Journal of Chromatography A, 1325 (2014) 49–55

Contents lists available at ScienceDirect

Journal of Chromatography A

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

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

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form 3 December 2013
Accepted 3 December 2013
Available online 15 December 2013

Keywords:
Derivatization
Headspace single-drop extraction
Short-chain fatty acids
Fatty acid ethyl esters

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).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.12.014

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