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Journal of Chromatography A, 976 (2002) 393–398

JOURNAL OF CHROMATOGRAPHY A

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Monitoring polychlorinated biphenyls in pine needles using supercritical fluid extraction as a pretreatment method

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Abstract

This paper reports on the first use of supercritical fluid extraction (SFE) of polychlorinated biphenyls (PCBs) from pine needles. Supercritical carbon dioxide was used as extraction fluid, and exhibited good extraction efficiencies and recoveries (>90%). GC–MS (selected ion monitoring mode) achieved both accurate identification and quantification of the PCBs. Compared with traditional time consuming multi-step sample preparation methods, SFE with carbon dioxide is easier to perform, and is a feasible alternative extraction procedure for the monitoring of PCBs in pine needle samples.
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Keywords: Supercritical fluid extraction; Plant materials; Environmental analysis; Polychlorinated biphenyls

1. Introduction

Polychlorinated biphenyls (PCBs) were manufactured under the trademark Aroclor from 1930 to 1970 by Monsanto (St Louis, MO, USA). They have been used as dielectric fluids, flame-retardants and industrial lubricating fluids [1,2]. Due to their persistence, toxicity and carcinogenic potential, concern over their presence in the environment has increased over the past two decades [1,3,4]. In a famous case in 1968, PCB-contaminated cooking oil caused a total of 1291 ''Yusho'' patients in western Japan [5–7]. Consequently, restrictions both on manufacturing and use were introduced into Europe and North America in the 1970s [2,5,8,9].

PCBs were first detected in eagles and herring in 1966 [10]. They were subsequently determined in many kinds of environmental matrices including water, air, plants, soils, animal and human tissue [6,7,11–16], etc. Many studies have been carried out on plant samples, with most of them on pine needles or lichen samples, because these two samples are very typical (they are found world-wide and can accumulate pollutants) [14,17–23]. There are no easy and simple sample pretreatment methods for PCBs. Traditional methods are time consuming and very difficult to perform, and have low recoveries. Generally, these sample preparation methods include a lot of steps, such as Soxhlet extraction, rotary evaporation, silica gel chromatography followed by gel permeation chromatography or Florisil column chromatography and silica gel fractionation, etc. [14,17,18]. These clean-up steps were used and reported in previous studies even as recently as 1998 [18,23]. For example, Ockenden reported using Soxhlet extraction, alumina/silica gel chromatography, gel permeation chromatography and silica gel fractionation as the sample preparation procedure [23]. Obviously, the more steps used, the more complicated the operation would be, and losses of analytes are highly probable. Even for an accom-

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PII: S0021-9673(02)01158-5

plished analyst in this field, if each step of the aforementioned five steps, can achieve 95% recovery, the overall recovery would still be only 77%. A simpler less tedious and easier sample preparation method is urgently needed.

Supercritical fluid extraction (SFE) became popular in the 1980s for the extraction of environmental pollutants [24–29]. For example, the technique has been applied to alkanes, aromatic hydrocarbons and phenols in air, pesticides, nicotine, dicyclohexylamine in liquid matrices, and herbicides, pesticides, phenols, etc. in soil and sediment matrices [26,29]. However, it appears no one has applied SFE to the extraction of PCBs from pine needles.

The aim of this study was to investigate the suitability of using supercritical fluid (carbon dioxide) extraction as an alternative to pre-treat pine needle samples in the analysis of PCBs. Comparisons between SFE and other methods in extracting PCBs from pine needle samples are made.

2. Experimental

2.1. Chemicals

PCBs used in this study were purchased from Ultra Scientific (North Kingstown, RI, USA) in the form of Aroclor 1242, 1248, 1254, and 1260. Stock prepared solution at 100 μg/ml concentrations in hexane (HPLC-grade, obtained from Fisher, Fair Lawn, NJ, USA). Anhydrous sodium sulfate was purchased from Goodrich Chemical Enterprise (Brecksville, OH, USA). Deionized water was produced with a Millipore Milli-Q system (Waters, Milford, MA, USA).

2.2. Pine needle sample collection and preparation

The pine needle samples were collected from three different locations within the Botanic Gardens, Singapore. Since height is the most important factor that influences pollutant concentration [30], samples in this study were collected at approximately 1.6 m above ground level. All samples were stored in solvent-cleaned glass jars with aluminum foil-lined lids, and were frozen on return to the laboratory until extraction.

