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Short communication

Focused microwave aqueous extraction of chlorophenols from solid matrices and their analysis by chromatographic techniques

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Abstract

Open-vessel focused microwave (FMW) extraction with a purely aqueous carbonate solution was used for the extraction of chlorophenols from various solid matrices. After SPE on C_{18}-bonded silica, the analytes were determined as such by LC–UV or, as their acetyl derivatives, by GC–ECD. The FMW aqueous extraction is efficient and rapid and no organic solvents are used. PCP was detected in several solid samples, with recoveries of 101–115% (RSD, 2–4%) relative to Soxhlet extraction. Similar recoveries were obtained for the other chlorophenols for spiked samples.

Keywords: Focused microwave extraction; Microwave aqueous extraction; Chlorophenols; Solvent-free extraction

1. Introduction

In order to prevent environmental pollution, analytical procedures using small amounts of organic solvents are increasingly being recommended [1]. One good example is the development of pressurized hot-water extractions [2]. In this study, an extraction procedure, which does not require organic solvents, is used for solid samples [3]. To this end, open vessel FMW device is used—a technique that works at atmospheric pressure and provides uniform, powerful and intensive heating [4,5]. FMW device and techniques have been discussed by Budzinski et al. [6] and have been applied successfully for various classes of analytes [6–8]. Because the extraction is carried out at the boiling point of the solvent, over heating and undesired side reactions are largely avoided. In most FMW procedures, organic solvents are used for the extraction; however the consumption is only 10–20% of that in Soxhlet or liquid–liquid extractions.

Chlorophenols are high-priority pollutants and several of them are classified as carcinogens [9–11]. Today, consumer goods are increasingly screened for pentachlorophenol (PCP) and tetrachlorophenols (TeCP). Continuous Soxhlet extraction is used to isolate these and other chlorophenols from solid samples in many laboratories. To overcome the problems associated with Soxhlet extraction (solvent and time consumption), MW-based extraction techniques were developed. MW extraction of chlorophenols has been reported, both with organic solvents and water–organic solvent mixtures [12,13]. Alonso et al. used FMW to extract chlorophenols with a mixture of organic solvent and water [14].

The present study discusses FMW extraction of chlorophenols from wood, leather and textiles using a purely aqueous carbonate solution and subsequent LC–UV or GC–ECD analysis. These cellulose and protein matrices have the tendency to swell in the aqueous alkaline extractant, which enhances the extraction efficiency.

2. Experimental

2.1. Reagents

Ammonium carbonate, sodium carbonate, potassium carbonate, acetone, acetic anhydride and triethylamine were of

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Table 1
Analytical performance data for determination of chlorophenols

No.	Chlorophenol	LOD (μg/g) in		Recovery (%) from	
		GC–ECD	LC–UV	Wood sample	Leather sample
1	2-MCP	0.5	0.2	89	86
2	3-MCP	0.5	0.4	91	85
3	4-MCP	0.9	0.4	92	93
4	3,5-DCP	0.4	1.4[a]	95	96
5	2,5-DCP	0.1	0.4	94	92
6	2,4-DCP	0.2	1.3[a]	97	102
7	2,6-DCP	0.3	0.9	95	95
8	3,4-DCP	0.1	1.3	96	94
9	2,4,6-TCP	0.1	1.2[a]	95	96
10	2,3,5-TCP	0.1	2.3[a]	98	96
11	2,4,5-TCP	0.1	1.7[a]	95	99
12	2,3,6-TCP	0.1	0.2	96	95
13	3,4,5-TCP	0.1	2.5[a]	98	99
14	2,3,4-TCP	0.8	3.0[a]	98	97
15	2,3,5,6-TeCP	0.1	1.1[a]	100	99
16	2,3,4,6-TeCP	0.1	1.0[a]	100	99
17	2,3,4,5-TeCP	0.1	3.5[a]	100	100
18	PCP	0.1	3.5[a]	101	102

MW extracted with 0.01% carbonate solution and ENVI-18 clean-up (for LC–UV); and derivatised before ENVI-18 clean-up for GC–ECD. Spiking at 1 μg/g (each analyte). RSD range 1–4% for wood and 2–4% for leather ($n = 5$).

