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Journal of Chromatography A, 858 (1999) 201–207

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Esterification of decanoic acid during supercritical fluid extraction employing either methanol-modified carbon dioxide or a methanol trap

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Received 29 April 1999; received in revised form 19 July 1999; accepted 21 July 1999

Abstract

The methylation of decanoic acid during the supercritical fluid extraction process was investigated. It was found that the majority of the methylation occurred not during the extraction, but during the collection step, in the presence of methanol. Increasing dynamic extraction time (and hence collection time) from 0 to 60 min increased acid to ester conversions from 2% to 4%. The addition of HCl as a catalyst to the reaction increased the reaction rate 10-fold, and resulted in 88% conversion to the methyl ester within 30 min, without any detrimental effects on the chromatographic system. Higher collection temperatures appeared to increase conversion, but decreased methyl ester collection efficiency.

Keywords: Supercritical fluid extraction; Derivatization, SFE; Methylation; Decanoic acid; Fatty acids

1. Introduction

An advantage of supercritical fluid extraction (SFE) involves the ability to perform derivatization reactions during the extraction. Field [1] has published an excellent review of derivatization reactions coupled with SFE, which includes a discussion of the theories and approaches to derivatization with SFE, as well as many applications. In addition to organics, organometallics have also been derivatized [2,3]. Hawthorne et al. [4] used trimethylphenylammonium hydroxide and boron trifluoride to enhance the extraction of microbial phospholipid fatty acids from whole cells and wastewater phenolics from water, both as their methyl esters.

Rochette et al. [5] investigated a variety of sample preparation methods to improve extraction recoveries of the pesticide (2,4-D) from soils. They used silylation, ion-pairing, methyl esterification and ionic displacement, finding the methyl esterification and ionic displacement to be the most promising for quantitative SFE work. Meyer and Kleibohmer [6] developed a rapid efficient method for the extraction of pentachlorophenol (PCP) from wood and leather products based on an in-situ derivatization method (acetylation with triethylamine and acetic anhydride). They found comparable results with conventional methods and SFE reduced analysis times from about two days to less than 3 h [7,8]. Lee et al. [9] also developed an in-situ derivatization method for determining PCP in soils.

Hills et al. [10] used a silylation reagent, tri-sil

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00818-3

concentrate, to derivatize samples of roasted coffee beans, roasted Japanese tea and marine sediment. They postulated that the derivatizing reagent not only made the compounds more soluble in the supercritical extraction fluid, but that it was involved in a competition for active matrix sites, resulting in displacement of the analyte from the matrix. King et al. [11] have reported the on-line derivatization of triglycerides under supercritical conditions. They used a solid catalyst, alumina pretreated with methanol, for the in-situ transesterification of the triglycerides. The methyl esters were then preferentially eluted via supercritical fluid chromatography (SFC) from the alumina catalyst.

All of these papers have involved the derivatization reaction as a result of the addition of materials to the extraction chamber. Kawakura and Hiata [12] have recently reported the methylation of carboxylic acids in only methanol-modified supercritical fluid carbon dioxide using a flow through system. They investigated the methylation of substituted benzoic acids and the use of a cation exchanger as a catalyst for the reaction. They were able to achieve a maximum conversion of just under 60% for *p*-nitrobenzoic acid. They were able to apply this technique to phenoxy acids retained on a solid-phase extraction disk, although under the investigated conditions, recoveries were not quantitative (less than 50%). All of this work used methanol-modified carbon dioxide and a liquid trap of methylene chloride.

The objective of our work was to investigate the methylation (with no derivatization agent) of decanoic acid that occurs during the normal SFE process, and to determine the effects of both extraction and collection parameters on the reaction. Factors such as the chemical nature of the extraction fluid, collection fluid, collection temperature, and the presence of a catalyst in the collection vessel were all considered. Such reactivity to fatty acid methyl esters may lead to errors in the analytical SFE of free fatty acids.

2. Experimental

2.1. Extraction

All extractions were performed using an Isco SFX 3560 (Lincoln, NE, USA) SFE system. Carbon dioxide with helium headspace (2000 p.s.i.; 1 p.s.i.= 6894.76 Pa) from Air Products and Chemicals (Allentown, PA, USA) was used as the extraction fluid, and HPLC-grade methanol (Fisher Scientific, Fairlawn, NJ, USA) was used as the extraction fluid modifier.

