Journal of Chromatography, 594 (1992) 309–315
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 850

Supercritical carbon dioxide extraction of resin and fatty acids from sediments at pulp mill sites

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(First received August 20th, 1991; revised manuscript received November 5th, 1991)

ABSTRACT

A rapid and efficient method for the extraction of resin and fatty acids commonly found in sediments collected from pulp mill locations was developed by using modified supercritical carbon dioxide. In the presence of a 1:1 mixture of methanol and formic acid, quantitative recovery of all acids except for palustric and neoabietic acids was achieved with a 5 min static and 10 min dynamic extraction with carbon dioxide at 365 bar and 80°C. Although the above two resin acids were only 40% recovered from spiked samples, these values were at least 250% better than those obtained by the classical Soxhlet technique. The cleaner supercritical fluid extract permitted a less stringent cleanup after the off-line derivatization of the acids, thus it further reduced analytical time and the use of solvent. An *in situ* extraction and on-line derivatization of the resin and fatty acids also proved feasible for the semi-quantitative screening of the toxic acids in sediments near pulp mill locations.

INTRODUCTION

A large number of environmental pollutants have been identified in the discharges from the pulp and paper industry. Chlorinated phenols, guaiacols, catechols, aliphatic neutrals and acids, as well as furans and dioxins have been identified from chlorobleaching mills [1,2]. Resin acids, natural products derived from wood and pulp, occur in effluent samples from every paper mill [3,4]. Many of the above chemicals are toxic to fish and have a life time long enough for bioaccumulation in aquatic organisms. Among them, resin acids and a few unsaturated fatty acids have been identified as the major components of effluents which contribute to the toxicity to fish [5–7]. The pulp and paper industry in Canada and elsewhere has implemented various techniques to detoxicate the effluents before they are discharged into the receiving waters. However, effluent levels of resin and fatty acids (RFAs) from those mills without an effective secondary (microbiological) waste treatment are so high that they can be acutely toxic to fish. Owing to their low solubilities, resin acids are readily adsorbed by sediments and are easily detected in samples downstream of the paper mills.

RFAs in sediments are extracted by using the classical Soxhlet technique with polar organic solvents [8,9]. In a recent study, we have found that, by addition of a trace amount of concentrated hydrochloric acid to the polar solvents, the recoveries of RFAs in sediments were improved by 200 to 300% [9]. However, the presence of a strong acid caused degradation of palustric and neoabietic acids into abietic acid. Therefore this techniques would produce biased low results for the above two unstable resin acids and biased high results for abietic acids in sediment samples.

Supercritical fluid extraction (SFE) has been applied to many organic pollutants in various environmental matrices [10–13]. In general, supercritical carbon dioxide produces good recoveries for non-polar compounds such as polychlorinated biphenyls [10]. However, for the extraction of more polar compounds, carbon dioxide modified by methanol or other polar solvent or supercritical

0021-9673/92/$05.00

nitrous oxide is required to improve the recoveries to a level comparable to Soxhlet extraction. Until recently, there were few reports on the supercritical fluid extraction of organic acid from sediments. This work describes the optimization of the extraction of resin and fatty acids from sediments using modified supercritical carbon dioxide.

EXPERIMENTAL

All resin acids (Table I) were obtained from Helix-Biotech Scientific (Vancouver, Canada) and used without further purification. Fatty acids and α-bromo-2,3,4,5,6-pentafluorotoluene (pentafluorobenzyl bromide, PFBBr) were purchased from Aldrich (Milwaukee, WI, USA). Stock solutions of individual resin and fatty acids were prepared in acetone at 1000 μg/ml and kept at −20°C in the dark. Spiking solutions of mixed RFAs also in acetone were stored at 4°C in the dark. A PFBBr solution was prepared by dissolving 1 g of the reagent in 20 ml of acetone and kept at −20°C until use.

All solvents used were distilled-in-glass grade supplied by Burdick & Jackson. SFC-grade carbon dioxide with a helium head pressure of 10500 kPa was purchased from Scott Specialty Gases (Troy, MI, USA).

Several river sediment samples were collected from different locations near an Ontario paper mill in September 1990. Among them, a sample obtained from a site approximately 2 km downstream of the mill was, as shown by previous analysis using Soxhlet extraction, contaminated with RFAs at levels typically found in paper mill sediments. This sediment was air-dried, crushed, homogenized and used for the development of the extraction method.

