Analytica Chimica Acta 636 (2009) 51–57

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Synchronous fluorescence determination of urinary 1-hydroxypyrene, β-naphthol and 9-hydroxyphenanthrene based on the sensitizing effect of β-cyclodextrin

Hong-Mei Yang[a], Yong-Sheng Wang[a,*], Jun-Hong Li[a], Gui-Rong Li[a], Ying Wang[a], Xuan Tan[a], Jin-Hua Xue[a], Xi-Lin Xiao[b], Rong-Hui Kang[a]

[a] College of Public Health, University of South China, Hengyang 421001, PR China
[b] College of Chemistry and Chemical Engineering, University of South China, Hengyang 421001, PR China

ARTICLE INFO

Article history:
Received 8 December 2008
Received in revised form 15 January 2009
Accepted 16 January 2009
Available online 23 January 2009

Keywords:
Synchronous fluorescence
1-Hydroxypyrene
β-Naphthol
9-Hydroxyphenanthrene
β-Cyclodextrin
Urine

ABSTRACT

A novel method for the simultaneous determination of 1-hydroxypyrene (1-OHP), β-naphthol (β-NAP) and 9-hydroxyphenanthrene (9-OHPe) in human urine has been established by using synchronous fluorescence spectrometry. It was based on the fact that synchronous fluorescence spectrometry can resolve the broad-band overlapping of conventional fluorescence spectra, which arise from their similar molecular structures. Only one single scan is needed for quantitative determination of three compounds simultaneously when $\Delta\lambda = 15$ nm is chosen. The signals detected at these three wavelengths, 369.6, 330.0 and 358.0 nm, vary linearly when the concentration of 1-OHP, β-NAP and 9-OHPe is in the range of 2.16×10^{-8}–1.50×10^{-5} mol L^{-1}, 1.20×10^{-7}–1.10×10^{-5} mol L^{-1} and 1.07×10^{-7}–3.50×10^{-5} mol L^{-1}, respectively. The correlation coefficients for the standard calibration graphs were 0.994, 0.999 and 0.997 ($n = 7$) for 1-OHP, β-NAP and 9-OHPe, respectively. The limits of detection (LOD) for 1-OHP, β-NAP and 9-OHPe were 6.47×10^{-9} mol L^{-1}, 3.60×10^{-8} mol L^{-1} and 3.02×10^{-8} mol L^{-1} with relative standard deviations (R.S.D.) of 4.70–6.40%, 2.80–4.20%, 3.10–4.90% ($n = 6$), respectively. The method described here had been applied to determine traces of 1-OHP, β-NAP and 9-OHPe in human urine, and the obtained results were in good agreement with those obtained by the HPLC method. In addition, the interaction modes between β-cyclodextrin (β-CD) and 1-OHP, β-NAP or 9-OHPe, as well as the mechanism of the fluorescence enhancement were also discussed.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that have received considerable attention because of their carcinogenic, teratogenic and mutagenic effects [1–3]. Biological monitoring of PAHs is useful for the estimation of health risks due to PAHs exposure, and is usually performed by the measurement of PAHs hydroxides in urine samples [4,5]. In 1987, Jongeneelen et al. developed the high-performance liquid chromatograph (HPLC) method for the determination of 1-hydroxypyrene (1-OHP), which is the main metabolite of pyrene and is regarded as the most sensitive biomarker used for the assessment of internal exposure to PAHs [4,6]. However, pyrene is only one of the hundreds of PAHs and background urinary 1-OHP levels are influenced by some factors such as cigarette smoking, diet, and industrial pollution, therefore, simply using 1-OHP as a biomarker has been called into question [7,8]. Naphthalene and phenanthrene are predominant PAHs compounds in occupational exposure, whose metabolites, β-NAP and 9-OHPe, present in urine together with 1-OHP. The determination of these metabolites to estimate the risk of PAHs exposure has been carried out [7,9]. So, developing an effective, simple, and rapid method for the simultaneous determination of 1-OHP, β-NAP and 9-OHPe is of great importance.

Analysis of 1-OHP, β-NAP and 9-OHPe is usually performed by using high-performance liquid chromatography with fluorescence detection (HPLC-FD) [4,7–9], gas or liquid chromatography coupled to mass spectrometry (GC/LC–MS) [5,10–12]. These methods have several disadvantages such as high cost, complex sample preparation procedures and long analysis time. From the analytical point of view, the metabolites of PAHs have very good fluorescence properties under UV–vis excitation and their determination can be carried out by fluorescence spectrometry. However, to solve the selectivity problem in the multicomponent analysis, which arises from the overlapping of the broad-band spectra of structurally similar components, these compounds must go through a pre-separation

* Corresponding author. Tel.: +86 734 8281595; fax: +86 734 8281771.
E-mail address: yongsheng.w@tom.com (Y.-S. Wang).

0003-2670/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2009.01.039

step, which is time-consuming for routine analysis and in some cases requires special and expensive instrumentation [9–10]. Since first introduced by Lloyd [13] in 1971, the synchronous fluorescence spectrometry (SFS) has been known as a powerful tool for simultaneous analysis of multicomponent samples without pre-treatment of samples [14,15]. The synchronous fluorescence technique consists in the measurement of spectra at the constant difference between the positions of emission and excitation monochromators ($\Delta\lambda$), so the technique can be performed by selecting the optimum $\Delta\lambda$ for particular compounds and recording the synchronous signal of the mixture at the suitable wavelengths. In recent years, SFS technique was successfully applied to drug [16–18], biochemistry [19], environment [20–23] and food analysis [24] and so on.