[a] 50 μl (10 μl in other cases) injected to improve detectability.

analytical grade; HPLC-grade methanol, ethyl acetate and hexane were procured from Merck (Mumbai, India). Individual chlorophenols as found in Table 1 and ENVI-18 RP solid phase extraction (SPE) material were purchased from Supelco (Bellefonte, USA), HPLC-grade water purity was prepared using Milli-Q apparatus of Millipore, (Bedford, MA, USA).

A stock solution of a mixture of chlorophenols at 1000 mg/l each was prepared using methanol; from this stock, working standard solutions were prepared daily. The working standards for LC were prepared in the range 0.10–10 μg/ml using methanol; and for GC in the range 0.01–1.00 μg/ml using hexane.

2.2. Equipment

A focused microwave system, model Soxwave 100 with a programmer was purchased from Prolabo (Fontenay-sous-Bois, France). The model works at atmospheric pressure and has 300 W capacity with an available power range of 30–150 W and a frequency of MW radiation of 2450 MHz. Borosilicate glass open extraction vessel was fitted with a solvent collector and an overhead Graham's water condenser as discussed by Budzinski [6]. A Julabo cooling water circulator (model FE1800, Seelbach, Germany) was used to support the water condenser.

An Alliance 2695 LC system with an auto injector and a diode array UV detector (DAD UV), Model 996 and Millennium 32 software version 3.0, from Waters Instruments (Milford, MA, USA) was used. A 250 × 2 mm I.D. column with 5 μm particles, Purosphere STAR (C_{18}) was from Merck (Darmstadt, Germany). A gas chromatograph model Auto system XL, equipped with an electron capture detector (ECD) and operated with a Turbo Chrome Navigator workstation was purchased from Perkin–Elmer (Norwalk, CT, USA). A mid-polar capillary DB-17, column of 30 m × 0.32 mm I.D., and 0.25-μm film thickness (J. & W. Scientific, Folsom, CA, USA) was used for GC separation.

2.3. Methods

2.3.1. Sample preparation

Soil free from chlorophenols, was air-dried, pulverized and sieved to a grain size of 2 mm. A total of 100 g of soil was mixed with acetone until the sample was completely soaked to form a slurry. 25 g slurried soil were spiked with an appropriate volume of standard solution to achieve 1 μg/g level of analyte. The contents were mixed well for over 3 h. The bulk of the solvent was evaporated at room temperature by thorough manual shaking. The sample was left for 48 h in a fume-hood to dry completely and aged for 1 week at room temperature. The prepared soil sample was stored in a refrigerator at 4 °C until analysis.

Wood and leather samples were prepared as powders of 100–200 mesh and 1 g of each sample was taken for analysis. The spiking was done by adding standard solution equivalent to 1 μg (100 μl of 10 μg/ml) to this weighed portion, the contents were mixed thoroughly for 1 h and taken for extraction. The extractions of textile and paper samples were done with 1 g of finely cut (1–2 mm^2) pieces. Samples such as dyestuff were analysed as 1 g portions after breaking down any lumps if present.

2.3.2. Soxhlet extraction

The conventional Soxhlet continuous solvent extractions were employed for solid samples using acetone as the solvent. 150 ml acetone was allowed for 60 recycles to ensure complete recovery of chlorophenols from solids. After the extraction, the solvent was rotary evaporated to near dryness. The residue was dissolved and made up to 5 ml with methanol. This solution was directly used for LC analysis; where as 1 ml of this solution was derivatised for GC analysis. The derivatisation was achieved by adding 0.5 ml acetic anhydride, 1 ml triethylamine and 10 ml hexane to 1 ml of sample solution taken along with 25 ml of 1% an aqueous carbonate solution in a separating funnel (50 ml) and then by shaking the contents vigorously in a mechanical shaker for 30 min. The hexane layer was collected separately in a 25 ml volumetric flask. LLE was repeated in a similar way with an additional 10 ml of hexane and the final volume adjusted to 25 ml with hexane. The extract was dried over anhydrous sodium sulphate.

