
7	30	20	7	1	3.60	3.81	6.89	9.58
8	30	20	8	1	3.37	3.67	6.75	8.83
9	30	20	7	1.4	4.06	4.35	7.70	10.08
10	30	20	7	2	5.51	5.82	9.51	10.72

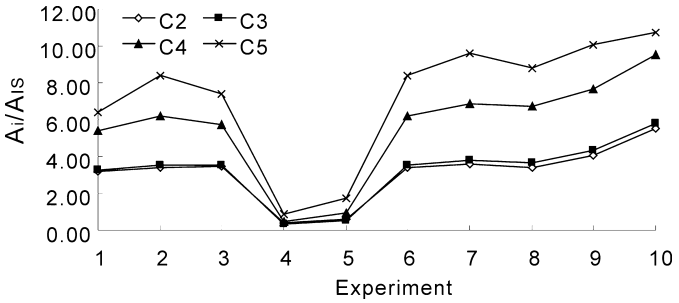

Fig. 2. The relative peak areas of each C_2–C_5 ethyl ester compared to the internal standard under different extraction conditions. C_2: ethyl acetate (362.1 μg/mL); C_3: ethyl propionate (252.9 μg/mL); C_4: ethyl butyrate (102.1 μg/mL); C_5: ethyl valerate (97.1 μg/mL).

and, as such, dibutylphthalate was chosen to be the drop solvent for HS-SDME in this study.

Our previous studies have shown that the extraction efficiencies of C_2–C_5 ethyl esters increase with greater agitation speed [21,22]. Hence, the highest stirring speed possible was used throughout all the extraction procedures.

Other parameters that can affect the extraction efficiency were optimized based on the relative peak areas obtained from 10 experimental trials. Extraction conditions for these experiments are listed in Table 1. Ten working solutions of C_2–C_5 ethyl esters at concentrations of 50.7 (C_2), 35.4 (C_3), 14.3 (C_4), and 13.6 mg/mL (C_5) were used in this optimization procedure. Fig. 2 shows the relative peak areas of each C_2–C_5 ethyl ester compared with the internal standard under different extraction conditions. As shown in Fig. 2, the conditions of experiment 10 resulted in the highest relative peak areas of C_2–C_5 ethyl esters. It is also evident that larger extraction drop volumes result in higher relative areas of the target analytes (e.g., experiments 7, 9, and 10), but that the chromatographic resolution is not as good when the drop volume drop is 2 μL (Fig. 3). The repeatability of experiment 9 was tested (Table 2) and showed that the repeatability is not good when the drop volume is 1.4 μL. Consequently, we choose a drop volume of 1 μL.

During our experiments, we found that the quantity of ethanol used in the working solution can affect the extraction efficiency.

Table 2
Ratios between peak areas of each target and the internal standard (A_i/A_{IS}) of three replicates of HS-SDME when the dibutylphthalate drop volume is 1.4 μL.

Analyte	A_{ij}/A_{IS}			RSD (%)
	1	2	3	
C_2	4.06	2.72	5.17	25.20
C_3	4.35	2.94	5.42	23.98
C_4	7.70	5.39	9.32	21.58
C_5	10.08	7.59	11.63	17.03

The amount of ethanol in the working solution is related to the volume of the derivatization production solution added as a spike to the extraction bottle. As such, the extraction quality is related to the volume of the derivatization production solution added to the sample solution. Table 3 lists the results of six parallel experiments in which the extraction conditions and concentration of the working solutions were identical, but the quantity of ethanol was varied. These results show higher extraction efficiency when the quantity of ethanol is decreased. For the convenience of the operation, we used a derivatization production solution volume of 60 μL to obtain the best extraction efficiency.

In summary, the optimum extraction parameters were: 1 μL drop volume, 30% (w/v) NaCl concentration, 20 min extraction time, 7 mL working solution or sample solution (relative to a 12 mL bottle volume), and 60 μL derivatization production solution.

