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Direct determination of chlorophenols in landfill leachates by solid-phase micro-extraction–gas chromatography–mass spectrometry ☆

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Abstract

Landfill leachates represent a serious environmental concern with regard to trace priority pollutants introduced into the aquatic environment. From the analytical point of view, they constitute complex matrices because of their high organic matter content and competition with the trace analytes in the extraction procedure. Although the use of SPME to extract chlorophenols in leachates has already been described in several publications, the limited number of chlorophenols restricts this analysis field of application. This paper presents a new analytical methodology to determine 13 chlorophenols and phenol by SPME–GC–MS in landfill leachates. The overall analysis was performed in 90 min and the detection limits range from 0.005 μg/l (pentachlorophenol) to 2.5 μg/l (phenol). Reproducibility, expressed by the coefficient of variation of repeated extractions at different concentration levels of the analytes, was on average inferior to 10%. Recovery, evaluated by standard addition to leachates, was 86.2% on average. Pentachlorophenol, 2,3,4,5-tetrachlorophenol and 2,3,4,6-tetrachlorophenol were the sole analytes detected at nanogram level in the landfill leachates analysed.

Keywords: Landfill leachates; Solid-phase microextraction; Chlorophenols

1. Introduction

Chlorophenols are highly toxic, poorly biodegradable and present carcinogenic and recalcitrant properties [1]. A typical example is the contamination of a Finnish aquifer by sawmill waste, in which chlorophenol concentrations exceeded regulatory levels. Epidemiological studies revealed an increased occurrence of cancer in the local population, presumably because of chlorophenol contact in drinking water [2].

Chlorophenols are widely used as wood preservatives, in paper, herbicide and pesticide industries. These applications often lead to wastewater and ground water contamination, which can threat public health when contamination of drinking water sup-

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plies occurs. They are thus included in both U.S. Environmental Protection Agency and European Union list of priority pollutants. The E.U. has established a maximum limit to the presence of pentachlorophenol in inland surface waters, estuary waters, internal coastal waters other than estuary and territorial waters of 2 μg/l [3]. EPA has set a maximum contaminant level of 1 μg/l of pentachlorophenol in drinking water [4].

Traditional disposal of solid residues in landfills makes the detection of chlorophenols in the resulting leachates a serious environmental problem. Leachates are complex aqueous matrices with high organic content, which require a specific extraction procedure in order to avoid analysis interferences. Because the chlorophenols exhibit different behaviour in terms of acidity and polarity, different interactions between matrix and analytes occur and the experimental conditions defined to analyse a restricted set of chlorophenols may not be adequate to analyse other molecules of the same group. This study, in the sense that it integrates a large range of chlorophenols, represents an advance urgently needed in the environmental field.

All chlorophenols are solid, except for 2-chlorophenol (2CP), which is a liquid with a melting point of 9 °C. The melting point of the other chlorophenols ranges from 33 to 191 °C. Phenol and 2-chlorophenol are hydrophilic compounds in comparison with tri-, tetra- and pentachlorophenol. Solubility in water at 25 °C ranges from 14 mg/l (pentachlorophenol) to $8{,}24\times10^4$ mg/l (phenol), and vapor pressures are lower for higher molecular mass compounds (Table 1).

Achieving nanogram levels of detection, for a large range of chlorophenols in a complex matrix, where organic matter exists at mg/l concentrations, is not an easy task.

The determination of chlorophenols in liquid samples has been traditionally done by gas chromatography with ECD or FID detectors after liquid–liquid extraction [5,6], or by HPLC after SPE [7]. On one hand, liquid–liquid extraction procedure is time consuming and uses large amounts of organic solvents, which are expensive with respect to waste disposal. One the other hand, the SPE procedure can be quite lengthy with a series of stages including drying and conditioning and even though requiring smaller amounts of organic solvents, can be expensive with the cartridges usually disposed of after one to four extractions [7]. Considering that the matrix that constitutes landfill leachates is often more complex than the one in effluents, this study intends to develop and validate a new methodology that includes the alternative solid-phase microextraction (SPME) [8–15], which is a fast, solvent-free methodology and is the only sample preparation procedure before analysis.