2.3. Supercritical fluid extraction

Supercritical fluid extraction was carried out using a Jasco (Tokyo, Japan) pump (PU 980) and Jasco 880-81 backpressure regulator (up to 500 kgf/cm^2). A CE ThermoQuest (Rodano, Italy) Trace series general GC oven was used as the temperature control unit.

The extraction fluid was pure CO_2. Flow-rates of 1.0, 1.5, 2.0, 2.5 and 3.0 ml/min were evaluated. The extraction temperature of this experiment was set to 120 °C (60, 80, 100, 120 and 140 °C were initially evaluated with restrictor temperature at 60 °C and pressure at 200 atm. For optimization, extraction durations of 30, 40, 50 and 60 min were evaluated. Finally, the optimized conditions were selected for the real sample analysis, i.e. flow-rate at 2.5 ml/min, extraction time of 50 min and oven temperature 120 °C.

The collection vial was about 12 cm long and test-tube like with a tapered end and a cone-socket cap, so it could be connected tightly to the back pressure regulator. No solid phase trap or liquid solvent was needed to trap the extract. Finally, the extract was collected using about 3 ml of HPLC-grade hexane and then pre-concentrated to 0.5–1 ml by nitrogen blow down. This extract was used for GC–MS analysis.

Pine needle samples (ca. 3 g), together with 4 g anhydrous sodium sulfate, were ground with mortar and pestle. Each sample was divided into four parts, spiked with 100 μg/l Aroclor 1242, 1248, 1254 and 1260, respectively. For supercritical CO_2 extraction, the stainless steel extraction cell was filled with 2.0 g anhydrous sodium sulfate. Then an accurately weighed sample was added to the cell. Another 2.0 g anhydrous sodium sulfate was added. Addition of the anhydrous sodium sulfate was to ensure a better distribution of extraction fluid and increase the sample surface area. The same processing procedure was applied to real world non-spiked pine needle samples.

2.4. Chromatographic analysis

A DB-1 (100% dimethylpolysiloxane) column (30 m×0.25 mm I.D., 0.25 μm) was purchased from

J&W Scientific (Folsom, CA, USA). The QP5000 GC–MS system was from Shimadzu (Tokyo, Japan).

The temperature programme was as follows: initial temperature 120 °C, held for 1 min, then increased at a rate of 5 °C/min to 280 °C and held for 3 min.

The injector temperature was set at 280 °C and the MS interface temperature at 300 °C. Carrier gas (helium) flow-rate was 1.2 ml/min. Typically, 1 μl of extract was injected under splitless mode.

3. Results and discussion

After extraction, 100 μg/l octachloronaphthalene (OCN) was added to the pre-concentrated extract (0.5–1 ml) as internal standard. For analysis of unspiked (genuine) pine needles, the extract was concentrated to ca. 0.1 ml.

In order to obtain optimized SFE conditions, parameters such as oven temperature, CO_2 flow-rate and extraction duration time were studied.

3.1. Effect of temperature of extraction

Previous work indicated that pure CO_2 at high temperature could yield high recoveries of many semi-volatile pollutants [31–33]. Our results demonstrated that temperature does play an important role in PCB extraction. With extraction time held constant at 40 min, CO_2 flow-rate fixed at 2.0 ml/min, and restrictor temperature at 60 °C, the relationship between oven temperature (varied at 60, 80, 100, 120 and 140 °C) and recoveries of Aroclor 1260 (spiked at 100 μg/l) were investigated. As shown in Fig. 1, as the temperature increased, the recovery of Aroclor 1260 increased correspondingly. At 120 °C, the recovery reached 95%; there was no further improvement at a temperature of 140 °C.

3.2. Effect of CO_2 flow-rate

The effect of CO_2 flow-rate was studied by fixing the oven and restrictor temperatures at 120 and 60 °C, respectively, and the extraction time at 40 min. Triplicate extractions were carried out at flow-rates of 1, 1.5, 2, 2.5 and 3 ml/min. Recovery was based on the amount of spiked Aroclor 1260 (100 μg/l) in each pine needle sample. Fig. 2 shows the relationship between CO_2 flow-rate and recoveries of Aroclor 1260.

As shown in Fig. 2, in the same period of time, the higher the CO_2 flow-rate, the greater the recovery. While it can be predicted that a higher flow-rate would cause faster extraction, a leveling-off is observed when the flow-rate is >2.5 ml/min. Also, with increase in the flow-rate, system pressure will increase correspondingly, which is undesirable for the extraction system. A CO_2 flow-rate of 2.5 ml/min was therefore selected as the optimum.

