CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 42, Issue 12, December 2014
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2014, 42(12), 1723–1728.

RESEARCH PAPER

Simultaneous Determination of Seven Metabolites of Polycyclic Aromatic Hydrocarbons in Human Urine by Online Solid Phase Extraction-High Performance Liquid Chromatography

YOU Fan[1,2], ZHU Lan[2], HE Ling[2], RAN Liang-Ji[3], JIN Yan[3], SUN Cheng-Jun[1,*]

[1] West China School of Public Health, Sichuan University, Chengdu 610041, China
[2] Sichuan Center for Disease Control and Prevention, Chengdu 610041, China
[3] Thermofisher Scientific (China) Corporation, Shanghai 201203, China

Abstract: A high performance liquid chromatographic method for simultaneous determination of seven metabolites of polycyclic aromatic hydrocarbons (PAHs), including 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 2-hydroxyphenanthrene, 4-hydroxyphenanthrene, 1-hydroxypyrene and 6-hydroxychrysene in human urine, was developed coupled with online solid phase extraction by use of a double ternary liquid chromatography system with a fluorescence detector. The target compounds were online concentrated on the Turboflow Cyclone solid phase extraction column, and then transferred by a six-way valve to the Hypersil Green PAH column for separation with acetonitrile and water as mobile phase at a flow rate of 1.0 mL min^{-1} and 35 ºC. The analysis was completed in 20 min. Under the optimized chromatographic conditions, the method showed good linearity ($r \geq 0.999$) in the range of 5–2000 ng L^{-1} or 50–20000 ng L^{-1} with the LODs of 0.5–15 ng L^{-1} and the recoveries of 80.7%–110.7%. The proposed method was successfully applied to the detection of metabolites of polycyclic aromatic hydrocarbons in urine from several smokers and non-smokers. The concentrations of 2-hydroxynaphthalene, 1-hydroxynaphthalene, 2-hydroxyfluorene, 2-hydroxyphenanthrene, 4-hydroxyphenanthrene and 6-hydroxychrysene in the smoker urine were much higher than those in non-smoker.

Key Words: Polycyclic aromatic hydrocarbon; Metabolites; Human urine; Solid phase extraction; High performance liquid chromatography

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are discovered as a category of environmental organic carcinogenic compounds at the earliest[1,2]. PAHs exist extensively in airborne particles, smoked food and barbecues. The smoke of tobacco also contains high levels of PAHs. PAHs are diverse and widespread in the environment. PAHs enter the human body mainly through the respiratory tract, the gastrointestinal tract and skin, and then transformed to DNA adducts in the body. Therefore, PAHs are the most important carcinogenic compounds[3]. Many researchers have studied the biomarkers for comprehensive assessment of human exposure to PAHs ever since the 1980s[4]. Monohydroxy PAHs (OH-PAHs), the biomarkers of PAH exposure, were investigated most frequently[5]. Metabolites of high boiling point PAHs are mainly excreted through feces and less than 1% is excreted through urine, so their concentrations in urine are quite low[6]. PAH metabolites in urine commonly used as biological monitoring indexes are mainly OH-PAHs originated from PAHs with less than 4 cycles such as pyrene, naphthalin, phenanthrene and chrysene[5–7].

Received 31 August 2014; accepted 23 October 2014
* Corresponding author. Email: sunchj@scu.edu.cn

DOI: 10.1016/S1872-2040(14)60789-1

The levels of PAHs metabolites in urine are very low, as well as the urine matrix is very complicated. The impurities in urine may injure chromatographic columns, so the samples should be subjected to a clean-up procedure before injection for analysis. Currently available sample pretreatment method is usually offline solid phase extraction (SPE). However, it is tedious and time-consuming, and the reproducibility is also unsatisfactory. Furthermore, SPE columns are usually for single use, so its labor and time costs are rather high. Online SPE is a fully automated sample pretreatment method developed in recent years and it allows enrichment and purification in one step. SPE columns can be used for many times with good reproducibility and easy automation and the online SPE technique was used in analysis of trace organic compounds in foods, environmental and biological samples[8–10].