Table 1
Some physicochemical properties of phenol and the 13 chlorophenols studied

Compound	Water solubility 25 °C (mg/l)	Vapor pressure 25 °C (mmHg)	pK_a
P	8.24×10^4	0.35	9.99
2CP	1.13×10^4	2.53	8.56
24DCP	4.5×10^3	0.1155	7.89
26DCP	1.9×10^3	0.033	6.79
235TCP	90.09	0.022	–
246TCP	800	0.008	6.23
245TCP	1200	0.0075	7.4
236TCP	450	0.00246	5.8
234TCP	97.46	0.00246	–
345TCP	64.49	0.00246	7.84
2346TeCP	28.69	0.000339	6.35
2345TeCP	54.9	0.000339	5.14
2356TeCP	23	0.000666	5.22
PCP	14	0.00011	4.7

Several studies have described SPME extraction techniques to determine chlorophenols in aqueous samples. Buchholtz and Pawliszyn [16] analysed, by SPME–GC–FID, 11 phenols in wastewaters and concluded that low pH levels and saturated salt conditions increased compound extraction efficiency, proving that it is possible to apply this extraction technique to more complex matrices. Barták and Cáp [14] investigated SPME for determination of four chlorophenols contained in a standard mixture. The best results were obtained with a polyacrylate coated fiber by sampling in the headspace. The extraction time was 60 min, the sample was acidified and salt was added in order to obtain the best recovery. Alpendurada [15] studied the influence of parameters like salt addition and sample pH on the extraction of seven chlorophenols and phenol in aqueous samples. Finally, in a study by Lee et al. [17], SPME

extraction procedures were optimised to extract chlorophenols from urine samples.

There are still very few studies to determine chlorophenols in complex matrices like leachates. Lee et al. [18] evaluated SPME coupled to GC–MS to determine five chlorophenols in landfill leachates. SPME experimental procedure was optimised with a polyacrylate coated fiber at pH 1, extraction time of 40 min and desorption in GC injector at 290 °C for 2 min although this study represents an important contribution to the determination of chlorophenols in complex matrices, it is applied to a limited number of chlorophenols.

The present study widens the field of application of recent analytical techniques, such as SPME and GC–MS, to a greater number of chlorophenols, with different physicochemical properties, in landfill leachates.

2. Experimental

2.1. Chemicals

Thirteen chlorophenols plus phenol–phenol (P); 2-chlorophenol (2CP); 2,4-dichlorophenol (24DCP); 2,6-dichlorophenol (26DCP); 2,3,5-trichlorophenol (235TCP); 2,4,6-trichlorophenol (246TCP); 2,4,5-trichlorophenol (245TCP); 2,3,6-trichlorophenol (236TCP); 2,3,4-trichlorophenol (234TCP); 3,4,5-trichlorophenol (345TCP); 2,3,4,5-tetrachlorophenol (2345TeCP); 2,3,5,6-tetrachlorophenol (2356TeCP); 2,3,4,6-tetrachlorophenol (2346TeCP) and pentachlorophenol (PCP), were obtained from a EPA 8040A Phenol Calibration Mix (Supelco), containing 24 phenols with an individual concentration of 500 μg/ml in isopropyl alcohol. Water was G Chromasolv from Riedel. In SPME extractions water was used as a solvent, pH was adjusted with H_2SO_4 p.a. from Riedel and to saturate the samples Na_2SO_4 p.a. from Riedel was used.

A working standard containing the 13 chlorophenols plus phenol at a concentration of 500 μg/l was prepared in isopropyl alcohol. Before extraction, sample pH was adjusted and salt was added until saturation.

