LPME with in situ derivatization and GC-MS for trace analysis of chlorophenols in water samples

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Introduction

Chlorophenols are said to be one of the indicators for monitoring dioxins, since there is a significant correlation between the amount of chlorophenols and dioxins, which are generated by garbage incineration[1]. Moreover, if chlorine processing of the phenol in tap water is carried out, chlorophenols are generated and the nasty smell becomes another problem. Furthermore, the estrogenic activity of 2,4-dichlorophenol has been extensively evaluated by *in vitro* assays[2]. Then, the monitoring of chlorophenols in an environmental medium is an important issue.

Many analytical methods for the determination of chlorophenols in water samples have been reported including gas chromatography–mass spectrometry (GC-MS). However, GC-MS was initially used for the determination of phenol compounds even though derivatization was required. The derivatization leads to sharper peaks and hence to better separation and higher sensitivity for the phenols. However, the derivatization faces the risk of contamination and hence an overestimation of chlorophenols concentration. In order to overcome these problems, *in situ* derivatization has been developed, which involves the simple addition of a reagent to a liquid sample.

Recently, a new solvent microextraction (SME) [3]technique that uses single drop by microsyringe was developed. The technique is known as single-drop microextraction (SDME)[4]or liquid phase microextraction (LPME)[5].

The aim of this study is to determine trace amounts of chlorophenols in water samples by LPME with *in situ* derivatization, followed by GC-MS. The developed method was applied for the determination of chlorophenols in river water samples.

Methods and Materials

Reagents:

2,4-Dichlorophenol (DCP), 2,4,6-trichlorophenol (TrCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP) of environmental analytical grade and acetic acid anhydride for trace analysis were purchased from Kanto Chemical, Inc. (Tokyo, Japan). 2,4-Dichlorophenol-d_4, 2,4,6-trichrolophenol-$^{13}C_6$, 2,3,4,6-tetrachrolophenol-$^{13}C_6$ and pentachlorophenol-$^{13}C_6$ were purchased from Hayashi Pure Chemical, Inc. (Osaka, Japan). Other reagents and solvents were of pesticide or analytical grade and purchased from Wako Pure Chemical, Inc. (Osaka, Japan). The water purification system used was Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA). The EDS polisher was a new filter purchased from Millipore, Japan.

Standard solutions

Standard solutions (1.0 mg ml^{-1}) of DCP, TrCP, TeCP and PCP were prepared as required by the addition of purified water. Calibration was performed daily for all samples with a surrogate standard.

Instrument:

Microsyringe for 10 µl was purchased from SGE Japan Inc.. For the extraction, 20 ml headspace vials from Agilent Technologies (Palo Alto, CA, USA) were used. GC-MS analysis was performed using an Agilent 6890 gas chromatograph with a 5973 mass-selective detector (Agilent Technologies).

GC-MS conditions:

Injection was performed in the splitless mode and was set to 250 ^{o}C. The separations were conducted on a DB-5MS fused silica column (30 m x 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies). The oven temperature was programmed to increase from 100 ^{o}C to 280 ^{o}C (held for 4 min) at 10 ^{o}C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.2 ml min^{-1}. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron ionization (ionization voltage: 70 eV). For SIM, corresponding ions were monitored (*m/z* 162 and 164 for the acyl derivative of DCP and *m/z* 196 and 198 for the acyl derivativeof TrCP, m/z 230 and 232 for the acyl derivative of TeCP and m/z 266 and 268 for the acyl derivative of PCP. The underlined number is the *m/z* of the ion used for quantification.).

Sample preparation:

Ten milliliters of river water sample was placed in a headspace vial containing surrogate standard. Then, 1 M potassium carbonate solution (1 ml) for pH adjustment, acetic acid anhydride (100 µl) as the derivatization reagent, and a stir bar were added. The stirring was performed for 1 min. Then, the ultrasonication was performed for 3 min. The vial was crimped with a Teflon-coated silicone septum. A 10 µl microsyringe was used for LPME. Before extraction, the syringe was rinsed with acetone followed by toluene 10 times to avoid carryover and air bubble formation. Four microliters of toluene was withdrawn into the syringe. The syringe needle tip was held 5 mm below the surface of a sample solution. LPME was performed at room temperature for 90 min while stirring at 1000 rpm. After the extraction, 2 µl of extract was carefully withdrawn into the syringe. The extract was then injected into the GC-MS system.