2.3.3. MW extraction

The solid samples found positive of chlorophenolic residues (by Soxhlet extraction) were chosen for MW

extraction with 25 ml of ammonium carbonate extractant. Before extracting solid samples, an optimum extraction condition was studied using aqueous ammonium carbonate of 0.01–10% concentrations. The optimization of experimental conditions was studied, by varying MW power from 10–40% of 300 W at constant time. Then, the influence of time on the extraction was studied by varying the time from 5–30 min at a constant power.

After the extraction, the MW extraction vessel was allowed to cool to room temperature before the condenser was removed. Then the aqueous extract was filtered through a glass micro fiber filter, made up to 50 ml with carbonate solution and taken directly for LC analysis. For analysis by GC–ECD, a 10 ml portion of the extract was acetyl derivatised as described in Section 2.3.2.

2.3.4. SPE clean-up for LC analysis

SPE clean-up and enrichment of chlorophenols have been reported elsewhere [15–19]. On completion of the MW extraction of a solid sample, the whole carbonate extract of about 25 ml along with 2–3 ml of 0.01% carbonate solution washings, was adjusted to pH 2.5 with 10% sulphuric acid and then passed through a 0.5 g ENVI-18 cartridge previously conditioned with methanol, at a flow rate of ca. 2 ml/min. The elution was done with 5 × 1 ml of ethyl acetate. This was filtered through 0.45 μm PTFE filter and used for LC analysis. The LODs for the various chlorophenols by LC-DAD UV are given in Table 1.

At first, the recovery of chlorophenols was ascertained by ENVI-18 SPE with the standard solution of chlorophenols. For this, the standard solution of chlorophenols (1 μg of each congener) was spiked into 25 ml of ammonium carbonate extractant, adjusted to pH 2.5 with H_2SO_4 and passed through ENVI-18 cartridge.

2.3.5. SPE cleaning for GC analysis

For GC analysis, acetyl derivatives of chlorophenols are preferred. If the SPE clean-up discussed in Section 2.3.4 is done, chlorophenols are collected into an organic phase of very low volume that is difficult to handle for derivatisation. Hence the derivatisation was done first, followed by SPE using 0.5 g of ENVI-18. SPE clean-up for acetyl derivatives of chlorophenols [18] using C_{18} RP cartridge was discussed earlier. The recovery of acetylated chlorophenols is much higher than for chlorophenols by a C_{18} SPE.

The recovery of acetyl derivatised chlorophenols was studied with a standard solution of chlorophenols. This was done by adding 1 ml of standard solution of chlorophenols (1 μg of each chlorophenol) to 25 ml of 0.01% potassium carbonate extractant and carrying out derivatisation in Section 2.3.2 but omitting hexane. After derivatisation, the extract was passed through ENVI-18 cartridge at 2 ml/min. Elution was done using 5 × 1 ml of *n*-hexane. The extract was used for GC–ECD analysis. The solid sample extracts after MW carbonate extraction and acetyl derivatisation, were cleaned-up with ENVI-18 and analysed by GC–ECD.

2.3.6. LC analysis

RP–LC with UV detection of these analytes was reported earlier [15–17]. In this study, a gradient elution programme was used with methanol and water (containing 0.3% formic acid to adjust the pH to 2.5) as solvents. Methanol was at 50% initially, then linearly increased to 90% in 40 min, with a further 10 min hold. The flow rate was 0.20 ml/min initially, and then it was raised to 0.35 ml/min on linear gradient in 40 min. From there it was increased to 0.40 ml/min in 10 min. 10 μl sample was used for injection (50 μl was used for some poorly responding analytes as referred in Table 1); detection was at 280 nm. The LC separation took 40 min.

2.3.7. GC–ECD analysis

A temperature gradient programme was employed which is as follows: the oven temperature was initially at 80 °C for 2 min, then raised to 220 °C at the rate of 15 °C/min and held at 220 °C for 10 min. Finally it was raised to 275 °C at 15 °C/min. The electron capture detector (ECD) and injection temperatures were maintained at 375 and 250 °C respectively while the carrier gas was set at a constant pressure mode of 8 psi. The separation was done on a DB-17 column, a mid-polar capillary column. High purity nitrogen was used as carrier gas and as detector make-up gas. 2 μl of samples were injected by split-less mode. The separation took 30 min.