2.2. Equipment and experimental conditions

2.2.1. SPME equipment and experimental conditions

An 85-μm polyacrylate (PA) fiber (Supelco Cat. No. PN 57304) and a SPME fiber holder (Supelco Cat. No. PN 57330-U) were used. The fiber was conditioned in the GC injector for 1 h at 250 °C. Whenever needed this procedure was repeated for fiber cleanup. Previous to the sample extraction, blank runs were performed to look for fiber contamination. The vial capacity was 4 ml, handling 2 ml of sample. The temperature and stirring velocity (750 rpm) were controlled during extraction.

Optimised extraction conditions were: immersion sampling at 40 °C for 60 min, with an 85-μm PA-coated fiber, saturated salt conditions and sample pH$<$2.

2.2.2. Chromatographic equipment and experimental conditions

Analysis was performed with a GC–MS Varian 3800 Saturn System with an ion-trap detector and split/splitless injection port. The column was a CP Sil 8 CB/MS (30 m×0.25 mm×0.25 μm) from Supelco. Carrier gas was helium at 1 ml/min. Injector was operated in a splitless mode and the temperature was 280 °C. After the extraction, the fiber was introduced in the injector at 280 °C for 3 min with the split valve closed. The fiber desorption time in the injector was 3 min.

The GC–MS transfer line was maintained at 45 °C and the detector at 160 °C. The oven temperature was initially set at 40 °C held for 4 min, then programmed to 220 °C at a rate of 12 °C/min. The total analysis time of a single run was 20 min. The ionisation mode was electron ionisation with electron energy of 70 eV. Data was acquired in full scan mode, for quantitative analysis purposes.

2.3. Quantification

Chlorophenols were quantified by peak area using external standard method. Calibration curves were obtained with standards extracted in the same conditions as samples were.

3. Results and discussion

3.1. Optimisation of SPME operating conditions

Experimental conditions, like extraction temperature and time, pH, salt addition, extraction mode (fiber immersed or in the headspace) and desorption time in the GC injector, were previously optimised before validating the analytical method. Stirring the system at a constant rate was also important in order to maintain reproducibility of the process and to generate continuously fresh surface, therefore destroying the static layer resistant to mass transfer. In subsequent experiments assays were conducted in duplicate.

3.1.1. Extraction in headspace versus immersion

Experiments with aqueous samples spiked with chlorophenols, with concentration of 50 μg/l, were conducted in order to study the influence of the sampling mode on the quantity of chlorophenols extracted. On one hand, in case of analysing only 2CP, 24DCP, 26DCP, 235TCP, 246TCP, 245TCP, 236TCP, 234TCP headspace would be better than immersion. One the other hand, the results obtained show that headspace sampling does not allow the detection of 2345TeCP, 2346TeCP, 2356TeCP, 345TCP and PCP (Fig. 1). These results may be explained regarding the low vapour pressure values presented by these compounds (Table 1). Considering the 13 chlorophenols altogether it was decided to use immersion sampling, as it proved to be a suitable technique in order to enable extraction for most of compounds.

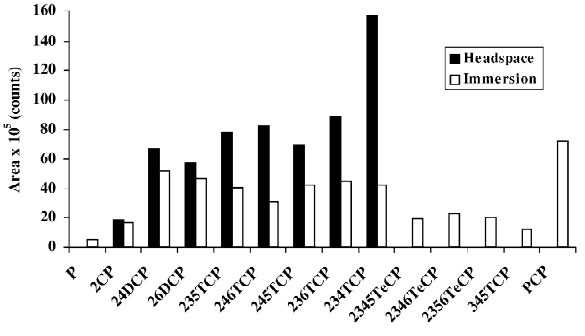

Fig. 1. Comparison of extraction extent (expressed by peak area) between headspace and immersion sampling (85 μm PA fiber, temperature = 70 °C; extraction time = 60 min; standard = 50 μg/l; pH = 2, sample saturated with salt).

3.1.2. Effect of temperature

Results obtained when studying the effect of five different temperatures, 30, 40, 50, 60 and 70 °C, in SPME extraction yield revealed that an extraction temperature of 40 °C represented the best compromise between the extraction yield and the amount of each chlorophenol extracted.