3.3. Evaluation of the headspace single-drop microextraction method

Validation of the HS-SDME method for the quantitative analysis of SC-FAEEs, including factors such as the linearity of the calibration curve, reproducibility, and detection limits, was performed under the optimized conditions described in the previous section. The working solutions for the calibration study were prepared by spiking boiled pure water with the stock solution over the concentration ranges of 0.158–189.1, 0.024–28.26, 0.0079–9.49, and 0.003–3.56 μg/mL for the C_2, C_3, C_4, and C_5 acid ethyl esters, respectively. The calibration curves were constructed based on the ratios of the peak area of each of the ethyl esters to the internal standard (A_{Cn}/A_{IS}) versus the corresponding concentration ratios (C_{Cn}/C_{IS}). Linearity was evaluated in terms of the correlation coefficients of the regression equations of the calibration curves. Table 4 shows that the calculated calibration curves exhibit good linearity for all the ethyl esters, with correlation coefficients in the range of 0.9965 to 0.9992.

The reproducibility of our method was evaluated by five replicate extraction experiments. The working solutions were spiked with SC-FAEEs at concentrations of 217.3 (C_2), 35.4 (C_3), 14.3 (C_4), and 13.6 μg/mL (C_5). The relative standard deviations (RSD %) listed in Table 4 range from 2.23% to 3.63%, demonstrating the excellent reproducibility of the method.

The detection limits under optimal conditions for the C_2–C_5 acid ethyl esters were determined by diluting the working solutions until the responses of the analytes during GC analysis were an order of magnitude greater than the signal-to-noise ratio, with the corresponding analyte concentrations taken to be the detection limits. This showed that our method has low detection limits, being ca. 0.158, 0.024, 0.0079, and 0.0030 μg/mL for the C_2–C_5 acid ethyl esters, respectively. Given the conversion relationships between C_2–C_5 acids and their ethyl esters, the method detection limits for the C_2–C_5 acids can be calculated as being 0.11, 0.017, 0.0060,

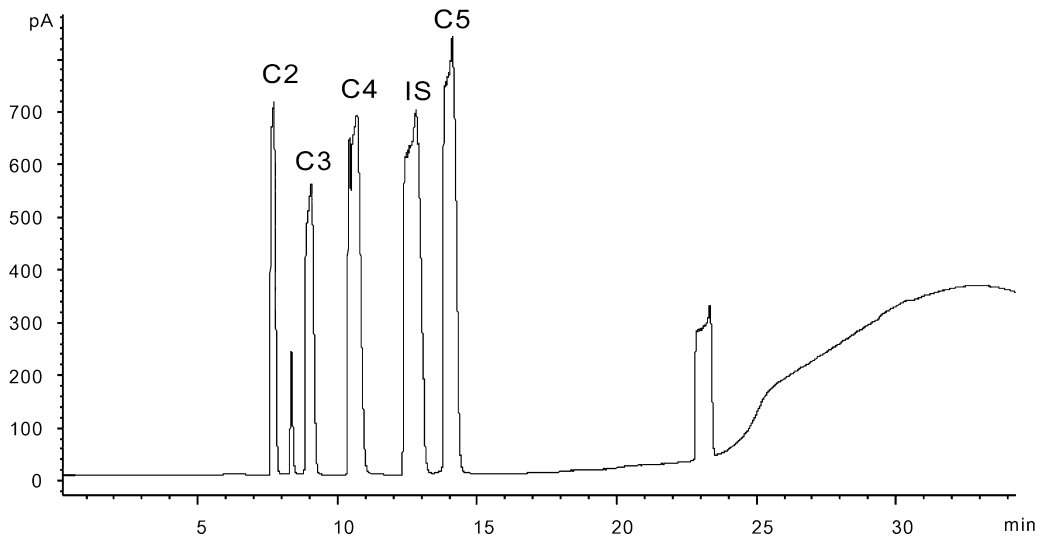

Fig. 3. The resolution chromatogram demonstration when the volume of the extraction solvent drop is 2 μL. C_2: ethyl acetate, C_3: ethyl propionate, C_4: ethyl butyrate, IS: methyl valerate, C_5: ethyl valerate.