3. Results and discussion

3.1. Influence of carbonate concentration

In order to study the influence of carbonate on the recovery of chlorophenols from solid samples, 0.001–10.0% aqueous ammonium carbonate were tried. The samples which originally contained PCP (by Soxhlet) were used. When only pure water was employed for the extraction under the identical experimental conditions, the recovery was 65% for PCP. For 0.001% carbonate, the recovery only increased to 67.5%. The maximum recovery was found with 0.01% carbonate, then declined with higher concentrations of carbonate and was found to be lowest for 10% carbonate. This trend was also found for all other solid samples. Carbonate solutions also serve to keep chlorophenols in their ionized forms in which volatility is low; thus the loss of analytes at higher temperatures is prevented. The reason for the decreasing recovery with increasing carbonate concentration should be the increased ion strength which hinders MW propagation.

3.2. Optimization of MW extraction

The main issue in deciding the sample size is the concentration of analytes in a sample. Environmental samples are likely to carry toxic residues at low concentrations and especially soil samples required to be larger in size. Leather

and textile samples which undergo more swelling than soil, consume more aqueous phase causing a decreased volume of the extractant; hence, higher sample size will cause problems. For the optimization of the volume of extractant and the sample size, soil and leather were chosen as examples. A soil sample was spiked with PCP at 0.5 μg/g for sample weights of 1, 2 and 5 g and a leather sample containing PCP at 1.3 μg/g, were studied in the same way with 15–50 ml of 0.01% carbonate extractant. When 15 ml extractant was employed, the recovery of PCP from soil was observed higher for 1 g (90%) than for 5 g (81%). A similar observation was found for leather. For 20 ml extractant, the recovery for 1 g was improved to 95%. On increasing the extractant volume to 25, 30 and 40 ml, the recovery reached the maximum at 25 ml (96%) remained constant up to 30 ml (95%) and then declined at 40 ml (91%). Hence, the optimal extractant volume was 25 ml. When the influence of sample size in 1–5 g range on recovery was studied no noticeable effect was observed for 25 ml or higher volumes however for 20 ml or lower it was noticeable. This should be due to excessive heat generated in the latter case.

MW power was varied from 10 to 40% of the maximum allowed 50% limit. Increased recoveries were observed up to 30% but on a further power increase to 40%, the recovery decreased probably due to excessive heat generation. Accordingly, 20–30% MW power was chosen. Varying the time from 5 to 30 min at constant power and determining the recovery of PCP from a leather sample on MW power and duration of extraction is shown in Fig. 1. Obviously, 30% MW power for 30 min duration is a proper choice. A similar result was found for all other test samples. The other chlorophenols were not present in the solid samples; hence a mixture of these analytes was spiked to achieve a 1 μg/g concentration of each of them. They were extracted with 0.01% carbonate, acetyl-derivatised and analysed by GC–ECD. The results for a wood and a leather sample are shown in Table 2.

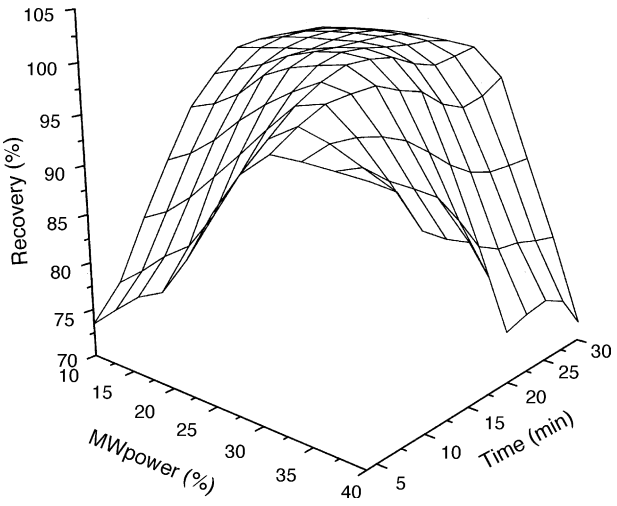

Fig. 1. Correlation of MW power (%), duration of extraction and recovery of PCP present originally in a solid sample (leather).