Two opposing effects were observed when the extraction temperature was increased. For most of the compounds studied, an increase in the extraction temperature up to 40 and 50 °C increased the extraction yield. For temperatures higher than this, a decrease in the extraction yield was observed. This somewhat contradictory effect may be justified, because increasing extraction temperature improves the mobility of the molecules through the phases, increases the extraction rate and therefore shortens the time needed to reach equilibrium but, simultaneously, there is a decrease in the distribution constant and the amount of chlorophenol extracted may decrease [11].

3.1.3. Extraction time

In order to select the extraction time, the influence of this parameter was studied on the extraction yield (Fig. 2). The results obtained show that the higher the extraction time, the higher the amount of chlorophenol extracted. For extraction time higher than 60 min the extraction extent, for most of the compounds, did not improve significantly. Therefore, an extraction time of 60 min represents the best compromise between the extraction yield and the amount of each chlorophenol extracted. Results obtained agree with those reported by Buchholtz and Pawlisyn [16], who established an extraction time of 60 min when using a PA fiber, pH 2, and with salt addition, for determination of P, 2CP, 24DCP, 246TCP and PCP in sewage samples.

3.1.4. Effect of pH and ionic strength

The influence of pH in the extraction extent, within the range 2–6, was evaluated. Experiments were done using an aqueous standard with chlorophenol concentration of 50 μg/l, an 85-μm PA fiber,

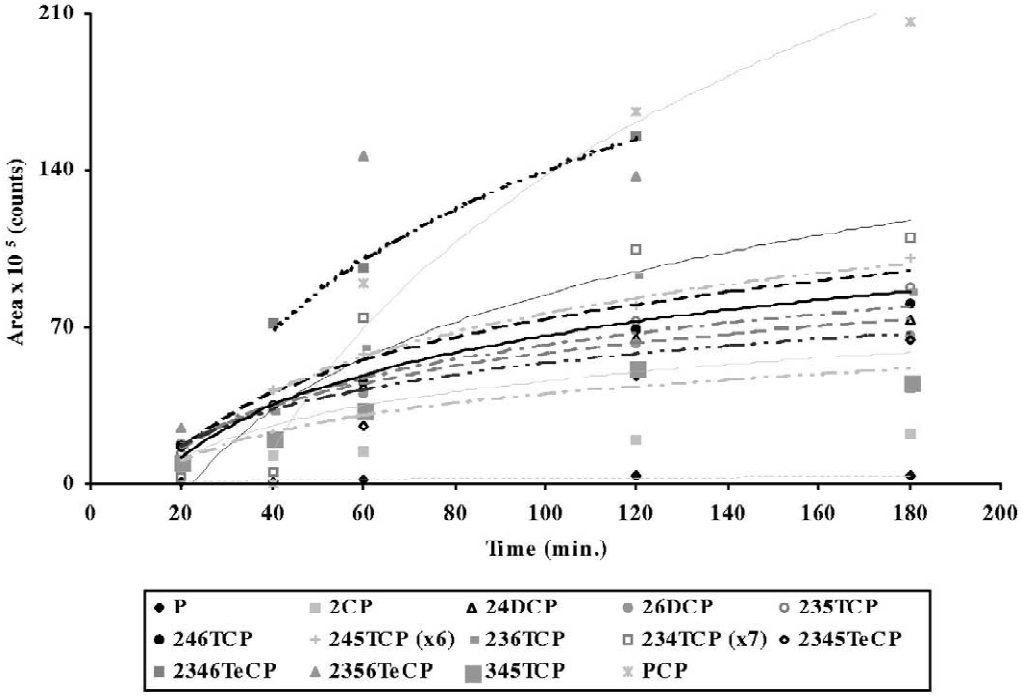

Fig. 2. Influence of extraction time in the extraction extent (85 μm PA fiber, temperature = 40 °C, immersion sampling, average concentration standard = 50 μg/l; pH = 2, sample saturated with salt).

extraction temperature of 40 °C, immersion sampling, extraction time of 60 min and sample saturated with salt. It was verified that the lower the pH value, the higher the improvement in the amount of chlorophenols extracted, particularly those whose pK_a is lower than 7 (Table 1), like 2346TeCP and PCP. At low pH values, the chlorophenols neutral form will be transferred to their ionic form, which has a higher affinity to the fiber. For these reasons, pH<2 was chosen.