Supercritical carbon dioxide extraction of the sediment was performed with the Hewlett-Packard 7680A extraction module and the available 7.0-ml stainless-steel thimbles and caps. Instead of using a restrictor to depressurize the supercritical fluid and deposit the extract into a test tube containing an organic solvent, the HP 7680A extractor employs an unique nozzle/trap assembly [14]. The nozzle allows instant depressurization of the carbon dioxide, and at the same time permits the decoupling of flow and pressure; thus, the density can be set independent of the flow of the fluid. During the dynamic extraction stage, the SFE extract is deposited onto a packed trap made of octadecylsilane (ODS) material. At the end of the extraction, the analytes are then rinsed off the trap with a predetermined amount of solvent into a glass vial and the extract is ready for analysis, cleanup, or further workup. The operation is fully automated from the point where the thimble is placed into the extraction chamber and on.

To minimize contamination and plugging of the bottom cap by the sample, two circles of Whatman GF/C filter paper of the same diameter as the thimble were cut by pressing the edge of the thimble against the paper and placed above the bottom cap after it was screwed in. A sediment sample typically of 500 mg was weighed and 25 μl of water and 300 μl of modifier added directly to the sample. In some cases, the sample was placed in between two layers of

TABLE I

IUPAC NAMES FOR SELECTED RESIN ACIDS

Structures of these resin acids are given in ref. 9.

Common name	IUPAC name
Pimaric acid	8(14),15-Pimaradien-18-oic acid
Sandaracopimaric acid	8(14),15-Isopimaradien-18-oic acid
Isopimaric acid	7,15-Isopimaradien-18-oic acid
Palustric acid	8,13-Abietadien-18-oic acid
Dehydroabietic acid	8,11,13-Abietatrien-18-oic acid
Abietic acid	7,13-Abietadien-18-oic acid
Neoabietic acid	8(14),13(15)-Abietadien-18-oic acid
14-Chlorodehydroabietic acid	14-Chloro-8,11,13-abietatrien-18-oic acid
12,14-Dichlorodehydroabietic acid	12,14-Dichloro-8,11,13-abietatrien-18-oic acid

Celite of 200 mg each (see later discussion). A 5 min static and a 10 min dynamic extraction was carried out at a chamber temperature of 80°C using supercritical carbon dioxide of 0.80 g/ml density (approximate pressure 365 bar) at a flow-rate of 2.0 ml/min. The ODS trap was maintained at 15°C with cryogenic carbon dioxide during the extraction stages. Following the extraction, the RFAs were collected in glass vials by eluting the trap, which was then warmed up to 40°C, with two 1-ml aliquots of acetone. The entire extraction cycle completed in *ca.* 35 min.

The acetone extract containing the RFAs were combined and reduced to 1 ml before the acids were converted into their pentafluorobenzyl (PFB) esters as described before [9]. After solvent exchange into light petroleum (b.p. 30–60°C), the derivatized products were cleaned up on a 5 cm 5% deactivated silica gel column prepared in a 20 cm × 0.7 cm I.D. disposable Pasteur pipet. Following the application of the derivatized extract to the column prewashed with 2 ml of light petroleum, the column was eluted with 5 ml of dichloromethane–light petroleum (5:95, v/v) and then with 7 ml of dichloromethane–light petroleum (1:1). The last fraction was saved and the solvent exchanged into 5 ml of isooctane for analysis by gas chromatography–electron-capture detection using a 30 m × 0.25 mm I.D. DB-17 column [15].

Gas chromatographic conditions: oven temperature was held initially at 70°C for 0.75 min and programmed to 160°C at 30°C/min, then to 290°C/min at 2°C/min. Injector and detector temperatures were 250 and 300°C, respectively. Helium was used as the carrier gas and the column head pressure was 105 kPa. Samples of 2 μl were injected in the splitless mode with a valve time of 0.75 min.

