Talanta 119 (2014) 396–400

Contents lists available at ScienceDirect

Talanta

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

Direct analysis of eight chlorophenols in urine by large volume injection online turbulent flow solid-phase extraction liquid chromatography with multiple wavelength ultraviolet detection

Feng Guo [a], Qian Liu [a], Jian-bo Shi [a,*], Fu-sheng Wei [a,b], Gui-bin Jiang [a]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] China National Evironmental Monitoring Center, Beijing, 100012, China

ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form
23 October 2013
Accepted 1 November 2013
Available online 19 November 2013

Keywords:
Turbulent flow chromatography
Online solid-phase extraction
Chlorophenols
Urine

ABSTRACT

A novel method for determining eight chlorophenols (CPs) by large volume injection online turbulent flow solid-phase extraction high performance liquid chromatography in urine samples was developed. An aliquot of 1.0 mL urine sample could be analyzed directly after centrifugation. The analytes were preconcentrated online on a Turboflow C18-P SPE column, eluted in back-flush mode, and then separated on an Acclaim PA2 analytical column. Major parameters such as SPE column type, sample loading flow rate and elution time were optimized in detail. Eight CPs from monochlorophenol to pentacholophenol were measured by multiple-wavelength UV detection at four different wavelengths. The limits of detection (LODs) were between 0.5 and 2 ng/mL. The linearity range was from the limit of quantification to 1000 ng/mL for each compound, with the coefficients of determination (r^2) ranging from 0.9990 to 0.9996. The reproducibility of intraday and interday relative standard deviations (RSDs) ranged from 0.6% to 4.5% ($n=5$). The method was successfully applied to analyze eight CPs in urine samples. Good recoveries, ranging from 76.3% to 122.9%, were obtained. This simple, sensitive and accurate method provides an alternative way to rapidly analyze and monitor CPs in urine samples, especially for matters of occupational exposure.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Chlorophenols (CPs) have been widely used as raw materials or intermediates for dyes, fungicides, pesticides, insecticides, and herbicides [1–3]. They are also used in the leather- and wood-preservation industries [4]. In addition, tap water chlorination may produce CPs [5]. These compounds are potential estrogens or carcinogenic [6–8] to human health, and may interfere with oxidative phosphorylation and inhibit ATP synthesis [1]. Substances such as 2-chlorophenol, 2, 4-dichlorophenol, 2, 4, 6-trichlorophenol, and pentachlorophenol have been regulated as priority pollutants by the U.S. Environmental Protection Agency (EPA). Highly chlorinated phenols are persistent [9,10]. Many countries and international organizations [11,12] have limited their maximum concentrations in drinking water. CPs can easily enter the human body via dermal, dietary, or aqueous absorption [13], and are partially excreted via urine. Studies on human or environmental exposures to these highly toxic compounds are ongoing [14–16]. Since human exposures to these compounds can be assessed by measuring them in urine, CPs in urine were frequently analyzed and monitored as xenobiotic indicators for metabolism studies or occupational exposures [17–19].

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are commonly used methods to measure CPs in environmental [5,9,20–22] and biological samples [23–25]. HPLC is more convenient and more robust than GC [26,27] because it does not require complicated derivatization. Fully automated online solid-phase extraction (SPE) coupled liquid chromatography methods [28–30] with simple pretreatment can further simplify the operations and release the operators from tedious work, so they are useful for rapidly screening controlled pollutants in diverse fluid samples.

The online Turboflow column is a kind of novel columns for online preconcentration, which is very effective for direct and fast analyzing complicated biological fluids, such as urine, serum and saliva [31–33]. Currently, this technique is mainly used for analysis of drugs and biomarkers. By using turbulent flow liquid chromatography tandem mass spectrometry (LC–MS/MS), the terbinafine in plasma have been directly analyzed, and the throughput was significantly improved [34]. The turbulent flow LC–MS/MS method was also successfully applied for simultaneous analysis of a broad

* Corresponding author. Tel./fax: +86 10 62849129.
E-mail address: jbshi@rcees.ac.cn (J.-b. Shi).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.006

range of controlled drugs in urine in a short cycle time [35]. In addition, the applications of turbulent flow column in analyzing drugs and toxins in wine, milk, and meat production were also developed. However, only a few studies [36,37] refer to its application for environmental pollutants.

The aim of this work was to develop a fully automated method for directly measuring eight CPs in urine samples with large volume injection online turbulent flow solid-phase extraction high performance liquid chromatography (SPE-LVI-HPLC). A large volume (1.0 mL) urine sample could be injected directly after centrifugation. Eight CPs, including two monochlorophenol isomers, three dichlorophenol isomers, one trichlorophenol, one tetrachlorophenol, and one pentachlorophenol were trapped on the Turboflow C18-P column, eluted in back-flush mode, and further separated on an Acclaim PA2 analytical column. The proposed method has been successfully applied to analyze twenty urine samples of healthy adults.

2. Experimental section

2.1. Chemicals and materials

Standards for 3-chlorophenol (3-CP, 99%), 2,4-dichlorophenol, (2,4-DCP, 99%), 3,4-dichlorophenol (3,4-DCP, 99%), and 2,4,6-trichlorophenol (2,4,6-TCP, 98%) were purchased from Acros Organics (Geel, Belgium). 4-Chlorophenol (4-CP, 100%) was purchased from AccuStandard (New Haven, CT). 2,3-Dichlorophenol (2,3-DCP, 98%) was purchased from Alfa Aesar. 2,3,5,6-Tetrachlorophenol (2,3,5,6-TeCP, 98%) was purchased from Sigma (St. Louis, MO). Pentachlorophenol (PCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Their molecular structures are shown in Fig. 1.

