Journal of Chromatography A, 1193 (2008) 79–84

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Application of liquid–liquid–liquid microextraction and ion-pair liquid chromatography coupled with photodiode array detection for the determination of chlorophenols in water

Che-Yi Lin, Shang-Da Huang*

Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan

ARTICLE INFO

Article history:
Received 6 November 2007
Received in revised form 28 March 2008
Accepted 31 March 2008
Available online 8 April 2008

Keywords:
Chlorophenols
Liquid–liquid–liquid microextraction
Ultraviolet spectrum of chlorophenolate anion
Ion-pair liquid chromatography

ABSTRACT

A method termed as liquid–liquid–liquid microextraction was utilized to extract chlorophenols from water. The extracted chlorophenols, present in anionic form, were then separated, identified, and quantitated by ion-pair high-performance liquid chromatography with photodiode array detection (HPLC/DAD). For trace chlorophenol determination using HPLC/DAD, the chlorophenolate anion provides a better ultraviolet spectrum for quantitative and qualitative analyses than does uncharged chlorophenol. This is due to the auxochromic effect of the phenolate anion. In the study, experimental conditions such as organic phase identity, acceptor phase volume, sample agitation, extraction time, acceptor phase NaOH concentration, donor phase HCl concentration, salt addition, and UV absorption wavelength were optimized. Relative standard deviations (RSD, 2.3–5.4%), coefficients of determination (r^2 0.9994–0.9999), and detection limits (0.049–0.081 ng mL^{-1}) of the proposed method were investigated under the selected conditions. The method was successfully applied to analyses of reservoir and tap water samples, and the relative recoveries of chlorophenols from the spiked reservoir and tap water samples were 94.1–100.4% and 87.8–101.2%, respectively. The proposed method is capable of identifying and quantitating each analyte to 0.5 ng mL^{-1}, confirming the HPLC/DAD technique to be quite robust for monitoring trace levels of chlorophenols in water samples.

1. Introduction

The use of some bactericides, herbicides, antiseptics, dyes, and wood preservatives results in the release of chlorophenols in the environment [1–3]. In particular, the reaction of phenol with chlorine produces chlorophenols during tap-water chlorination [4,5]. Because of their toxicity chlorophenols are regulated by numerous countries and international organizations [6–8].

The toxicity of pentachlorophenol is indicated by the very low maximum contaminant level (1 ng mL^{-1}) and maximum contaminant level goal (0 ng mL^{-1}) set for drinking water in the USA, and by a Fresh Water Guideline of 0.5 ng mL^{-1}) set by Canada for the protection of aquatic life. Chlorophenols cannot be determined by gas chromatography (GC) without derivatization. Their relative polarities and low vapor pressures cause response loss and peak tailing in GC. There is a tendency to use liquid chromatography (LC) for chlorophenols analysis. The performance of chlorophenols determination using solid-phase extraction (SPE) with LC coupled to mass spectrometry (MS) has been found to be good; the lowest concentration range yielding good linearity is 0.1–2 ng mL^{-1} and the detection limit is 0.4–30 ng L^{-1} [9]. Implementation of SPE requires expensive equipment, and the eluent volume must be adjusted to a small value for injection into the LC. In 1999 Pedersen-Bjergaard and Rasmussen [10] used a novel technique, a static liquid–liquid–liquid microextraction (LLLME). pH adjustment was used to control the charges of the various analyte species as the target compounds were extracted from the donor phase (the water sample) into an organic phase and then extracted into an aqueous acceptor phase inside the lumen of a hollow fiber. The enriched acceptor phase was directly injected into either a HPLC or a capillary electrophoresis (CE). The LLLME technique, which requires minimal solvent, is environmentally friendly, low in cost, and simple to use. The LLLME technique combined with LC was applied to determine chlorophenols in water [11,12]. To achieve acceptable performance in the determination of chlorophenols (lowest linear concentration range of 1–2 ng mL^{-1}, detection limit of 0.1–5 ng mL^{-1}) requires 72 h of extraction time if one is using static LLLME combined with HPLC/DAD [12]. In 2006 Chen et al. [13] demonstrated a dynamic LLLME technique that utilized automated movement of the acceptor and donor phases and termed the technique LLLME/AMADP. In this technique the aqueous acceptor phase was moved back and forth between the fiber lumen and the syringe barrel while the

* Corresponding author. Tel.: +886 3 572 1194; fax: +886 3 573 6979.
E-mail address: sdhuang@mx.nthu.edu.tw (S.-D. Huang).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.03.082

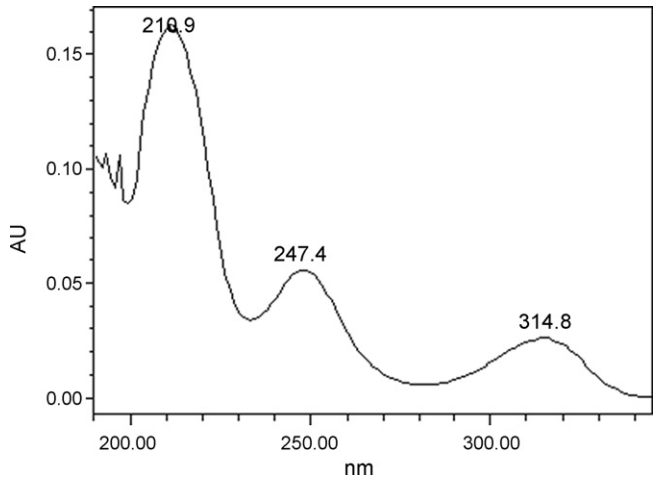

Fig. 1. UV absorption spectrum of 2,4,5-trichlorophenolate anion at 20 μg mL^{-1} concentration.

aqueous donor phase was moved back and forth between the fiber lumen and the bulk solution. Extraction efficiency is enhanced by use of this dynamic mode of extraction.