RESULTS AND DISCUSSION

Supercritical carbon dioxide extraction of RFAs from sediments

RFAs in sediment were poorly extracted by supercritical carbon dioxide. Even at the maximum extraction chamber temperature of 80°C and a fluid density of 0.80 g/ml, only a small amount of palmitic acid yet no resin acids were recovered from the reference sample after a 5 min static and another 10 min dynamic extraction at a flow-rate of 2 ml/

TABLE II

EFFECT OF MODIFIER ON THE RECOVERY OF RFAs IN SEDIMENT

	Recovery (%)[a] with modifier					Soxhlet (μg/g)
	None	Methanol	Acetic acid	Formic acid	Methanol–formic acid	
Palmitic	3	63	71	74	94	18.0
Stearic	<1	64	69	73	94	6.4
Oleic	<1	65	78	82	94	6.8
Linoleic	<1	73	68	88	90	13.9
Pimaric	<1	44	90	87	102	12.7
Isopimaric	<1	33	67	84	88	40.2
Palustric	<1	117	204	141	267	4.6
Abietic	<1	37	64	73	89	52.7
Dehydroabietic	<1	44	76	84	102	65.8
Neoabietic[b]	<1	(1.9 μg/g)	(3.8 μg/g)	(0.7 μg/g)	(4.4 μg/g)	<0.1
Chlorodehydroabietic[c]	<1	23	71	81	89	48.9
Total fatty acids[d]	3	65	70	80	92	55.3
Total resin acids[e]	<1	34	66	79	94	232

[a] All SFE recoveries were relative to Soxhlet results.

[b] The recoveries of neoabietic acid under various SFE conditions were given in μg/g since this acid was not recovered by the Soxhlet method.

[c] Sum of 12- and 14-chlorodehydroabietic acids.

[d] Sum of the fatty acids listed together with lauric, myristic, linolenic and eicosanoic acids.

[e] Sum of the resin acids listed together with sandaracopimaric and dichlorodehydroabietic acids.

min. Addition of 300 μl of methanol improved the recoveries of the total fatty and resin acids to 65 and 34%, respectively, of the Soxhlet values (Table II). Analogous to the fact that the presence of an acid substantially improved the recovery of RFAs in the Soxhlet extractions, the SFE recovery of RFAs was also greatly improved by the presence of 300 μl of acetic acid in the sample. Using a stronger acid such as formic acid further enhanced the recovery of total RFA to *ca.* 80%, however, it was noted that the recoveries of palustric and neoabietic acids were lower when the stronger acid was used. The use of dichloroacetic acid, 10% hydrochloric acid in methanol and a 1:1 mixture of acetic acid and methanol as modifiers all proved to be less effective than formic acid for the extraction of all RFAs, although a mixture of methanol–formic acid (1:1) provided the best recovery of RFAs in sediment. The effect of each modifier on the recovery of the major RFAs in sediments is shown in Table II.

The effect of the amount of modifier used on the recovery was also studied. Based on a 500 mg sample size, 300 μl of methanol–formic acid (1:1) was found to produce the optimal recovery of RFAs. Smaller amounts such as 100 or 200 μl of the modifier were insufficient and a larger volume such as 500 μl did not further improve the recovery.

Other factors affecting the recovery of RFAs in sediments

At the early stage of our work, extraction of sediments was carried out at either 50 or 60°C. Within the working temperature range of 40 to 80°C for the HP 7680A, recovery of RFAs from the reference sediment was found to increase with increasing chamber temperature. Therefore all subsequent extraction were done at a temperature of 80°C. Note that supercritical carbon dioxide of the maximum density attainable by the HP 7680A at each temperature was used in each case so that highest extraction efficiency could be achieved.

The moisture content of a sample also plays an important role in the extraction of RFAs from sediments. Our results indicated that the best recovery of RFAs was obtained from samples containing 5 to 10% moisture content. If freeze-dried sediments were used, a reduction of 25 to 40% in the recovery of the RFAs was observed. However, an addition of 5% (w/w) of water to the dry sediment prior to the extraction would bring the recovery back to quantitative. The enhancement in RFA extractability in this case could be attributed to the increase in acidity due to the presence of water in supercritical carbon dioxide.

It was also observed that, an improvement of *ca.* 10% in the recovery of RFAs was achieved by sandwiching the sediment with 200 mg layers of Celite and spiking each layer with half the amount of modifier. Presumably the slightly better recovery was attributed to the longer retainment of the modifier with solids during the dynamic extraction stage. While longer extraction times (both static and dynamic) did not further improve the recovery, shorter extraction times resulted in incomplete recovery of RFAs.

Under the optimized conditions, a second extraction of the sediment with fresh modifier recovered less than 5% of additional RFAs, indicating that the first extraction was essentially complete.

Cleanup of derivatized extracts

In comparison to the exhaustive but often nonselective Soxhlet extraction, supercritical carbon dioxide extraction of RFAs from sediment produced a much smaller amount of coextractives in the extract. The cleaner extract enabled us to employ a

TABLE III

RECOVERY OF RESIN AND FATTY ACIDS FROM FORTIFIED SEDIMENT SAMPLES BY SUPERCRITICAL FLUID EXTRACTION

Recoveries and standard deviations were calculated from replicate determinations of six identical samples.