3.3. Effect of extraction duration time

The CO_2 flow-rate was fixed at 2.5 ml/min, and the extraction temperature at 120 °C, while extractions were carried out for 30, 40, 50 and 60 min, respectively.

Obviously, the longer the extraction time, the better the recovery would be. However, considering

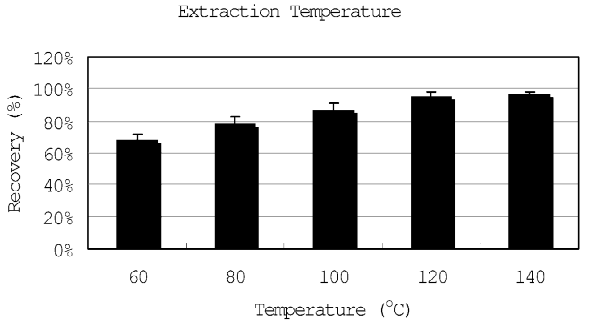

Fig. 1. Profile of extraction temperature and recoveries, at constant extraction time (40 min) and CO_2 flow-rate (2.0 ml/min).

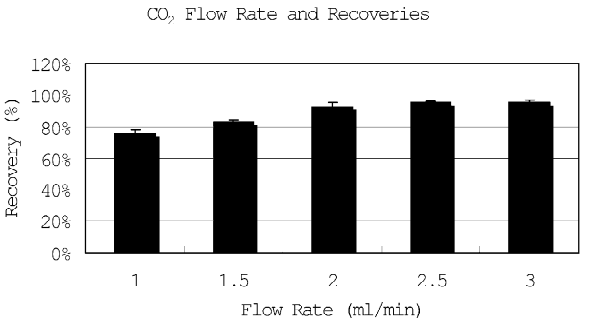

Fig. 2. Profile of CO_2 flow-rate and recoveries, at constant extraction time (40 min) and extraction cell temperature 120 °C.

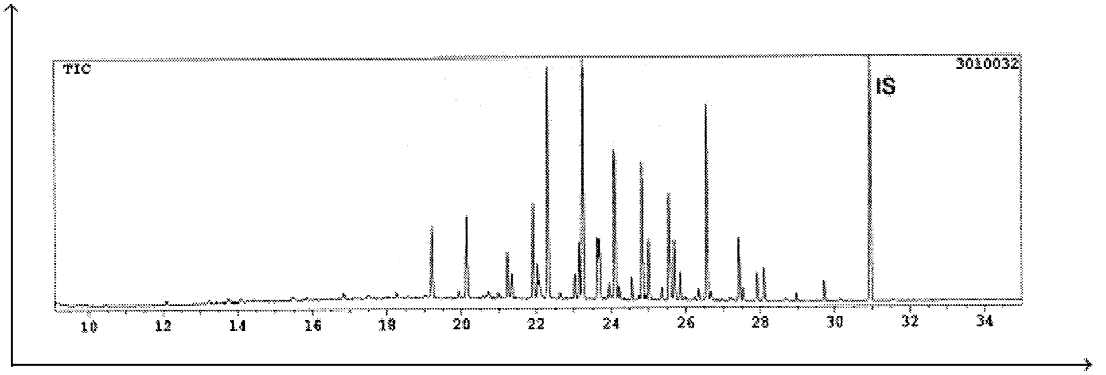

Fig. 3. Mass chromatogram of Aroclor 1260 and internal standard OCN. Analytical conditions are given in the text; *x*-axis in min.

that more interfering materials would be extracted as well, 50 min was finally selected as the extraction time.

In this work we did not focus on individual PCB congeners, but mainly on determining total PCBs in the form of the Aroclor series. Quantitative recovery measurements of PCBs were performed using the sum of PCB congener peak areas after a five-point linear calibration curve from the corresponding gravimetrically prepared Aroclor standards. All results were corrected by the internal standard method.

Fig. 3 shows a full-scan mass chromatogram of Aroclor 1260 with internal standard OCN. It is clear that the separation is rapid (less than 35 min) and complete.