Table 3
Ratios between peak areas of SC-FAEE and the internal standard (A_i/A_{IS}) using HS-SDME with different volume of ethanol.

Number	NaCl (W/V) (%)	Extraction time (min)	Solution volume (mL)	Drop volume (μL)	Quantity of ethanol (μL)	A_i/A_{IS}			
						C_2	C_3	C_4	C_5
1	30	20	7	1	500	2.17	2.02	3.47	5.33
2	30	20	7	1	250	2.64	2.66	4.79	7.44
3	30	20	7	1	200	2.9	2.82	4.95	7.44
4	30	20	7	1	100	3.35	3.33	5.77	8.01
5	30	20	7	1	50	3.53	3.55	6.18	8.49
6	30	20	7	1	0	3.6	3.81	6.89	9.58

and 0.0024 μg/mL, respectively. Compared with our previous study [15], the method detection limits of C_2–C_5 acids in our present study are 2.8, 4.0, 5.0, and 8.5 times lower, respectively.

The exact volume of the derivatization mixture is unknown in our experiments, and some variability of the sample volume taken from the derivatization system is inevitable. Toluene was thus used as a volume correction standard and the amount of each of the ethyl esters in the products can be calculated by determining the amount of toluene spike in each mixture. A calibration plot of toluene was prepared over a concentration range of 0.0031–3.68 μg/mL. Table 4 shows that the correlation coefficient (R^2) of the calibration curve is 0.9999, representing excellent linearity.

3.4. Efficiency of the whole pre-preparation procedure

Ethylbenzene was used as a model compound to evaluate the efficiency of the whole procedure, including the RICO reaction, SCFA derivatization, HS-SDME, and GC quantification. First, the ethyl side chain on the benzene ring of ethylbenzene was oxidized to propanoic acid (C_3). The product was then derivatized and determined by HS-SDME coupled with the GC analysis. Five replicate experiments were conducted as described above. Concentrations of the C_3 ethyl ester and toluene in the sample solution can be calculated based on their calibration curves and the known concentration of methyl valerate (IS). As the C_3 acid ethyl ester and toluene were in the same aqueous solution and were diluted simultaneously to the same volume, the mass ratio of C_3 acid ethyl ester and toluene is equal to the ratio of their concentrations, as follows:

$$\frac{m_{C_3}}{m_{TOL}} = \frac{C_{C_3}}{C_{TOL}} \quad (1)$$

where m_{C_3} and m_{TOL} represent the amount of C_3 acid ethyl ester and toluene, respectively, and C_{C_3} and C_{TOL} are the concentrations of C_3 acid ethyl ester and toluene in the sample solution, respectively.

As the amount of spiked toluene is known, the actual amount of C_3 acid ethyl ester can be obtained from Eq. (1). The quantity of C_3 acid produced from RICO processing of ethylbenzene can then be calculated from Eq. (2) and the theoretical amount of C_3 acid can be calculated from Eq. (3) and the added ethylbenzene weight. Thus, the conversion efficiency of the RICO process and the derivatization reaction is reflected by the ratio of the determined amount of C_3 acid to its theoretically calculated value. Our

Table 4
Quantitative results of HS-SDME.