RFA	Recovery (%)	
	Fortification 10 μg/g	Fortification 1 μg/g
Palmitic	99 ± 7	94 ± 10
Stearic	97 ± 7	85 ± 7
Oleic	94 ± 7	105 ± 5
Linoleic	88 ± 8	107 ± 7
Pimaric	91 ± 6	98 ± 6
Isopimaric	90 ± 7	95 ± 3
Palustric	38 ± 8	35 ± 4
Abietic	90 ± 10	98 ± 10
Dehydroabietic	108 ± 5	104 ± 8
Neoabietic	36 ± 7	40 ± 5
Chlorodehydroabietic	89 ± 6	96 ± 9

smaller (0.8 g *vs.* 5.0 g) column for sample cleanup after the derivatization [9], and thus it further improves the saving in time and the amount of solvent used. This cleanup step effectively removed interferences deriving from sediment coextractives as well as the blank of the SFE-grade carbon dioxide which was found to contain various amounts of impurities from all samples provided by three different suppliers. The purity problem of supercritical carbon dioxide for electron-capture detection has also been reported lately [16].

Method performance

With the exception of palustric and neoabietic acids, the recoveries of other major fatty and resin acids found in pulp mill sediments from spiked samples were better than 85% at fortification levels of 10 and 1 μg/g (Table III). Recoveries for palustric and neoabietic acids were between 35 and 40% at the same levels, presumably due to degradation of these two acids under acidic extraction conditions. It should be noted that, with Soxhlet extraction, the recoveries were even poorer for palustric (5 to 15%) and neoabietic (<5%) acids [9]. This extraction procedure has been successfully applied to sample sizes from 25 mg to 1 g. Larger sample sizes were not tried since with a 1 g sample, a detection limit of 0.05 μg/g [9] can easily be achieved for most monitoring purposes.

In situ extraction and derivatization of RFAs in sediments

Although derivatization analysis of polar organics enjoys many advantages such as improved chromatographic properties and enhanced detector response of the derivatives, this approach is more cumbersome because of the extra step. Therefore, an ideal method would be one which combines the extraction and derivatization steps into one. In our case, experimental conditions had to be modified since the SFE conditions and the esterification reaction with the PFBBr reagent were incompatible with each other. The esterifying agent reacts with acids and methanol and the reaction requires a base to catalyze the formation of esters. This problem was solved by replacing the methanol–formic acid modifier with 250 μl of 5% solution of the PFBBr reagent in acetone and 50 μl of triethylamine. An initial extraction was attempted by using a 10 min static and 5 min dynamic extraction time. The

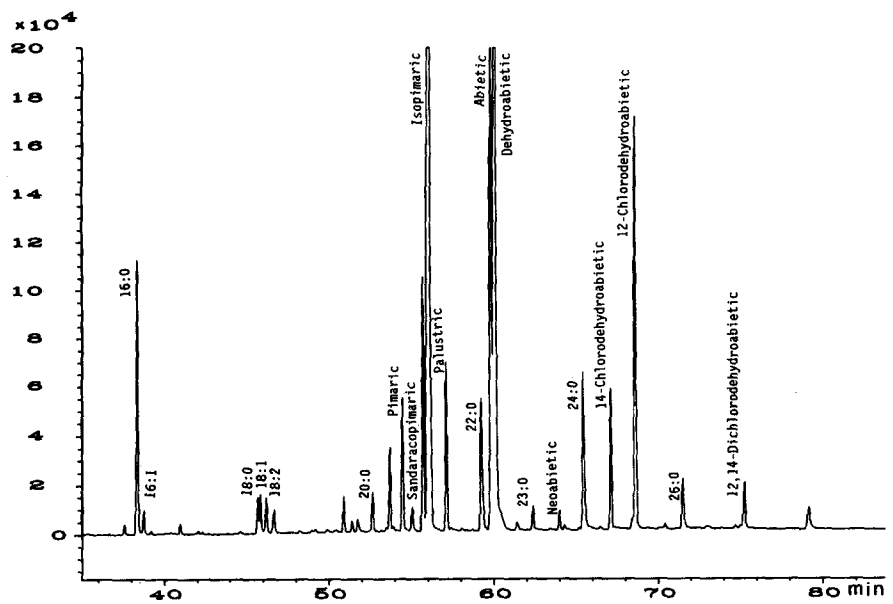

Fig. 1. Gas chromatography–electron-capture detection of the RFA PFB esters in the reference sediment sample as recovered by the *in situ* extraction/derivatization route. Splitless injection (2 μl) onto a 30 m × 0.25 mm I.D. DB-17 column. See Experimental section for conditions.