Approximately 14 g of Ottawa Cement Testing Sand (Fisher Scientific, Houston, TX, USA) was placed in a 10-ml Isco special high crystalline polymer extraction vessel. The sand was used as received, with no clean-up steps or preliminary extractions performed. A solution of approximately 10 mg/ml decanoic acid (Fisher Scientific) in HPLC-grade methylene chloride (Fisher Scientific) was made, and 100 μl of the solution was spiked onto the Ottawa sand. The sand was allowed to air dry prior to extraction. The volume of the collection solvent was held constant at 10 ml, there was no static extraction time, and the dynamic extraction time was 10 min. No solvent replenishment of the trapping solvent was performed to replace losses which occurred during the course of the extraction. The collection solvents were HPLC-grade hexane, methanol and methylene chloride, all from Fisher Scientific.

The SFE conditions used for this study were a fluid pressure of 340 atm, an extraction temperature of 40°C, a restrictor temperature of 80°C, a liquid flow-rate of 1.0 ml/min, along with variable dynamic and static extraction times and collection temperatures (1 atm=101 325 Pa). A pressurized (~30 p.s.i.) collection mode was used for all experiments.

2.2. Extract analysis

After the extraction was completed, the collection vial was removed from the extractor, solvent was added to a volume of 10 ml. Then 100 μl of an internal standard solution [5 mg/ml tetradecane in methylene chloride (Fisher Scientific)] was added. To establish an equivalent 100% recovery, 100 μl of the same spiking solution and 100 μl of the internal standard solution were added to an empty collection vessel, and the volume was adjusted to 10 ml. A portion of the solution was transferred to an amber

autosampler vial for analysis. Each standard was injected four times and the response factors were averaged in order to calculate recoveries by the internal standard method.

In those cases where actual SFE was not performed, 100 μl of the spiking solution was deposited into a collection vial containing 10 ml of the collection solvent. The SFE program was then run on an empty extraction thimble, and the decompression of the fluid occurred in the spiked collection solvent.

All extracts were analyzed using a Hewlett-Packard (Little Falls, DE, USA) HP 5890 GC system equipped with a split/splitless capillary column inlet system which was maintained at 275°C. A 30 m× 0.25 mm I.D., 0.25 μm d_f DB-5 (J&W Scientific, Folsom, CA, USA) fused-silica capillary column was used for the separation. Grade 5.0 helium (BOC, Murray Hill, NJ, USA) was used as the carrier gas and the flame ionization detector was maintained at 325°C. The GC temperature program contained a 2 min initial temperature of 80°C, followed by a ramp of 10°C/min to a final temperature of 180°C which was maintained for 3 min. For all of the samples, 1 μl was injected using a HP 7673 (Hewlett-Packard) automatic injector in the splitless mode.

Identification of the eluted chromatographic peaks was also performed for several samples using GC–mass spectrometry (MS). These samples were analyzed using a Hewlett-Packard HP 5890 Series II GC system equipped with a split/splitless capillary column inlet system which was maintained at 280°C. A 30 m×0.25 mm I.D., 0.25 μm d_f DB-5-MS (J&W Scientific) fused-silica capillary column was used for the separation. Mass spectra were obtained by directly interfacing the GC system with a HP 5972 (Hewlett-Packard) mass-selective detector. Grade 5.0 helium (BOC) was used as the carrier gas and the transfer line was maintained at 280°C. The GC temperature program employed a 3 min initial temperature of 60°C, followed by a ramp of 10°C/min to a final temperature of 300°C which was maintained for 10 min. For all of the extract solutions, 1 μl was injected using a HP 7673 (Hewlett-Packard) automatic injector in the splitless mode. The mass spectra were obtained by electron impact (EI) ionization at 70 eV. The ion source temperature was set at 280°C and the masses scanned were from 30 to 550 u. All data were analyzed using a Hewlett-Packard ChemStation equipped with the Wiley library of mass spectral data.

