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Stir bar sorptive extraction with in situ derivatization and thermal desorption–gas chromatography–mass spectrometry for determination of chlorophenols in water and body fluid samples

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Abstract

A new method, stir bar sorptive extraction (SBSE) with in situ derivatization and thermal desorption (TD)–gas chromatography–mass spectrometry (GC–MS), which is used for the determination of trace amounts of chlorophenols, such as 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TrCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and pentachlorophenol (PCP), in tap water, river water and human urine samples, is described. The derivatization conditions with acetic acid anhydride and the SBSE conditions such as extraction time are investigated. Then, the stir bar is subjected to TD followed by GC–MS. The detection limits of the chlorophenols in tap water, river water and human urine samples are 1–2, 1–2, and 10–20 pg ml^{-1} (ppt), respectively. The calibration curves for the chlorophenols are linear and have correlation coefficients higher than 0.99. The average recoveries of the chlorophenols in all the samples are higher than 95% (R.S.D. < 10%) with correction using added surrogate standards, 2,4-dichlorophenol-d_5, 2,4,6-trichlorophenol-$^{13}C_6$, 2,3,4,6-tetrachlorophenol-$^{13}C_6$ and pentachlorophenol-$^{13}C_6$. This simple, accurate, sensitive and selective analytical method may be applicable to the determination of trace amounts of chlorophenols in liquid samples.

Keywords: Chlorophenols; Stir bar sorptive extraction (SBSE); Thermal desorption (TD)–GC–MS; In situ derivatization

1. Introduction

Chlorophenols are used as materials of the product for industry and agricultural chemicals. Moreover, chlorophenol is one of the indicators of dioxin generation. It has been reported that there is a correlation is between the amount of chlorophenol and that of dioxin, both of which are generated by garbage incineration [1]. In addition, the processing of phenols in tap water by chlorination leads to the generation of chlorophenols, which are responsible for the unfavorable smell. The estrogenic activity of 2,4-dichlorophenol (2,4-DCP) has been extensively evaluated by in vitro assays [2]. On the other hand, chlorophenols are usually detected in urine because of the intake of food and water containing them or the metabolism of other chlorinated substances present in the environment [3]. It is for these reasons that the effects of chlorophenols on the environment and human health have become a controversial issue. In order to assess environmental and human exposure to chlorophenols, a reliable and sensitive analytical method is required. Many analytical methods are available for the determination of chlorophenols in environmental water [4–9]. There are also a number of reports of the measurement of chlorophenols in human urine samples to assess human exposure [10–14]. Thus, the measurement of chlorophenols in various liquid samples is performed by

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different analytical methods. Moreover, almost all the previous methods were required for the time and effort for sample preparations. In this regard, the determination of chlorophenols in various liquid samples by only one simple analytical method is desired. However, to our knowledge, no such study has been performed so far.

Many analytical methods for the determination of chlorophenols in various samples have been reported, such as liquid chromatography (LC) with ultraviolet detection (UV), electrochemical detection (ED) and mass spectrometry (MS) [5–10,14]. However, LC has low resolution and is frequently affected by the sample matrix. On the other hand, gas chromatography (GC) was initially used for the determination of phenol compounds including chlorophenols, even though derivatization was required [4,12,13]. The derivatization leads to sharper peaks and hence to better separation of and higher sensitivity for the phenols. However, the derivatization procedure requires much time and effort. In order to avoid this problem, in situ derivatization was developed, which involves the simple addition of a reagent into a liquid sample.

Such analytical procedures as liquid–liquid extraction (LLE) [5] and solid-phase extraction (SPE) [4,7–10] have been developed for the determination of phenolic xenoestrogens. However, LLE requires large volumes of organic solvents and additional clean-up steps, and although SPE requires small volumes of organic solvents, the manual concentration of large volumes of samples takes 8–10 h. Recently, solid-phase micro extraction (SPME) has been successfully used for the determination of chlorophenols in water and urine samples [6,11]. However, the sensitivity of the above methods remains low. Because SPME with polydimethylsiloxane (PDMS) is by nature an equilibration technique that is based on the partitioning of an analyte between the stationary phase and the aqueous sample, the enrichment is dependent on the distribution coefficients of the analyte in the two phases. Therefore, water/PDMS phase ratio is very important for sorptive extraction. The limited enrichment on the SPME fiber is mainly due to the volume of the PDMS phase (typically 0.5 μl or less), and increasing the volume of PDMS relative to the aqueous matrix is expected to markedly increase the enrichment of the analyte. Recently, a new sorptive extraction technique that uses a stir bar coated with PDMS was developed [15] and is known as stir bar sorptive extraction (SBSE). Its main advantage is its wide application range that includes volatile aromatics, halogenated solvents, polyaromatic hydrocarbons, polychlorinated biphenyls (PCBs), pesticides, odor compounds and organotin compounds [15–33]. SBSE has been applied successfully in biological samples. [24–29]. Moreover, SBSE with in situ derivatization has been used in the analysis of phenolic compounds [26–32].

The aim of this study was to determine trace amounts of chlorophenols in different liquid samples by SBSE with in situ derivatization and thermal desorption (TD)–GC–MS. The developed method was applied to tap water, river water, and human urine samples.

2. Experimental

2.1. Materials and reagents

2,4-Dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TrCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-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_5 (2,4-DCP-d_5), 2,4,6-trichlorophenol-$^{13}C_6$ (2,4,6-TrCP-$^{13}C_6$), 2,3,4,6-tetrachlorophenol-$^{13}C_6$ (2,3,4,6-TeCP-$^{13}C_6$) and pentachlorophenol-$^{13}C_6$ (PCP-$^{13}C_6$) were purchased from Hayashi Pure Chemical Inc. (Osaka, Japan). *E. coli* β-glucuronidase (25,000 units/0.4 ml) and *H. pomatia* sulfatase (3540 units ml^{-1}) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Prior to use, the β-glucuronidase was added to 0.1 M ammonium acetate (2.1 ml) to make a total concentration of 10,000 units ml^{-1}. Other reagents and solvents of pesticide or analytical grade were purchased from Wako Pure Chemical, Inc. (Osaka, Japan). The water purification system was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Standard solutions

Concentrated solutions (1.0 mg ml^{-1} in acetonitrile) of 2,4-DCP, 2,4,6-TrCP, 2,3,4,6-TeCP and PCP were prepared as required by the addition of purified water. Calibration was performed daily for all samples with a surrogate standard.

2.3. Instrumentation

Stir bars coated with a 500 μm-thick PDMS layer (24 μl; Twister™: a magnetic stirring rod is placed inside a glass jacket and coated with PDMS) were obtained from Gerstel (Mülheim an der Ruhr, Germany). The stir bars were conditioned for 4 h at 300 °C in a flow of helium. The stir bars could be used more than 50 times with appropriate re-conditioning. For the extraction, 10 and 20 ml headspace vials from Agilent Technologies (Palo Alto, CA, USA) were used. TD was performed with a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS A autosampler and a Gerstel CIS 4 programmable temperature vaporization (PTV) inlet. GC–MS was performed with an Agilent 6890N gas chromatograph equipped with a 5973N mass-selective detector with an ultra ion source (Agilent Technologies).

2.4. TD–GC–MS conditions

The TDS 2 temperature was programmed to increase from 20 °C (held for 1 min) to 280 °C (held for 5 min) at 60 °C min^{-1}. The desorbed compounds were cryofocused in the CIS 4 at −150 °C. After the desorption, the CIS 4 temperature was programmed to increase from −150 to 300 °C (held for 10 min) at 12 °C s^{-1} to inject the trapped compounds into