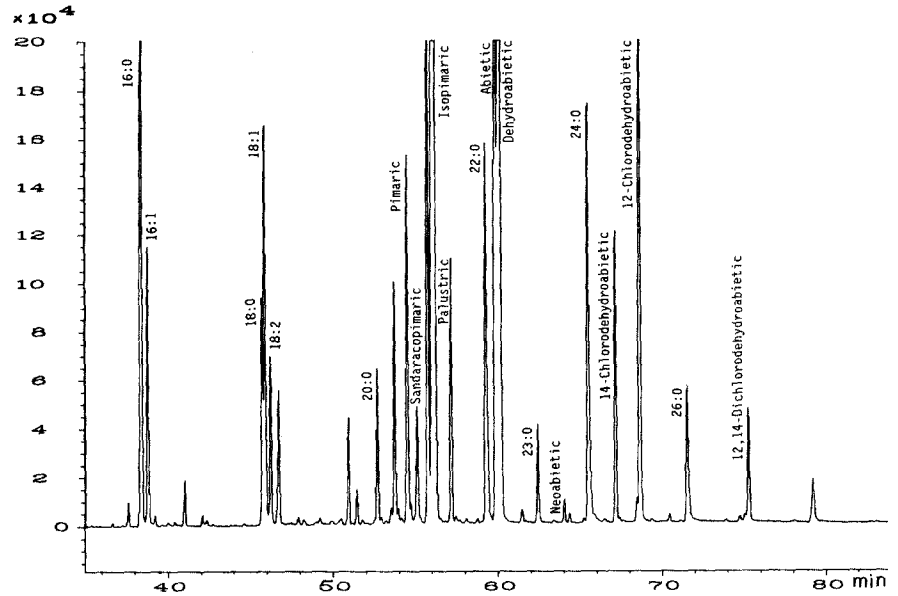

Fig. 2. Gas chromatography–electron-capture detection of the RFA PFB esters in the reference sediment sample as recovered by the off-line derivatization route. Conditions as in Fig. 1.

derivatized RFA extract was then eluted from the ODS trap by isooctane, cleaned up on a silica gel column and solvent exchanged as described previously before gas chromatography–electron-capture detection. Although the recoveries of RFAs by this *in situ* method (Fig. 1) were only 35–45% of the off-line derivatization technique (Fig. 2), the results nevertheless indicated that the *in situ* derivatization was feasible for the determination of RFAs in sediments. The lower recoveries were not unexpected since acetone was a less effective modifier than the methanol–formic acid (1:1) mixture for the extraction of RFAs and also the complete conversion of the acids into their PFB esters required 1 to 2 h at 60°C. Indeed, by extending the static extraction time from 10 to 60 min, the recoveries were improved to *ca.* 60% for the RFAs by comparison to the best off-line SFE extraction and derivatization results. However, the amounts of palustric and neoabietic acids extracted by the *in situ* method were proportionally higher, since these two acids are less stable under acidic conditions. Doubling the amounts of PFBBr and triethylamine only improved the recovery of RFA by another 5 to 10%. Further extension of the static extraction time is impractical since the sample throughput would be severely reduced.

CONCLUSIONS

Most RFAs commonly found in sediments downstream of pulp and paper mill locations are quantitatively extracted by supercritical carbon dioxide in the presence of methanol and formic acid as modifiers. Although the recoveries of the unstable palustric and neoabietic acids are *ca.* 40% as indicated by the recovery experiments for the spiked sediments, the SFE results of the above two acids are at least 250% better thant he Soxhlet values on both spiked and naturally contaminated samples. Because of the feasibility of a rapid, one-step *in situ* extraction and derivatization of RFAs, this technique is most suitable for the semi-quantitative screening of the toxic RFAs in sediments for quick sample turn around time.

ACKNOWLEDGEMENTS

The authors are grateful to Hewlett-Packard (Canada) Ltd. for the generous loan of the HP 7680A

supercritical fluid extractor module. We also thank W. Pipkin of Hewlett-Packard, Avondale, PA, USA, for helpful discussions.

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