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Development of a solid phase microextraction-gas chromatography–mass spectrometry method for the determination of pentachlorophenol in human plasma using experimental design

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Abstract

A new method, headspace solid-phase microextraction (HS-SPME) with in situ derivatization and gas chromatography–mass spectrometry (GC–MS), which was used for the determination of trace amount of pentachlorophenol (PCP) in human plasma was presented. The acetylation derivatization reaction was firstly optimized using a Doehlert design. Then a series of parameters relevant to the headspace SPME procedure, including fiber coating, extraction temperature, extraction time and salt addition, were optimized using a two-level full factorial design expanded further to a central composite design. The validation of method showed that the optimized method had good linearity ($R^2 = 0.999$) within the concentration ranges 0.1–50.0 ng ml^{-1}, and was sensitive with the limit of detection of 0.02 ng ml^{-1}. Intra- and inter-day precision for pentachlorophenol in human plasma samples were not greater than 11.9% and 12.6%, respectively. The proposed method, to our knowledge, describes the first application of HS-SPME with GC–MS for analysis of PCP in blood plasma sample. Application of the method to real human plasma samples, PCP was successfully detected in some cases at concentration levels 1.2–6.3 ng ml^{-1}.
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Keywords: SPME; GC–MS; Doehlert design; Central composite design; Pentachlorophenol; Human plasma

1. Introduction

Pentachlorophenol (PCP) is a broad spectrum biocide which has been used extensively world-wide as herbicide, germicide and wood preservative in industry, agriculture, and private household for decades. In china, PCP and its salt were used extensively as a molluscide to control snail-borne schistosomiasis. Because of its relatively high hydrophobicity and environmental persistence, PCP is readily bioaccumulated. The US Environmental Protection Agency (EPA) has listed PCP as a priority pollutant based upon its toxicity and widespread distribution in the environment. The general population may become exposed to PCP through inhalation of contaminated air, ingestion of contaminated water and food, and dermal contact with contaminated leather or wood products. The observed toxic effects of PCP on human health include symptoms associated with uncoupling of oxidative phosphorylation, liver effects, impaired immune function, etc. (WHO, 1987). As a member of environmental endocrine disruptors, nM level of PCP could interfere with the transport of thyroid hormones in human plasma (Ishihara et al., 2003).

PCP was found to be mainly retained in blood and only to a lesser extent in adipose tissue (WHO, 1987). In order to assess human exposure to PCP, some analytical methods have been developed for the determination of PCP in the human blood samples (Gerhard et al., 1999; Heudorf et al., 2000, 2003; Sandau et al., 2000; Hovander et al., 2002). These

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existing methods are effective but have their limitations. In these methods, the sample preparation procedures remain to use the conventional liquid-liquid extraction (LLE) technique. The LLE technique, as a conventional sample pretreatment procedure, is time-consuming, labor-intensive and multi-stage operations. Each step will introduce the possible losses of analytes and sample contamination (Theodoridis et al., 2000). And it requires the use of large amounts of organic solvents which are often expensive, toxic, carcinogenic and hazardous to the environment. Waste disposal of solvents is an additional problem, adding extra cost to the analytical procedure. These problems can be avoided by Solid phase microextraction (SPME) technique, which is a relatively recent technique integrating sampling, extraction, concentration and sample introduction into a single solvent-free step (Ulrich, 2000). SPME technique offers several advantages over the conventional LLE technique, such as high extraction efficiency, simplicity, solvent-free extraction and easy automatization for GC instrument. In addition, while using LLE sample pretreatment procedure, a large amount of sample is necessary because of its low extraction efficiency. However, a large amount of biological sample especially blood sample is often hard to be acquired, and when using a large sample, background influences are more likely to have an adverse effect on the analysis (Namera et al., 1999). The headspace solid phase microextraction (HS-SPME) is an ideal sample preparation method for the analysis of biological specimens, as interference from the matrix is reduced, thus yielding improved sensitivity and using less amount of sample (Mills and Walker, 2000). To date a few applications of SPME have indicated a good potential for the analysis of blood samples (Queiroz and Lancas, 2004; Shah et al., 2006). On the other hand, prior to capillary gas chromatography, PCP are commonly transformed into less polar derivative that gives improved peak shape, resolution and sensitivity. Herein, acetic anhydride was utilized as derivatization reagent instead of diazomethane with toxicity and explosive nature used in existing methods (Sandau et al., 2000; Heudorf et al., 2000, 2003).

In this paper, a HS-SPME procedure with in situ derivatization coupled to capillary gas chromatograph-mass spectrometry (GC–MS) was developed for quantification of PCP in human plasma. Various factors affecting the efficiency of the SPME extraction and derivatization steps were optimized using the chemometrics approaches (Jeffwu and Hamada, 2000; Salafranca et al., 2003; Domeño et al., 2005). Finally, the analytical method proposed was validated and applied to real human plasma samples.

2. Experimental

2.1. Reagents and materials

Pentachlorophenol (99%) and 2,4,6-tribromphenol (TBP, 99%) used as an internal standard (IS) were purchased from sigma–aldrich Co. (St. Louis, MO, USA), methanol and acetone from sigma–aldrich Co. (St. Louis, MO, USA) were pesticide grade solvents. Acetic anhydride, sodium chloride (NaCl) and potassium carbonate (K_2CO_3) were also supplied by sigma–aldrich Co. (St. Louis, MO, USA). Distilled water was processed through a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Preparation of standard solutions and samples

Stock standard solutions of PCP and TBP were prepared separately by dissolving an appropriate amount of each compound in methane to achieve a concentration of 1.0 mg l^{-1}. All stock solutions were stored at −20 °C. Working standard solutions of the compounds were obtained by serial dilution of the stock standard solution with methanol and stored at 4 °C until analysis.

Plasma samples were prepared by adding 50 μl of methanol to 0.5 ml plasma to release the possibly bonded target compounds (Krogh et al., 1997). And then 600 μl of acetone was added to precipitate the plasmatic proteins (Shah et al., 2006). Plasma was centrifuged for 5 min at 10000 rpm. The supernate was transferred to a new tube and concentrated to 0.5 ml under a gentle stream of nitrogen. The PCP-free blank plasma samples were spiked with different amounts of working standard solutions in order to prepare the samples used for the different studies.

2.3. GC–MS conditions

Preliminary studies were performed to optimize the chromatographic conditions. All analyses were performed using a Trace Ultra gas chromatograph interfaced to a Trace DSQ quadrupole mass spectrometer (Thermo Electron Corp., USA). The GC–MS was operated with a transfer line temperature of 250 °C, and an ionization source temperature of 250 °C. Chromatographic separation was achieved using a DB-5MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA). The gas chromatograph equipped with a split/splitless injection port was operated at 250 °C. The samples were injected in the splitless mode and then the splitter was opened after 1 min. The optimal gas chromatograph separation conditions were programmed as follows: initial temperature 60 °C for one minute, then from 60 °C to 190 °C at a rate of 10 °C min^{-1}, subsequently from 190 °C to 230 °C at a rate of 8 °C min^{-1}, finally from 230 °C to 280 °C at a rate of 15 °C min^{-1} and the final temperature was held for 3 min. Helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1}.

The mass spectrometer was operated in the positive-ion electron impact (EI) mode using an ionizing energy of 70 eV and an emission current of 100 μA. Full-scan data was obtained with a mass range of *m*/z 40–500 amu. The qualitative ions selected for the compound studied were: *m*/z 264, 266, 268 (PCP) and *m*/z 328, 330, 332 (IS). The quantitative determination was carried out using selected

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