Up to now, the methods reported for analysis of OH-PAHs includ high performance liquid chromatography coupled with a fluorescence detector (HPLC-FLD)[11,12], gas chromatography-mass spectrometry (GC-MS)[13–15] and liquid chromatography-mass spectrometry (LC-MS)[16–20]. GC-MS needs a tedious derivatization procedure, and LC-MS needs expensive instrument. HPLC-FLD is a common analytical instrument in most chemical laboratories, by which the derivatization procedure is not necessary for analysis of OH-PAHs. Compared with LC-MS method, it has equivalent even higher sensitivity when it combines with online concentration technique. In this study, a double ternary liquid chromatographic system was performed. The target analytes in urine were online enriched and purified using Turboflow Cyclone SPE columns on the basis of rapid turbulent flow chromatography. By isolating and removing large molecule impurities prior to chromatographic analysis, not only the service time of SPE columns were prolonged, but also the cross contamination was reduced. A few reports were available on rapid separation of 7 PAH metabolites using a Hypersil Green PAH column. Urine samples of smokers and non-smokers were analyzed, and a comparison with the reported methods[11,12,20] was conducted. The proposed method exempts conventional SPE purification, enrichment and concentration procedure, and the urine samples can be directly injected for chromatographic analysis after enzymatic hydrolysis, centrifugation and filtration. The experimental results showed that the method was simple, inexpensive, accurate and precise. Furthermore, the method was more sensitive than the reported methods[11,12], and suitable for the determination of OH-PAHs in urine samples.

2 Experimental

2.1 Instruments and reagents

An Ultimate 3000 double ternary liquid chromatographic system equipped with a FLD 3400 fluorescence detector was purchased from Thermo Fisher Scientific, USA. Hypersil Green PAH column (150 mm × 4.6 mm, 5 μm, Thermo Fisher Scientific, USA) was used for separation and Turboflow Cyclone column (50 mm × 1.0 mm, Thermo Fisher Scientific, USA) was used for online SPE.

1-Hydroxynaphthalene (≥ 99.0%), 2-hydroxynaphthalene (≥ 99.0%), 1-hydroxypyrene (≥ 99.0%), 2-hydroxyphenanthrene (10 ng μL^{-1} in acetonitrile) and 4-hydroxyphenanthrene (10 ng μL^{-1} in acetonitrile) were purchased from Dr. Ehrenstorfer GmBH (Germany). 6-Hydroxychrysene (100%) was purchased from AccuStandard (USA). 2-Hydroxyfluorene (98%), *β*-glucuronosidase (Type B-1, ≥ 1000000 units g^{-1}) and phenol sulfatase (Type H-1, ≥ 10000 unit g^{-1}) were obtained from Sigma-Aldrich (USA). Acetonitrile and methanol (HPLC grade) were obtained from Fisher (USA).

0.5 M acetic acid-sodium acetate buffer solution was prepared by dissolving 20.5 g of anhydrous sodium acetate in 500 mL of ultrapure water and its pH was adjusted to 5.0 with glacial acetic acid.

A mixed hydrolase solution containing 600 U mL^{-1} of *β*-glucuronosidase and 14 U mL^{-1} of phenol sulfatase was prepared freshly before use by dissolving 60 mg of *β*-glucuronosidase and 140 mg of phenol sulfatase in 100 mL acetic acid-sodium acetate buffer[11,20].

2.2 Standard solutions

Stock solutions, intermediate solutions and mixed standard solutions were prepared in methanol using OH-PAHs standard substances or standard solutions. Then the standard series were prepared in 40% methanol, as listed in Table 1.

Table 1 Preparation of standard solution of OH-PAHs

OH-PAHs	Stock solution ($mg\ L^{-1}$)	Intermediate solution ($\mu g\ L^{-1}$)	Mixed standard solution ($\mu g\ L^{-1}$)	Standard series concentration ($\mu g\ L^{-1}$)					
1-Hydroxynaphthalene	100	1000	200	0.050	0.50	1.00	2.00	5.00	10.0
2-Hydroxynaphthalene	100	1000	20	0.005	0.050	0.10	0.20	0.50	1.00
2-Hydroxyfluorene	100	1000	20	0.005	0.050	0.10	0.20	0.50	1.00
2-Hydroxyphenanthrene	10	—	20	0.005	0.050	0.10	0.20	0.50	1.00
4-Hydroxyphenanthrene	10	—	20	0.005	0.050	0.10	0.20	0.50	1.00
1-Hydroxypyrene	100	1000	20	0.005	0.050	0.10	0.20	0.50	1.00
6-Hydroxychrysene	100	1000	20	0.005	0.050	0.10	0.20	0.50	1.00