Analyte	Equation	R^2	Liner range (μg/mL)	RSD (%, $n=5$)	Detection limit (μg/mL)
C_2	$y = 30.536x - 0.0072$	0.9984	0.158–189.1	3.42	0.11
C_3	$y = 5.3347x + 0.0253$	0.9992	0.024–28.26	3.08	0.017
C_4	$y = 1.2406x + 0.0176$	0.9992	0.0079–9.49	2.23	0.006
C_5	$y = 0.6239x + 0.0077$	0.9965	0.003–3.56	3.63	0.0024
Toluene	$y = 0.3443x + 0.0025$	0.9999	0.0031–3.68		

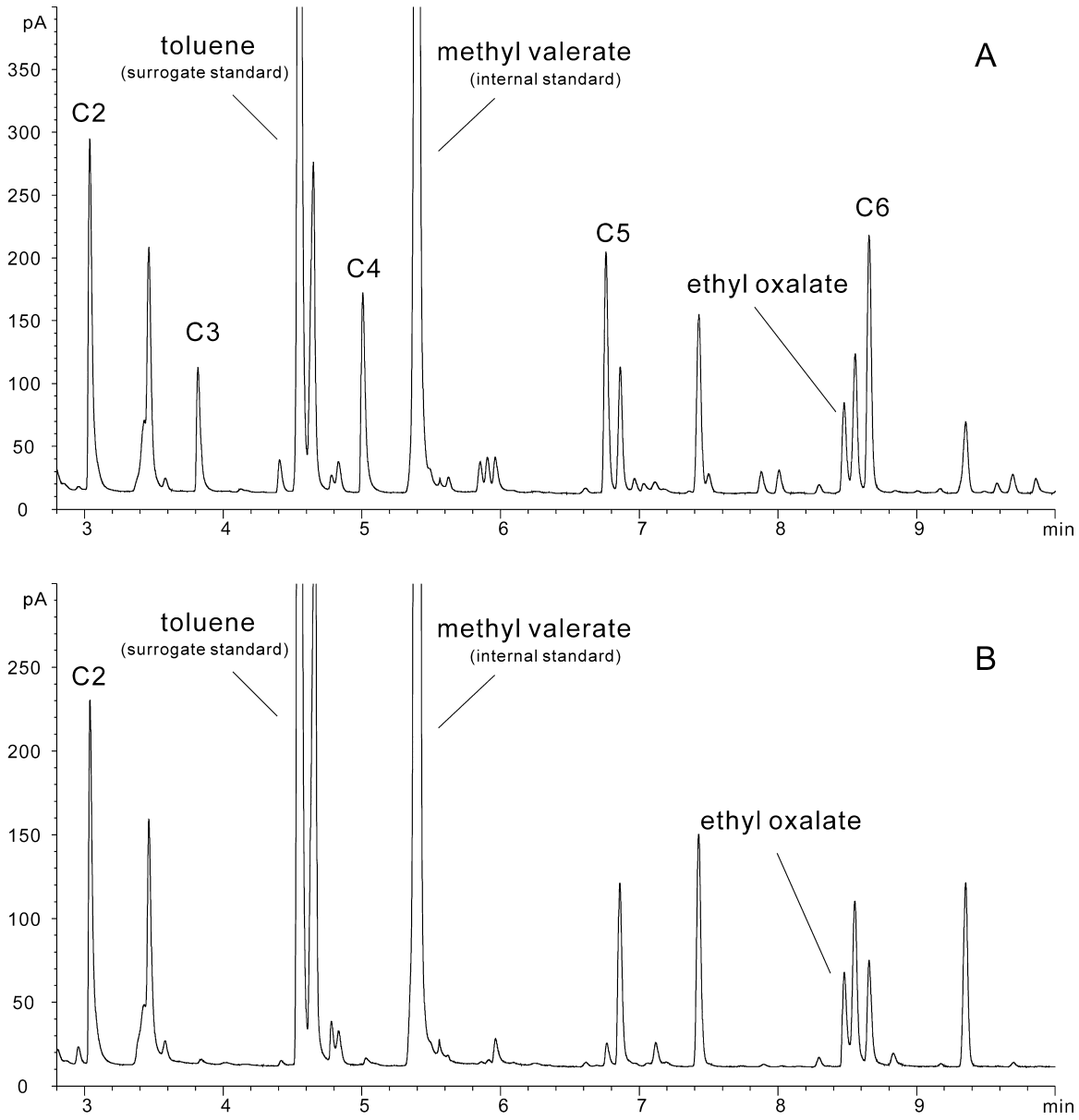

Fig. 4. Gas chromatograms of SC-FAEEs extracted by the optimized HS-SDME method from the RICO products of (A) type I kerogen with the Ro = 0.7%; (B) highly mature solid bitumen. C_2: ethyl acetate, C_3: ethyl propionate, C_4: ethyl butyrate, C_5: ethyl valerate, C_6: ethyl hexanoate.