3.3. Recoveries of chlorophenols by MW aqueous extraction

Standard solutions were spiked at different levels from 0.2–1.0 μg/g to each of the chosen solid matrices. The samples were analysed after MW extraction using the 0.01% carbonate and ENVI-18 cleaning method. The spike studies were carried out only with (at random) selected isomers. 3-MCP and 2,4-DCP were spiked only at 0.5 and 1.0 μg/g, as their LODs (in GC–ECD) are 0.5 and 0.25 μg/g, respectively; for 2,4,6 TCP, 2,3,4,6-TeCP and PCP with their LODs <0.1 μg/g, a still lower spiking level of 0.2 μg/g was feasible. The typical recoveries are given in Table 2. The general trend in the spiked recoveries for solid samples reveals that the spikes at lower concentrations give relatively less recovery. LC–UV chromatogram of a leather sample spiked at 1 μg/g is shown in Fig. 2. A similar result for a spiked wood sample using GC–ECD is shown in Fig. 3.

Table 2
Percent recovery of chlorophenols at different spike levels, for solid samples MW extracted with 0.01% aqueous carbonate, acetyl derivatised, cleaned over ENVI-18 and analysed by GC–ECD (RSD: $n = 3$)

Chlorophenol	Spike level (μg/g)	Soil		Leather		Wood	
		Recovery	RSD	Recovery	RSD	Recovery	RSD
3-MCP	0.5	92	4	92	4	85	5
	1.0	89	3	85	4	91	3
2,4-DCP	0.5	82	5	94	3	93	3
	1.0	90	5	102	2	97	4
2,4,6-TCP	0.2	85	6	89	5	81	6
	0.5	92	3	97	3	94	4
	1.0	93	4	96	4	95	3
2,3,4,6-TeCP	0.2	80	5	84	6	86	5
	0.5	91	5	90	4	90	4
	1.0	92	3	99	2	100	1
PCP	0.2	86	4	93	3	95	4
	0.5	91	5	95	5	92	5
	1.0	96	5	102	4	101	4

