Trace analysis of chlorophenols in river water samples by stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography-mass spectrometry

Migaku Kawaguchi[1], Yumiko Ishii[1], Noriya Okanouchi[1], Norihiro Sakui[1], Rie Ito[1], Koichi Inoue[1], Koichi Saito[2], Hiroyuki Nakazawa[1]

[1]Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[2]Dioxin Research Group, Saitama Institute of Public Health, Kamiokubo, Sakura-ku, Saitama 338-0824, Japan

Introduction

Chlorophenols are one on the indicators for monitoring dioxins. It is reported that correlation is between the amount of chlorophenols and dioxin 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 has been a problem. Furthermore, the estrogenic activity of 2,4-dichlorophenol as the chlorophenols has been extensively evaluated by *in virto* assays[2]. Then, the monitoring of chlorophenols in an environmental medium is 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 sorptive extraction technique that uses a stir bar coated with polydimethylsiloxane (PDMS) was developed[3]. The technique is known as stir bar sorptive extraction (SBSE). We already reported that determination of 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP) in river water[4] and body fluid samples[5] by using SBSE. In addition, SBSE with *in situ* derivatization has been successfully used in the determination of bisphenol A (BPA) in human body fluid samples[6] and phenolic xenoestrogens in river water samples[7].

The aim of this study is to determine trace amounts of chlorophenols in water samples by SBSE with *in situ* derivatization, followed by thermal desorption (TD)-GC-MS. The developed method was applied to determination of chlorophenols in river water samples.

Methods and Materials

Reagents:

2,4-Dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TCP) 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.

Instrument:

Stir bars coated with 500-µm-thick (24 µl) PDMS (Twister^{TM}:the magnetic stirring rod is incorporated in a glass jacket and coated with PDMS) were obtained from Gerstel (Mullheim an der Ruhr, Germany). Prior to use, the stir bars were conditioned for 4 h at 300 ^{o}C in a flow of helium. The stir bars could be used more than 50 times with appropriate re-conditioning. For the extraction, 20 ml headspace vials from Agilent Technologies (Palo Alto, CA, USA) were used. TD-GC-MS analysis was performed using a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS-A autosampler and a Gerstel CIS 4 programmable temperature vaporization (PTV) inlet (Gerstel) and an Agilent 6890 gas chromatograph with a 5973 mass-selective detector (Agilent Technologies).

GC-MS conditions:

The TDS 2 temperature was programmed to increase from 20 ^{o}C (held for 1 min) to 280 ^{o}C (held for 5 min) at 60 ^{o}C min^{-1}. The desorbed compounds were cryofocused in the CIS 4 at –150 ^{o}C. After the desorption, the CIS 4 temperature was programmed to increase from –150 ^{o}C to 250 ^{o}C (held for 10 min) at 12 ^{o}C s^{-1} to inject the trapped compounds onto the analytical column. Injection was performed in the splitless mode. The separations were conducted on a DB-5MS fused silica column (30 m x 0.25 mm i.d., 0.50 µm film thickness, Agilent Technologies). The oven temperature was programmed to increase from 60 ^{o}C to 300 ^{o}C (held for 4 min) at 15 ^{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 2,4-DCP and *m/z* 196 and 198 for the acyl derivative of 2,4,6-TCP, m/z 230 and 232 for the acyl derivative of 2,3,4,6-TCP 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.). A blank run of the stir bar was always performed after an analysis, and memory effects were not detected. Therefore, satisfactory analysis was possible under these thermal desorption conditions.

Sample preparation:

Ten milliliters of river water sample was placed in a headspace vial containing surrogate standard. Then, 1 M sodium carbonate solution (1 ml) for pH adjustment, and acetic acid anhydride (200 µl) as the derivatization reagent were added. The stir bar was added and the vial was crimped with a Teflon-coated silicone septum. SBSE was performed at room temperature for 30 to 180 min while stirring at 500 rpm. After the extraction, the stir bar was easily removed with forceps (due to magnetic attraction), rinsed with purified water, dried with lint-free issue and placed in a glass thermal desorption tube. The thermal desorption tube was then placed in the thermal desorption unit. Then, the stir bar was thermally desorbed in the TD system, and this was followed by GC-MS.

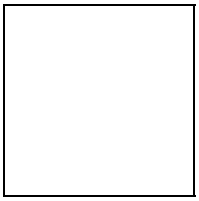
Results and Discussion

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:

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

where $\beta = V_w/V_{PDMS}$, V_{PDMS} the volume of PDMS and V_w the volume of water. The theoretical recoveries by SBSE were calculated on the basis of a 10 ml sample volume and a stir bar with a phase thickness of 500 µm (24 µl PDMS).



Time for and efficiency of SBSE with in situ derivatization:

An important parameter affecting SBSE 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 SBSE with *in situ* derivatization were determined by TD-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.

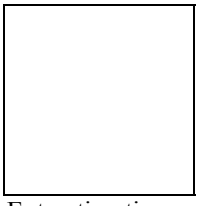
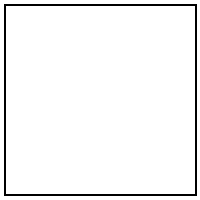


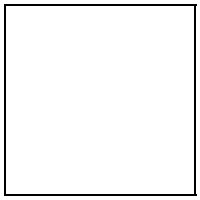
Fig. 1 Extraction time profiles

Validation of the method:

The calculated detection limits (LODs) of 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TCP and PCP in river water sample with *in situ* derivatization were 2, 2, 2 and 5 pg ml^{-1}, respectively, by SBSE-TD-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 10, 10, 10 and 20 pg ml^{-1} for 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TCP 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^2) higher than 0.999. The validation results are summarized in Table 2.



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 SBSE with *in situ* derivatization and TD-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 (Table 3). 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.



Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology.

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 Baltussen E., Sandra P., David F., Cramers C. (1999) J. Microcolumn Sep., 11, 737.
4 Kawaguchi M., Inoue K., Yoshimura M., Sakui N., Ito R., Nakazawa N. (2004) Anal. Chim. Acta, 505, 217
5 Kawaguchi M., Inoue K., Sakui N., Ito R., Izumi S., Makino T., Okanouchi N., Nakazawa H. (2004) J. Chromatogr. B, 799, 119

6 Kawaguchi M., Inoue K., Yoshimura M., Ito R., Sakui N., Okanouchi N., Nakazawa H. (2004) J. Chromatogr. B, 805, 41
7 Kawaguchi M., Inoue K., Yoshimura M., Sakui N., Okanouchi N., Ito R., Yoshimura Y., Nakazawa H. J. Chromatogr. A, *in press*