experimental results indicate that the conversion efficiency ranges from 101.0% to 128.8% (Table 5). In addition, the reproducibility (RSD%) of the five replicate experiments is 10.48%, indicating an acceptable reproducibility for the whole procedure.

4. Applications

Samples of kerogen and solid bitumen were used to examine the robustness of our modified procedure for determining the amount and composition of alkyl side chains attached to aromatic structures in natural samples. The kerogen sample was isolated from a late Proterozoic marine shale of the Xiamaling Formation, north China, and is type I organic matter with the vitrinite reflectance of 0.7% Ro. The other sample is a highly mature solid bitumen collected from the Panlongdong area in Sichuan Basin, China. The bitumen is considered to be the cracking residue of oils derived from a marine source rock [23,24]. The kerogen and solid bitumen samples were subjected to the optimal analysis conditions described above.

Table 5
Recoveries of ethylbenzene during RuO_4 oxidation and the derivatization.

Number	Theoretical value (μg)	Determined value (μg)	Recovery (%)	RSD (%)
1	3.48	4.48	129	10.48
2	3.48	3.56	102	
3	3.48	3.74	107	
4	3.48	3.51	101	
5	3.48	4.08	117	

C_2–C_5 acids and oxalic acid were detected in the kerogen, whereas only acetic and oxalic acid were detected in the solid bitumen (Fig. 4; Table 6), indicating that abundant side and bridge chains are present in the molecular structure of kerogen, but only small amounts of methyl and methylene are in the solid bitumen. Therefore, the RICO process coupled with esterification and HS-SDME can be used to obtain structural information for even highly mature kerogen and asphaltene samples.

Table 6
Levels of targets in the field sample using HS-SDME coupled with derivatization.

Analyte	Kerogen (μg/mol)	Asphaltene (μg/mol)
C_2	1498.0	215.3
C_3	117.4	–
C_4	39.3	–
C_5	21.6	–
2C	395.8	80.7

5. Conclusions

We have developed an improved sample pre-treatment method for the analysis of SCFAs in RICO products where derivatization of SCFAs was conducted prior to HS-SDME using ethanol and BF_3–diethyletherate catalysis. A volume of 1.0 μL of dibutylphthalate was chosen as the extraction solvent for the ethyl esters of SCFAs. All the extraction procedures were conducted under the highest agitation speeds (1000 rpm), and the other optimized extraction conditions were: 30% (w/v) NaCl concentration, 20 min extraction time, and 7 mL working or sample solution in 12 mL sample vials. The amount of the derivatization product spiked to the sample solution was also explored, and a volume of 60 μL was determined to be the suitable volume. Linear calibration curves and good reproducibility were obtained under the optimized extraction conditions, with correlation coefficients varying from 0.9965 to 0.9992 and the reproducibility (RSD) ranging between 2.23% and 3.63%. Ethylbenzene was used as a model compound to examine the performance of the whole procedure, including the RICO reaction, BF_3–diethyletherate derivatization, HS-SDME, and GC analysis. Five replicate experiments show that the conversion efficiency of ethylbenzene ranges from 101.0% to 128.8%, highlighting the efficiency of the conversion. The reproducibility (RSD) of the five replicate experiments is 10.48%, which indicates a satisfactory reproducibility for the whole procedure. Our method allows detection of the studied analytes at low concentrations (e.g., 0.11, 0.017, 0.0060, and 0.0024 μg/mL for C_2–C_5 acids, respectively) in aqueous phases, which is 2.8–8.5 times lower than the detection limits obtained in our previous study[21]. Compared with previous studies (e.g., [21]), our new method is inexpensive and simple, has lower detection limits, and does not rapidly degrade the column or GC instrument. Therefore, this method is recommendable for the analysis of SCFAs in the RICO products of asphaltene or kerogen.