3. Results and discussion

This work was divided into preliminary scouting experiments to determine whether the majority of the methylation reaction was occurring during the SFE, collection, or the chromatographic process, and specific experiments to determine the effects of various parameters on the methylation. During the scouting experiments no internal standard was added to the collection vials, as only qualitative data were desired.

3.1. Effect of collection solvent

The first experiment involved the formation of the methyl ester of decanoic acid during SFE with non-modified carbon dioxide. After spiking the extraction vessel with decanoic acid, the SFE was performed and collection occurred in either methanol or hexane, during a 12 min extraction period. Obviously, hexane lacks the ability to provide the methylating agent necessary to from the methyl ester, and the presence of the fatty acid methyl ester (FAME) was not seen in the chromatograms resulting from these extractions, as seen in Fig. 1a. It should be noted that there is no evidence of a chromatographic peak for decanoic acid either, due to the activity of the chromatographic system itself. However, when methanol was used as the collection solvent a well shaped peak appeared in the chromatogram, as seen in Fig. 1b, indicating the formation of the methyl ester of decanoic acid, as confirmed by GC–MS. This indicated that the FAME formation took place during the collection step, since no methylating agent was available during the extraction step.

3.2. Effect of extraction fluid

The next preliminary experiment was designed to investigate if the methylation occurred more readily under favorable extraction or collection conditions. The same experiment as above was repeated using 20% methanol-modified CO_2 as the extraction fluid, and the resultant chromatograms are shown in Fig.