To study ionic strength effect, experiments were done with different salt (Na_2SO_4) concentrations, ranging from 1.1 to 7.7 g in 30 ml of an aqueous standard with concentration of 50 μg/l, an 85-μm PA fiber, extraction temperature of 40 °C, immersion sampling, extraction time of 60 min and pH<2. For most compounds, except 234TCP, 2345TeCP and 2356TeCP, salt addition decreases the solubility of compounds in water, especially the most polar ones, therefore improving the affinity to the fiber and increasing the extraction efficiency. On high molecular-mass chlorophenols, such as PCP, this effect was more significant while for phenol molecule there was little influence.

Several authors obtained similar results. Rodriguez et al. [7] stated that the efficiency of the extraction of phenols can be enhanced at low pH and in a saturated salt environment. Lee et al. [18] verified that the addition of KCl and a sample pH of 1 offered a better extraction yield, especially for high-molecular-mass chlorophenols. Buchholtz and Pawllszyn reported for P, 2CP, 24DCP, 246TCP and PCP positive effects in the extraction yield of both acid and salt used in combination [16].

3.1.5. Effect of the fiber desorption time in the GC injector

The results obtained when evaluating the effect of the fiber desorption time present great variability. Desorption time of 3 min was chosen considering that it corresponds to the maximum quantity of chlorophenols desorbed. Intermediate blank runs were performed and no remaining compounds at the fiber were detected.

3.1.6. Extraction efficiencies

For accounting extraction efficiencies, it was decided to use the calibration curves obtained with extracted standards in water (Table 2). Every assay was repeated at least six times at concentration levels of 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0 and 50.0 μg/l. Extraction efficiency was calculated as the ratio between the peak area obtained from the extracted standard and the corresponding peak area of the standard with the same concentration. The lowest values for the average extraction efficiencies were 0.3% for 2CP and 12.2% for P. These results can be justified by the fact that P and 2CP present lower values of the octanol partition coefficient K_{ow}, respectively, pK_{ow} 1.5 and 2.15, and therefore present the lower affinity for this polymeric phase. As the number of chlorine substituents on the molecule increases so does the chlorophenols affinity to the fiber and therefore compounds present higher extraction efficiencies.

3.2. Validation parameters of the analytical method

Chlorophenols were quantified by peak area, obtained from standard chlorophenol mixtures extracted in the same experimental conditions as the samples. The standard chlorophenol mixtures were diluted in water, from the working standard of 500 μg/l, in a range of concentrations from 0.1 to 200 μg/l. Detection limits were calculated according to Buchholz and Pawliszyn [16] (Table 3). The signal-to-noise ratio (S/N) of the lowest detectable concentration was compared to a S/N of 20. Reproducibility was determined by doing six consecutive extractions in three different days. This parameter was expressed by means of the coefficient variation, which was on average inferior to 10% (Table 3). 2346TeCP and 236TCP presented the lower values for reproducibility (Table 3).

Recovery was evaluated adding standard solutions at different concentrations to a leachate (spiked sample) (Table 2). It was calculated from the ratio between the peak area obtained from the extracted spiked sample and the expected one obtained from the extracted calibration curve. Recoveries were higher than 80% except for 26DCP, 235TCP, 246TCP and 234TCP. Recovery was 86.2% on average. Every assay was repeated at least two times at concentration levels 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 50.0, 100.0 and 200.0 μg/l (Table 2).