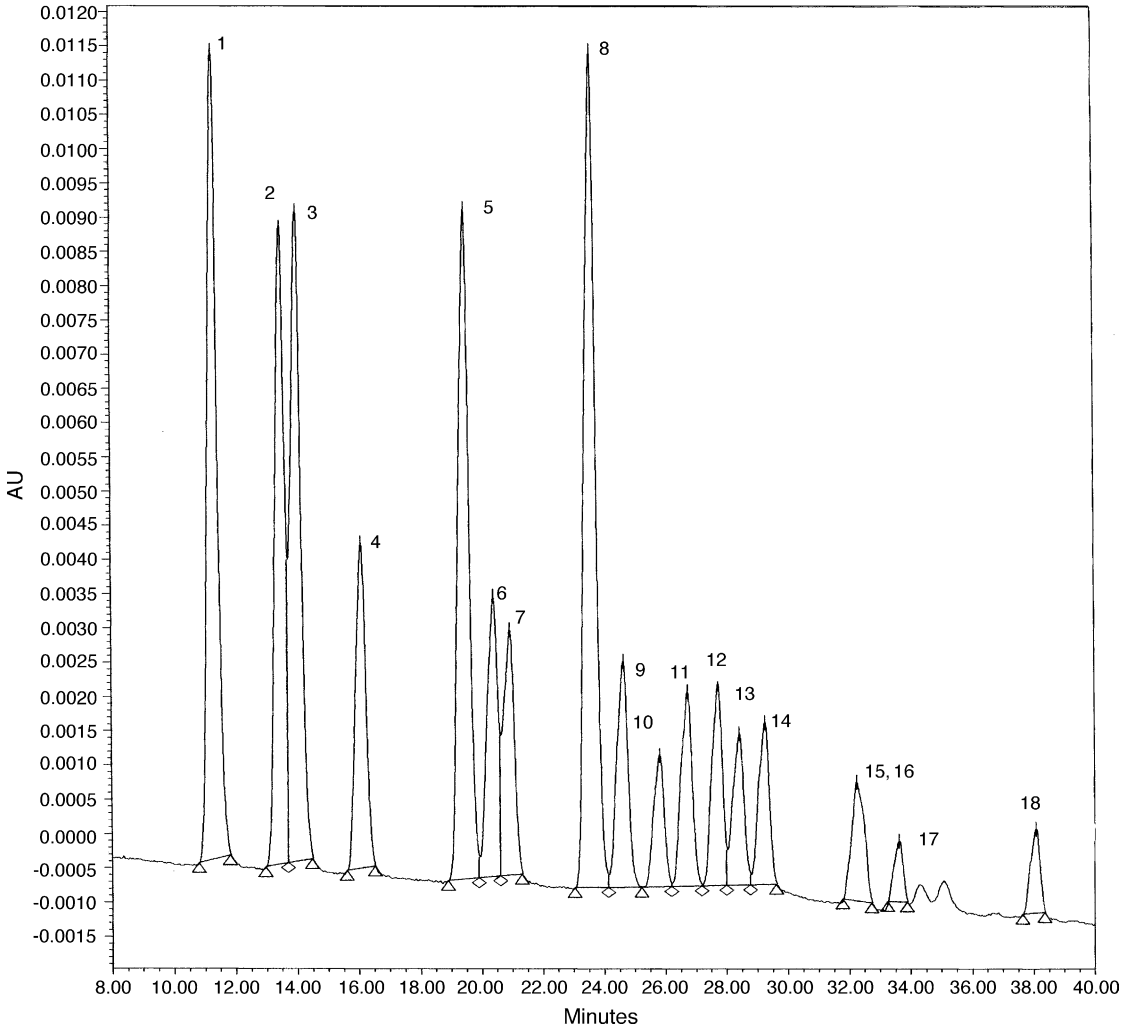

Fig. 2. LC–UV of a leather sample spiked (1 μg/g of each analyte) with standard mixture of chlorophenols except PCP that is originally present (50 μl were injected; detection at 280 nm): peaks (the analytes are referred by the number assigned in Table 1): (1) 1; (2) 3; (3) 2; (4) 9; (5) 5; (6) 6 ;(7) 4; (8) 7; (9) 10; (10) 13; (11) 12; (12) 8;(13) 14; (14) 11; and (15)–(18) are in the same order of Table 1.

3.4. SPE clean-up of extracts

The SPE recoveries using ENVI-18 were found to be ca. 82% for more polar analytes like 2-MCP and 102% for PCP, which is relatively non-polar. The SPE recoveries obtained for standard chlorophenols as their acetyl derivatives showed little mutual differences and ranged from 100% for 2-MCP to103% for PCP. The C_{18}-SPE material improves recovery of the acetyl- derivatised chlorophenols, especially for highly polar MCP isomers. The LODs for the various chlorophenols in GC–ECD after SPE enrichment are given in Table 1.

3.5. Analysis of real samples

Paper, soil (collected from an industrial site), adhesive tape, dyes and other solid samples mentioned earlier in this paper were taken for analyzing chlorophenols. PCP was the only chlorophenol found in many of these samples. Values ranged from 0.2 mg/kg in a soil sample to 115 mg/kg in a dyestuff. That is, the proposed method works over a wide range. The proposed FMW aqueous extraction and Soxhlet techniques produced close by similar results for the same samples, confirming the validity of the method. This is revealed from the data in Table 3, obtained for real solid samples

Table 3
GC–ECD analysis of PCP in real samples by different extraction techniques

Nature of sample	Soxhlet extraction using acetone		MW extraction using aqueous carbonate (SPE after acetyl derivatisation)	
	(mg/kg)	RSD (%)	(mg/kg)	RSD (%)
Wood	31	2	32	4
Textile	2	2	3	2
Leather	29	4	30	4
Paper	0.6	2	0.7	3
Dyestuff	115	1	117	2
Soil	0.2	4	0.2	3
Adhesive tape	19	3	20	2