Table 1 listed the recoveries of different Aroclor standards in the spiked pine needles. As shown in Table 1, recoveries are very good (90–95%). They are superior to traditional column chromatography-based methods (around 80% recovery) [14,18,22,23], that are time-consuming and labor-intensive and comprise five or more steps. A recently published work for example, used at least five steps for the pretreatment of pine needle samples [18]. Firstly, Soxhlet extraction was performed, the extract was then evaporated under vacuum. The third step was alumina/silica gel chromatography, followed by gel permeation chromatography. Finally, silica gel fractionation was carried out. This procedure consumed more organic solvents and took longer than SFE, with lower recovery. In addition, the operation is tedious and difficult even to an experienced chemist.

We observed the co-extraction of some interfering materials, probably terpenes and alkyl alcohols.

Table 1
Mean (%) recoveries and RSDs of PCBs extracted by SFE from three different spiked pine needle samples

PCB	Spiked level (μg/kg)	Sample 1		Sample 2		Sample 3	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Aroclor 1242	100	93.44[a]	6.17[b]	89.28	4.76	94.52	4.18
Aroclor 1248	100	95.44	7.27	94.67	6.73	89.92	4.85
Aroclor 1254	100	94.96	7.91	93.28	7.05	93.21	5.59
Aroclor 1260	100	95.68	5.54	96.67	6.28	94.72	8.02

SFE were performed with pure CO_2 at 120 °C and 200 kg/cm^2 for 50 min. Restrictor temperature = 60 °C. Recovery in % is based on the spiked value. Relative standard deviation is based on triplicate extractions.

[a] $N = 3$.

[b] Internal standard method was used for quantification. Analyses were carried out by GC–MS (selected ion monitoring).

Table 2
Main ions of Aroclor 1242, 1248, 1254 and 1260 monitored by GC–MS (SIM)

PCB	m/z
Aroclor 1242	186.05, 221.95, 258.00, 291.85, 325.75
Aroclor 1248	186.05, 257.95, 291.90, 325.85
Aroclor 1254	254.00, 291.95, 325.85, 327.80, 359.95
Aroclor 1260	325.90, 359.85, 393.95, 395.85

However, since GC–MS-SIM (selected ion monitoring) mode was used, the problem was not significant.

Under SIM mode, not only the sensitivity but also the selectivity were much improved. Under this mode, only those target PCBs were monitored. Generally 4–5 typical ion sets were monitored for each Aroclor mix to confirm its identity. Table 2 lists the main ions monitored for each Aroclor set.

The limits of determination (LODs) in this work ($S/N=3$) were 5–10 μg/l for Aroclor 1260. Thus, the LOD for each congener should be approximately 0.5–1 μg/l since each Aroclor mix contains at least 15 congeners.

To test the ruggedness of the method, we compared the results obtained at an interval of 2 days each. We found that the RSDs were quite close to those shown in Table 1, i.e. around 6–7%. This demonstrates that the method displays both good repeatability and reproducibility.

To evaluate the SFE method developed, three different real world pine needle samples were determined under the optimized experimental conditions. The PCB concentrations are shown in Table 3.

The results show that the concentrations of PCBs in the pine needles were low or below the detection limits, in comparison to samples analysed in Italy, UK, etc. [14,17,18,23].

4. Conclusion

For the first time, SFE with CO_2 was used to extract PCBs from pine needle samples. Results show that SFE with GC–MS (SIM mode) is a reliable technique to determine PCBs in these samples. The advantages are faster extraction, consumption of less organic solvent, and high extraction efficiency, etc.

Although some co-extractants were observed, they were in very small quantities, and the use GC–MS-SIM for analysis afforded a measure of selectivity for the PCBs.

The present work indicates that it is feasible to use SFE as an effective sample pre-treatment method in analysis of PCBs from pine needles.

Acknowledgements

The authors are grateful to the National University of Singapore for financial support of this work. X.R.Z. thanks the university for the award of a research scholarship.

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Table 3
SFE and GC–MS (SIM) analysis of PCBs in pine needle samples

Sample	Concentration (ng/g±SD)			
	Aroclor 1242[a]	Aroclor 1248[a]	Aroclor 1254[a]	Aroclor 1260[a]
Sample 1	ND[b,c]	ND	0.7±0.07	1.1±0.37
Sample 2	ND	ND	ND	ND
Sample 3	ND	0.6±0.24	ND	ND

[a] Different selective ion monitoring methods set up for the respective Aroclor sets were used to monitor the samples.
[b] ND, not detected (below 0.5 ng/g); $n=3$.
[c] Quantification based on internal standard calibration.

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