3.3. Leachate analysis

The samples were collected from one landfill in the north of Portugal. Leachates are complex ma-

Table 2
Extraction efficiencies obtained after SPME of a standard mixture and recoveries and extraction efficiencies obtained after SPME of a landfill leachate

Compound	% Extraction efficiency		Recovery (%)
	Aqueous standard	Spiked landfill leachate	
P	12.2±14.7	90.5±17.9	97.9±15.5
2CP	0.3±43.4	65.9±24.2	87.7±14.7
24DCP	34.5±13.5	52.5±25.4	97.7±23.9
26DCP	27.9±8.8	63.4±10.1	72.7±15.9
235TCP	38.8±8.2	49.6±22.7	64.5±12.9
246TCP	32.4±13.3	46.4±24.4	75.0±16.1
245TCP	37.1±8.2	49.3±23.8	94.4±16.8
236TCP	31.7±13.8	46.4±25.3	83.1±12.0
234TCP	30.8±9.2	48.3±24.3	78.5±13.5
345TCP	42.8±50.6	45.9±24.2	85.4±19.8
2346TeCP	48.1±14.7	51.3±37.6	93.0±23.7
2345TeCP	24.3±20.3	29.8±17.8	92.9±11.4
2356TeCP	20.5±60.1	67.0±13.3	89.8±10.0
PCP	22.8±25.5	41.3±30.7	95.9±12.0

Table 3
Validation parameters for the analytical method

Compound	R^2	Linearity range (μg/l)	DL (μg/l)	Average reproducibility C.V. (%) ($n=6$)
P	0.9999	50–200	2.5	7.0
2CP	0.9992	5–200	0.25	13.9
24DCP	0.9989	1–200	0.05	5.2
26DCP	0.9989	1–200	0.05	3.6
235TCP	0.9995	1–200	0.05	4.9
246TCP	0.9995	1–200	0.05	4.9
245TCP	0.9987	5–200	0.25	4.2
236TCP	0.9993	5–200	0.25	0.4
234TCP	0.9990	5–100	0.25	4.5
345TCP	0.9949	5–200	0.25	23.5
2346TeCP	0.9918	20–200	1	1.8
2345TeCP	0.9911	0.1–50	0.005	60.5
2356TeCP	0.9935	5–200	0.25	26.2
PCP	0.9930	0.1–200	0.005	26.6

DL, detection limits; C.V., coefficient of variation.