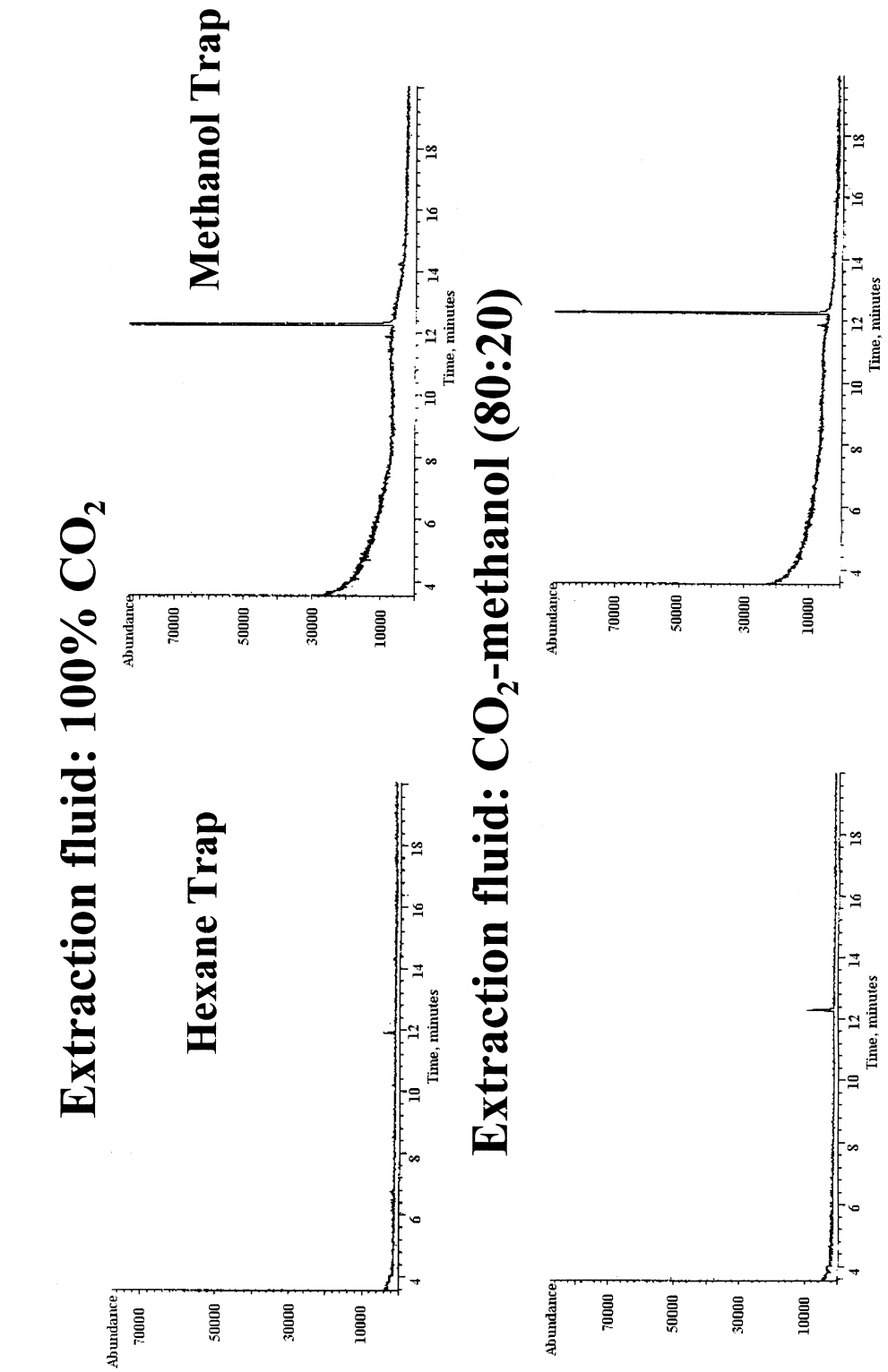

Fig. 1. Effect of extraction fluid on methyl decanoate formation.

1c and d. These chromatograms indicate that some formation of the methyl ester has occurred even when using hexane as a collection solvent, but it must be remembered that methanol was continually being added to the liquid trap during the course of the extraction, so that formation of the FAME could have occurred during either the extraction or collection step. Fig. 1d indicates that the majority of the methylation took place during the collection step. Though this work was done at a qualitative level it was obvious that a higher amount of the FAME was obtained when using methanol instead of hexane as a collection solvent, although it was not obvious if there was a greater amount of the FAME formed when using methanol-modified CO_2.

3.3. Effect of static extraction time when using methanol-modified carbon dioxide

If the FAME was being formed during the SFE, in addition to the collection step, it would follow that an increase in static extraction time; the period that decanoic acid and methanol are in contact with one another under supercritical conditions, should increase the amount of FAME formed. (For this experiment, a 3 min dynamic extraction was followed by the static extraction time period. This initial short dynamic extraction was performed to assure a reproducible extraction fluid composition. The static extraction time was always followed by a 30 min dynamic extraction). Results of these experiments indicated that there was no statistical increase or decrease (at the 95% confidence level) in FAME formation with increasing static time.

The clear indication at this point was that the majority of the reaction occurred during the collection step. To accomplish the separation of FAME formation during extraction from FAME formation during collection, subsequent experiments involved spiking the decanoic acid into the collection vessel, thus bypassing the extraction vessel completely. The CO_2 passed into the collection solvent after depressurization, at near atmospheric conditions.

3.4. Effect of dynamic extraction time

In order to determine the effect of dynamic extraction time on the formation of methyl decanoate, dynamic extraction time was varied from 0 to 60 min, in 15-min increments. A small amount of methyl decanoate was seen in the 0 min samples, probably formed during the injection of the trapping solvent into the gas chromatograph. The length of the blank extraction could affect the methyl ester formation in two ways. First, the reaction could be ongoing, and the increase in time would result in an increase in product formed. The second effect could be based on the fact that the addition of the carbon dioxide to the collection vessel results in a more acidic solution in the presence of residual water, serving to catalyze the reaction. There were no special precautions taken to remove water from either the supercritical fluid carbon dioxide or the methanol prior to use. The results of these experiments are shown in Table 1 and indicate that over the course of a 60 min extraction, the amount of methyl ester present is about twice that present at the beginning of the experiment. A plot of the natural logarithm of the methyl decanoate concentration versus time for the suspected pseudo-first-order reversible reaction yielded a reaction rate of 0.011 min^{-1} or 0.69 s^{-1}. Determination of the methyl ester concentrations indicated that there was 2% conversion (from acid to ester) at 0 min, which increased to 4.1% at 60 min.

Table 1
Effect of dynamic extraction time on methyl decanoate formation during SFE

Dynamic time (min)	Methyl decanoate concentration (mol/l)	Percent conversion to methyl decanoate
0	$1.16 \cdot 10^{-3}$	2.0 (4)[a]
15	$1.53 \cdot 10^{-3}$	2.6 (8)
30	$1.71 \cdot 10^{-3}$	3.0 (7)
60	$2.37 \cdot 10^{-3}$	4.1 (5)

[a] Numbers in parentheses are relative standard deviations (%), $n=3$.