The UV absorption spectra of aromatic hydrocarbons have three sets of characteristic bands that originate from $\pi \rightarrow \pi^*$ transitions. The two longer wavelength bands are the E2 band and the B band. Chlorophenols show a strong absorption at the E2 band (about 200 nm) and a rather weak absorption at the B band (about 300 nm). In HPLC utilizing an ultraviolet detection (UV) system, absorption close to 200 nm suffers from significant background absorption in the chromatogram. A trace determination using the E2 band is low in signal-to-noise ratio (S/N), hence the band is easily shifted or sunk in the background absorption. In addition, all aromatic hydrocarbons have marked E2 band absorption at about 200 nm, causing the chance of interference by non-target compounds to be significant. As to the B band, use of a weak absorption peak causes the quantitation of chlorophenols to be of poor sensitivity [14–16]. Thus the UV spectra of chlorophenols are not satisfactory for the quantitative determination of trace levels of these compounds by HPLC/UV and in qualitative identification from the analyte spectrum in HPLC/DAD. These defects can be overcome by LC/MS, but the instrument is expensive and uncommon. Therefore, an efficient and sensitive method was developed that converts the target chlorophenols to their ionic forms for analysis by ion-pair HPLC/DAD. The pH of the mobile phase used in HPLC must be greater than pK_a + 1 for the chlorophenolate anion to predominate. As with phenol [17], the auxochromic effect is greater for the chlorophenolate anion than for chlorophenol. The chlorophenolate anion differs from uncharged chlorophenol in (i) a shift of all characteristic bands to longer wavelength, (ii) a new characteristic band (the red shift characteristic band) at around 240–255 nm, and (iii) stronger B band absorption. As indicated in Fig. 1, the 2,4,5-trichlorophenolate anion spectrum exhibits two suitable UV characteristic bands for trace determination, at 247.4 nm (the red shift characteristic band) and at 314.8 nm (the enhanced B band).

In this study a method is developed using the LLLME/AMADP technique for chlorophenols extraction in water and the ion-pair HPLC/DAD technique for identifying and quantitating the extracted chlorophenols in anionic form.

2. Experimental

2.1. Reagents and samples

2-Chlorophenol (2-CP), 4-chloro-3-methylphenol (4-C-3-MP), and 2,4-dichlorophenol (2,4-DCP) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). 2,4,6-trichlorophenol (2,4,6-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), and pentachlorophenol (PCP) were obtained from Chem Service (West Chester, PA, USA). Tetrabutylammonium hydroxide solution was purchased from Fluka Chemical GmbH (Buchs, Switzerland). Methanol and acetonitrile (J.T. Baker, Philipsburg, NJ, USA) used for this study were HPLC grade. 1-Octanol, 1-nonanol, *p*-xylene, *o*-xylene, 4-nitro-*m*-xylene (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 1,2,4-trichlorobenzene (Janssen Chimica, Geel, Belgium) were also used. The solvent 1,2,4-trichlorobenzene as received contained acidic impurities, so was purified by partition separation; liquid–liquid extraction of 3 mL of 1,2,4-trichlorobenzene was carried out three times with 5 mL 0.1N NaOH followed by three subsequent extractions with 5 mL deionized water. The stock solutions of the chlorophenols were prepared by dissolving each chlorophenol in methanol to obtain 1 mg mL^{-1} solutions. Aliquots of these stock solutions were diluted with water to prepare standard working solutions of 100 ng mL^{-1} concentration. All other reagents and solvents used were of analytical reagent grade or LC grade unless otherwise stated.

A Q3/2 Accurel polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). The hollow fiber was cut into 4.0 cm segments, cleaned with acetone, and dried before use. Deionized water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Field samples, a reservoir and a tap water sample, were collected from Hsinchu county, Taiwan.

2.2. Instrumentation

An LC system assembled from modular components consisted of a Waters 2695 Separations Module and a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). Waters Empower Software was utilized to control the system and also to analyze data. A high pH tolerance column [18] (Waters XBridge C_{18}, 4.6 mm × 250 mm, particle size 5 μm) was used for the separation of the target compounds. The mobile phase was composed of 90% acetonitrile$_{(aq)}$ and 0.006 M tetrabutylammonium hydroxide$_{(aq)}$ at gradient compositions shown in Table 1. Mixtures of water and 90% acetonitrile$_{(aq)}$ with different ratios were used to clean the column for 15 min between successive injections. To equilibrate the column, the initial mobile phase gradient composition was eluted for 10 min before each injection. Detection was initially done at

Table 1
Composition of mobile phase in HPLC

Time (min)	Flow rate (mL min^{-1})	90% acetonitrile$_{(aq)}$	6 mM tetrabutylammonium hydroxide$_{(aq)}$
0	1	25	75
10	1	40	60
20	1	40	60
30	1	50	50
35	1	60	40