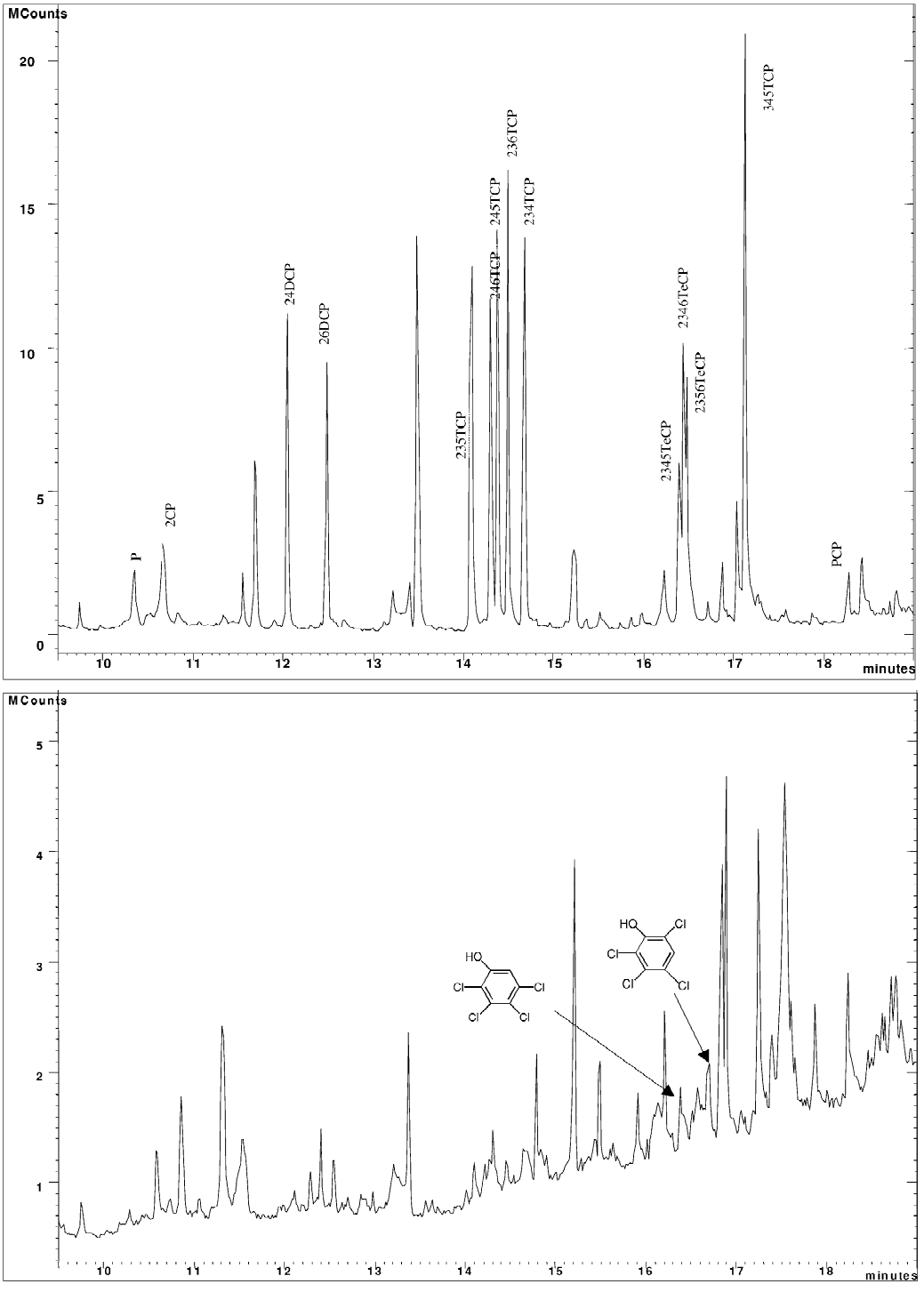

Fig. 3. GC-MS after SPME chromatograms using full scan mode (upper) extracted chlorophenols standard mixture in leachate(chlorophenol concentration = 100 μg/l); (lower) same leachate where 2345TeCP (0.11 μg/l) and 2346TeCP (0.06 μg/l) were detected.

trices and great care must be taken in order to avoid erroneous conclusions due to matrix effects. Extraction efficiencies using a leachate spiked with the standard chlorophenol mixture and applying the optimal experimental conditions, were calculated (Table 2). Fig. 3 shows two leachate chromatograms. The upper one shows a chromatogram of a leachate spiked with 100 μg/l of the chlorophenol standard mixture and the lower one shows a chromatogram, of the same leachate, where 2345TeCP (0.11 μg/l) and 2346TeCP (0.06 μg/l) were detected in a real sample. In this leachate sample PCP was also identified however, it was not possible to quantify this compound.

4. Conclusions

This study evaluated SPME combined with GC–MS for determining 13 chlorophenols in landfill leachates. SPME proved to be a suitable methodology to extract chlorophenols from leachate samples. An optimised methodology was developed and best results were obtained with an 85-μm PA-coated fiber, immersion sampling at 40 °C for 60 min and stirring (750 rpm), with saturated salt conditions sample pH$<$2 and desorption for 3 min at 280 °C.

The quantification method used calibration curves obtained from a standard chlorophenol mixture extracted in the same experimental conditions as the samples. Detection limits ranged from 0.005 to 2.5 μg/l and reproducibility was on average inferior to 10%. For chlorophenols with low values of K_{ow}, like P and 2CP, the use of a fiber of relatively non-polar nature would be desirable. Recovery was 86.2% on average.

Analysing landfill leachate samples the application of SPME–GC–MS system to the determination of chlorophenols was tested successfully.

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