Table 2
Effect of adding hydrochloric acid to the collection solvent

Amount of HCl added (mg)	Amount methyl decanoate (relative to standard containing no HCl)	Amount methyl decanoate (relative to standard containing HCl)
0	1.14	1.14
8	41.91	1.15
22	43.36	1.11
44	44.11	1.07

3.5. Effect of adding hydrochloric acid (HCl) to the collection vessel

The objectives of this experiment were to determine if it was possible to add small amounts of the mineral acid, HCl, to the collection vessel to catalyze the methylation reaction, and determine if the acid catalysis was more important than a longer reaction time with the weaker acid, resulting from the CO_2 and water present. Varying amounts of HCl were added to the collection vessel, with the dynamic extraction time being held constant at 30 min. The results, shown in Table 2, indicate that addition of the HCl greatly enhanced the amount of methyl ester formed and that larger changes in HCl concentration do not appreciably change the amount of ester formation. In Table 2, the second column indicates the amount of methyl ester present compared to a standard prepared in methanol (100 μl of 10 mg/ml decanoic acid in methylene chloride). The third column indicates the amount of methyl ester formed when compared with a standard prepared in a similar manner, except that the prescribed amount of HCl was added to the standard prior to chromatography. These columns indicate that the presence of HCl greatly enhanced methyl ester formation, but that the dynamic extraction time had little effect in comparison. A comparison of the conversion to the methyl ester for the uncatalyzed versus catalyzed reaction indicates that the reaction rate (when calculated for 0 and 30 min data) increases from 0.013 min^{-1} to 0.126 min^{-1}, or roughly a 10-fold increase. Conversion of the acid to the ester went from 3% for the uncatalyzed to 88% for the HCl catalyzed reaction. It should be noted that there was no evident deterioration in the chromatographic column when injecting the acid containing samples, though only a limited number of injections were made.

3.6. Effect of collection temperature

The last collection variable to be investigated was the collection temperature. The objective of this experiment was to determine if an increase in collection temperature would result in an increase in conversion to the methyl ester. Table 3 shows the results for this experiment, which indicate that the effect of collection temperature is difficult to discern. Though it appears that the amount of methyl ester decreased with increasing temperature (second col-

Table 3
Effect of collection temperature on methyl decanoate formation

Collection temperature (°C)	Methyl decanoate: amount relative to standard	Decanoic acid: amount relative to standard	Methyl decanoate/decanoic acid ratio
+40	0.75 (4)[a]	0.03 (98)	20.22 (5)
+20	0.64 (8)	0.11 (22)	5.99 (8)
0	0.83 (7)	0.29 (15)	2.93 (26)
−20	1.26 (3)	0.37 (16)	3.45 (19)

[a] Numbers in parentheses are relative standard deviations (%), $n=6$.

umn), the amount of fatty acid also decreased (third column), which indicated that the fatty acid was somehow being lost in the reaction. The methyl ester/acid ratio (fourth column) indicated a greater amount of methyl ester was present for the amount of acid present. Though these ratios are based in part on the fatty acid determinations, which have a large amount of error due to quantitative chromatographic problems, they do show a clear trend toward increasing conversion at higher collection temperature. It is quite possible that the methyl ester was being preferentially lost at the higher collection temperatures, due to its increased volatility.

4. Conclusions

The objective of this work was to investigate the methylation of decanoic acid occurring during the SFE process. The methylation was found to occur primarily during the collection process and was greatly enhanced (reaction rate increased almost 10-fold) by the presence of an acid catalyst (i.e., additional to any carbonic acid formed from the CO_2 and residual water.) Increasing reaction time and collection temperature also increased the conversion rate to the methyl ester, but very little in comparison to the catalyzed reaction. This work indicates that extraction and derivatization can be performed simultaneously for subsequent chromatographic analysis.

Acknowledgements

The authors would like to acknowledge the loan of the Isco SFX 3560 from the Isco/Suprex Corporation, and the donation of the SFE/SFC grade carbon dioxide from Air Products and Chemicals, Inc.

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