Stock solutions (2000 mg/mL) were prepared in methanol (MeOH) and stored in the dark at 4 °C. Working solutions were freshly prepared by diluting the stock solutions with water. HPLC grade Acetonitrile were all purchased from J.T. Baker (Phillipsburg, NJ). Ultrapure water produced from a Milli-Q system (Millipore, Billerica, MA) was used throughout. All reagents were of analytical grade unless otherwise noted.

2.2. Online SPE procedure and HPLC analysis

The UltiMate™ 3000 system (Thermo, USA) was controlled by Chromeleon® Chromatography Management Software (v. 6.80, Dionex, USA). This system consisted of a WPS-3000TSL autosampler with large-volume loop (2.5 mL) for injection, a TCC-3200 thermostated column compartment with a two-position, six-port (2P-6P) valve, a DGP 3600M dual-gradient pump, and a SRD 3600 solvent rack with integrated vacuum degasser.

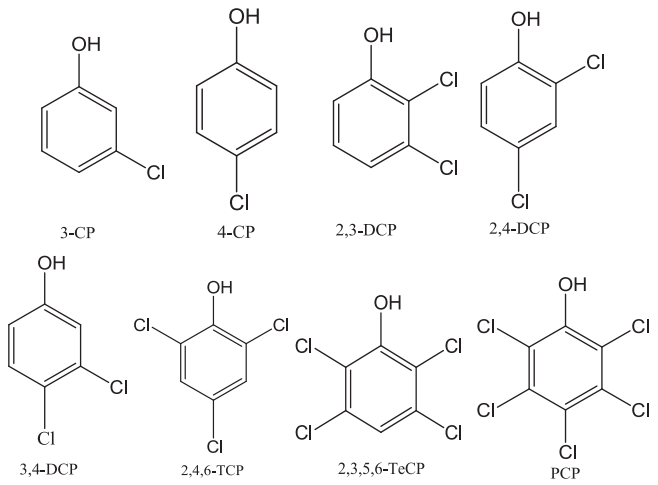

Fig. 1. Chemical structures of the eight CPs.

Table 1
Online SPE procedure, HPLC gradient elution and valve switching program.

Time ($_{min}$)	Pump 1			Pump 2			Valve position
	A (%)	*B* (%)	Flow rate (mL/min)	*A* (%)	*B* (%)	Flow rate (mL/min)	
−0.5	0	10	3.0	75	25	0.7	1
0.0	90	10	3.0	75	25	0.7	1
0.1	90	10	1.0	75	25	0.7	1
1.1	90	10	1.0	74	26	0.7	2
6.1	90	10	0.7	68	32	0.7	1
17.0	90	10	0.7	55	45	0.7	1
18.0	90	10	0.7	55	45	0.7	1
28.0	90	10	0.7	10	90	0.7	1
28.5	90	10	3.0	10	90	0.7	1
29.0	90	10	3.0	10	90	0.7	1
29.1	90	10	3.0	75	25	0.7	1
31.0	90	10	3.0	75	25	0.7	1

"−0.5" stands for sample loading time before the start time of baseline acquisition (recorded as "0").
Mobile phase: A, 25 mM HAc/25 mM NH_4Ac (1.45:1, v/v); B, ACN.

The setup and method procedures are similar to our previous study [38]. Briefly, five major steps were used: sampling, cleanup, SPE column regeneration, elution and HPLC separation. Each 1.0 mL sample was drawn by syringe from a 1.5 mL vial and pumped into the large-volume loop, and then delivered to the online SPE column (Turboflow C18-P, 60 μm, 1.0 × 50 mm, Thermo Scientific) with a high flow rate mobile phase (3 mL/min, 90% A) for the pump 1. After sampling, the flow rate was changed to 1 mL/min and kept for 1 min to remove matrix components concentrated on the SPE column together with the analytes. After cleanup, the valve was switched to elute the analytes from the SPE column to the analytical column by the mobile phase (25% B) for 5 min in back-flush mode. Then the valve was transferred back and the analytes were further separated on an analytical column (Acclaim® PA2, 3 μm, 3.0 × 150 mm, Thermo Scientific). Meanwhile, the SPE column was regenerated for next analysis. The mobile phase consisted of (A) 25 mM HAc/25 mM NH_4Ac (1.45:1, v/v) and (B) acetonitrile (ACN) for both pumps. The analytical column temperature was set at 40 °C. The online SPE procedure for the pump 1, schedules of valve switching, gradient elution and separation condition for the pump 2 are listed in Table 1. The SPE column was regenerated after each elution step in order to remove any residual contamination so that the method will be reproducible. Flushing with 10% B for 22 min was enough for this. Multiple wavelength UV detection (Dionex, USA) was used in quantifying CPs: 269 nm for 4-CP, 277 nm for 3-CP, 286 nm for 2,3-DCP, 2,4-DCP, 3,4-DCP and 2,4,6-TCP, and 303 nm for 2,3,5,6-QCP and PCP. At the same time, 3D scanning was used to obtain the spectrograms of the analytes and identify them.

3. Results and discussion

3.1. HPLC analysis

HPLC parameters were optimized to ensure proper resolution, symmetry, and adequate separation of the eight CPs. The analytical column was an Acclaim PA2 (3 μm, 3.0 × 150 mm, Thermo Scientific). With an acetonitrile/water mixture as the mobile phase, the Acclaim PA2 column resolved the analytes well. Slower elution from 25% (v/v) ACN to 45% (v/v) ACN in 17 minutes separated the eight CPs better, especially for the two groups of isomers.

Download English Version:

https://daneshyari.com/en/article/7681410

Download Persian Version:

https://daneshyari.com/article/7681410

Daneshyari.com