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References

[1] M. Vandenbroucke, C. Largeau, Org. Geochem. 38 (2007) 719.
[2] O.P. Strausz, T.W. Mojelsky, F. Faraji, E.M. Lown, Energy Fuels 13 (1999) 207.
[3] P.A. Peng, A. Morales-Izquierdo, A. Hogg, O.P. Strausz, Energy Fuels 11 (1997) 1171.
[4] P.A. Peng, J.M. Fu, G.Y. Sheng, Energy Fuels 13 (1999) 266.
[5] P.A. Peng, A. Morales-Izquierdo, E.M. Lown, O.P. Strausz, Energy Fuels 13 (1999) 248.
[6] O.P. Strausz, T.W. Mojelsky, E.M. Lown, Fuel 71 (1992) 1355.
[7] O.P. Strausz, T.W. Mojelsky, E.M. Lown, Energy Fuels 13 (1999) 228.
[8] A.L. Ma, S.C. Zhang, D.J. Zhang, Org. Geochem. 39 (2008) 1502.
[9] T.W. Mojelsky, T.M. Ignasiak, Z. Frakman, D.D. McIntyre, E.M. Lown, D.S. Montgomery, O.P. Strausz, Energy Fuels 6 (1992) 83.
[10] O.P. Strausz, E.M. Lown, Fuel Sci. Technol. Int. 9 (1991) 269.
[11] S. Murata, K. U-esaka, H. Ino-ue, M. Nomura, Energy Fuels 8 (1994) 1379.
[12] C. Schaeffer-Reiss, P. Schaeffer, A. Putschew, J.R. Maxwell, Org. Geochem. 29 (1998) 1857.
[13] Gy. Wittmann, H. Van Langenhove, J. Dewulf, J. Chromatogr. A 874 (2000) 225.
[14] M. Saraji, A.A.H. Bidgoli, J. Chromatogr. A 1216 (2009) 1059.
[15] X.P. Lee, T. Kumazawa, K. Kondo, K. Sato, O. Suzuki, J. Chromatogr. B 734 (1999) 155.
[16] A.L. Theis, A.J. Waldack, S.M. Hansen, M.A. Jeannot, Anal. Chem. 73 (2001) 5651.
[17] L. Tan, X.P. Zhao, X.Q. Liu, H.X. Ju, J.S. Li, Chromatographia 62 (2005) 305.
[18] P. Shahdousti, A. Mohammadi, N. Alizadeh, J. Chromatogr. B 850 (2007) 128.
[19] S. Shariati-Feizabadi, Y. Yamini, N. Bahramifar, Anal. Chim. Acta 489 (2003) 21.
[20] N. Bahramifar, Y. Yamini, S. Shariati-Feizabadi, M. Shamsipur, J. Chromatogr. A 1042 (2004) 211.
[21] Y. Li, Y.Q. Xiong, Q.Y. Liang, C.C. Fang, C.J. Wang, J. Chromatogr. A 1217 (2010) 3561.
[22] C.C. Fang, Y.Q. Xiong, Q.Y. Liang, Y. Li, P.A. Peng, Org. Geochem. 42 (2011) 316.
[23] C.L. Mou, Y.S. Ma, Q. Yu, T.L. Guo, Q.Y. Tan, L.Q. Wang, Pet. Geol. Exp. 27 (2005) 570.
[24] F. Hao, T.L. Guo, Y.M. Zhu, X.Y. Cai, H.Y. Zou, P.P. Li, AAPG Bull. 92 (2008) 611.