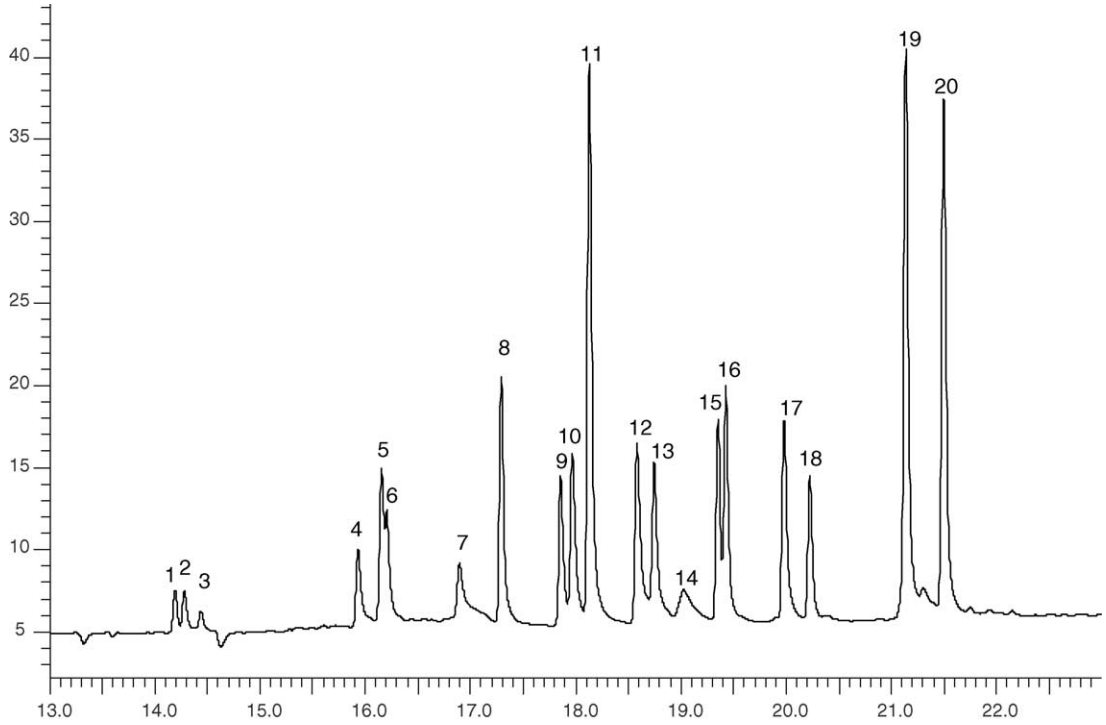

Fig. 3. GC–ECD chromatogram of a wood sample spiked (1 μg/g of each of the analytes) with standard mixture of chlorophenols except PCP that is originally present: peaks (the analytes are referred by the number assigned in Table 1) from (1) to (14) and (17) are the same order and (15) is 16, (16) is 15, (18) Hexachlorobenzene (injection marker), (19) is 18 and (20) Tetrachloroguiacol. (Internal standard).

which were extracted with 0.01% carbonate by MW, derivatised, cleaned by ENVI-18 and analysed by GC–ECD. PCP extracted from real samples gave recoveries of 101–115% (relative to Soxhlet solvent extraction) with RSDs of 2–4%. Both the efficiency of the technique and the RSD values are either similar to or better than with Soxhlet extraction, with good repeatability. With real-sample analyses, matrix effects are quite dominant and the ENVI-18 clean-up is found to give cleaner extracts.

4. Conclusions

This study supports the fact that aqueous extractants are as efficient as organic solvents for the MW extraction of chlorophenols, with the added advantage of the proposed method being more eco-friendly. In the proposed procedure, the role of the solvent is limited to a small volume for the SPE clean-up prior to the chromatographic analysis; that is, solvent consumption is significantly reduced. Besides, there is an additional advantage for vegetable and biological samples which swell in the aqueous extractant which should help to improved recoveries.

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