Results and Discussion

Theoretical recovery:

Table 1 shows log $K_{o/w}$ and the theoretical recoveries of the compounds investigated in this work. The $K_{o/w}$ values were calculated from the Log P predictor, which is available from Interactive Analysis Inc. (Bedford, MA, USA). Theoretical recoveries are calculated by the following equations:

Theoretical recovery = $K_{o/w}/\beta/(1 + K_{o/w}/\beta)$

where $\beta = V_w/V_e$, V_e the volume of extract solvent and V_w the volume of water. The theoretical recoveries by LPME were calculated on the basis of a 10 ml sample volume and 4 µl of toluene. The results revealed that the theoretical recoveries of chlorophenols were increased by the derivatization.

Table 1 Log Ko/w and theoretical recovery of chlorophenols and their derivative by LPME

Compound	Log $K_{o/w}$ [a]	Theoretical recovery (%)
DCP	2.80	20.2
DCP acetate	2.88	23.3
TrCP	3.45	53.0
TrCP acetate	3.52	57.0
TeCP	4.09	83.1
TeCP acetate	4.17	85.5
PCP	4.74	95.6
PCPacetate	4.81	96.3

[a] Log $K_{o/w}$ values for all compounds as calculated from "SRC KowWin", as well as calculated recoveries.

Time for and efficiency of LPME with in situ derivatization:

An important parameter affecting LPME was the extraction time. To optimize the extraction time, a 10 ng ml^{-1} standard solution of chlorophenols was used. The extraction time profiles (equilibration curves) of acyl derivative of chlorophenols in 10 ml standard solutions using LPME with *in situ* derivatization were determined by GC-MS, and are shown in Fig.1. The acyl derivative of chlorophenols reached equilibrium after approximately 90 min. This condition was therefore used for the determination of chlorophenols in liquid samples.

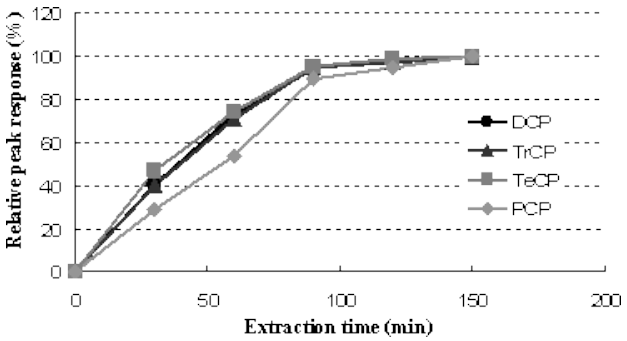

Fig. 1 Extraction time profiles

Validation of the method:

The calculated detection limits (LODs) of DCP, TrCP, TeCP and PCP in river water sample with *in situ* derivatization were 0.2, 0.5, 1 and 1 pg ml^{-1}, respectively, by LPME-GC-MS when the ratio of the compound's signal to the background signal (S/N) was 3. In addition, the calculated limits of quantification (LOQs) when S/N >10 were 1, 2, 5 and 5 pg ml^{-1} for DCP, TrCP, TeCP and PCP , respectively. The peak area ratios with respect to each surrogate standard were plotted and the response was found to be linear over the calibration range with correlation coefficients (r) higher than 0.999. The validation results are summarized in Table 2.

Table 2 Validation of LPME with in situ derivatization and GC-MS methods

Compound	LOD[a] (pg ml^{-1})	LOQ[b] (pg ml^{-1})	Correlation coefficient (r)
DCP	0.2	1	0.999 (1-1000)[c]
TrCP	0.5	2	0.999 (2-1000)
TeCP	1	5	0.999 (5-1000)
PCP	1	5	0.999 (5-1000)

[a] LOD: limit of detection (S/N = 3)

[b] LOQ: limit of quantification (S/N >10)

[c] Values in parentheses are the linear ranges of the calibration curves (pg ml^{-1}).

The recovery and precision of the method were assessed by replicate analysis (n = 6) of river water samples spiked at the 0.1 and 1.0 ng ml^{-1} level with the surrogate standard. Non-spiked and spiked samples were subjected to LPME with *in situ* derivatization and GC-MS. The recoveries were calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using calibration curves of standard solutions with surrogate standards. The average recovery was higher than 95 % (RSD < 10 %) for river water samples. Therefore, the method enables the precise determination of standards and can be applied to the determination of trace amounts of chlorophenols in river water samples.

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References

1 Uchimura T., Hafner K., Zimmermann R., Imasaka T. (2003) Appl Spectrosc., 57, 461.

2 Jones P.A., Baker V.A., Irwin A.J.E., Earl L.K. (1998) Toxicol. In Vitro, 12, 373.

3 Lucy C.A., Cantwell F.F. (1989) Anal. Chem., 61, 101.

4 Jeannot M.A., Cantwell F.F. (1996) Anal. Chem., 68, 2236.

5 He Y., Lee H.K., (1997) Anal. Chem., 69, 4634.