To our knowledge, we first report here about the simultaneous determination of 1-OHP, β-NAP and 9-OHPe by synchronous fluorescence spectrometry using constant-wavelength scan technique. But above all, it is necessary to select a kind of sensitizing agent for enhancing fluorescence signals of β-NAP and 9-OHPe because they only emit weak fluorescence. β-Cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of seven α-D-(+)-glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity and hydrophilic external surface [25]. For its special hydrophobic cavity structure, β-CD has the unique ability to form inclusion complexes with various guest molecules with suitable polarity and dimension [26–35]. The formation of these supramolecular complexes can alter the physicochemical properties of the guest molecules and considerable attentions have been focused on the luminescence application of β-CD, for instance, biomacromolecules [30], drug [31] and pesticides [32] were determined by using β-CD to enhance the fluorescence intensity of guest molecule. Therefore, we selected β-CD as a sensitizing agent to enhance the fluorescence intensity of 1-OHP, β-NAP and 9-OHPe through the formation of inclusion complexes.

The purpose of this study is to propose a SFS method for the simultaneous determination of 1-OHP, β-NAP and 9-OHPe in urine samples based on the sensitizing effect of β-cyclodextrin. The synchronous spectra were obtained by scanning both monochromators simultaneously at constant-wavelength differences of $\Delta\lambda$ = 15 nm for 1-OHP, β-NAP and 9-OHPe and the synchronous spectra of the three compounds could exhibit the maximum signals (λ = 369.6 nm for 1-OHP, λ = 330.0 nm for β-NAP, and λ = 358.0 nm for 9-OHPe) with minimum interferences of each other. The proposed method has additional advantages of less time-consuming and simple instrument because samples can be analyzed directly, without chromatographic separation procedure before detection or using expensive instruments [9]. Furthermore, the recovery, selectivity and limits of detection (LOD) of this method were better than those of other constant-energy [23] and constant-wavelength synchronous fluorescence methods [14]. The method is also easy to generalize in the analysis of 1-OHP, β-NAP and 9-OHPe in human urine. In addition, the sensitization mechanism was initially studied.

2. Experimental

2.1. Chemicals

All experiments were performed with analytical reagent grade chemicals and doubly distilled water. 1-OHP, β-NAP and 9-OHPe were purchased from Sigma. Their stock solutions (5.00×10^{-3} mol L^{-1}) were prepared by dissolving an appropriate amount of 1-OHP or β-NAP or 9-OHPe in methanol, then diluting to 100 mL with water, respectively. The concentration of working solution for 1-OHP is 5.00×10^{-6} mol L^{-1}, and for β-NAP and 9-OHPe is 1.00×10^{-5} mol L^{-1}. β-CD and Tris were purchased from Shanghai Chemical Reagent Company (Shanghai, China). β-CD working solution (0.055 mol L^{-1}) was prepared by dissolving β-CD in water. A 0.1 mol L^{-1} buffer solution of pH 7.0 was prepared by dissolving Tris in water and adjusting pH with 0.1 mol L^{-1} HCl.

2.2. Apparatus

All the fluorescence spectra were measured with a Hitachi 4500 spectrofluorimeter (Japan) equipped with a 1 cm quartz cell. The slit widths of both excitation and emission were kept at 5 nm, a PMT voltage of 700 V and a response time of 0.1 s. For synchronous excitation measurements, both excitation and emission monochromators were locked together and scanned simultaneously with a constant wavelength difference $\Delta\lambda = \lambda_{em} - \lambda_{ex}$.

HPLC (DionexP680, USA) equipped with UV detector was used for comparing of SFS and HPLC method. Samples were separated on a Diamonsil^{TM}C$_{18}$ column (150 mm × 4.6 mm, 5 μm, particle size). The column chamber temperature was set at 30 °C. The detection wavelength of the UV–vis detector was set at 280 nm. A pH meter (PB-20, Germany) was used for pH adjustment.

2.3. Pretreatment of urine samples

Urine samples were collected from three smokers including a cook, a boilersmith and a parking-worker, and one preschool child. Urine specimens were pretreated as the method reported by Ouyang et al. with modification [36]. After mixing completely, a 10 mL aliquot of the urine sample was hydrolyzed by 2 mL of 50% sodium hydroxide, and heated for 3.5 h in a boiling water bath away from light. After heating, the hydrolyzed sample was adjusted to pH 2–3 with concentrated HCl, and then filtered through a 125 mL separatory funnel. Filtrate was extracted twice with 6 mL of n-hexane each time, and the organic phase was evaporated to dryness at 30 °C under a gentle flow of nitrogen gas. The residue was redissolved in 0.2 mL of methanol water (1:1, v/v) and analyzed directly with the developed method and the HPLC method.

Several different extracting solvents for urine sample, such as chloroform, diethyl ether, dichloromethane, petroleum ether and n-hexane, were tested. Taking the extraction efficiency and foreign substance interference into account, we selected n-hexane for the extraction of 1-OHP, β-NAP and 9-OHPe in hydrolyzed samples.

2.4. General procedures

To a series of 10 mL test tubes, 2.00 mL of 5.50×10^{-2} mol L^{-1} β-CD, an appropriate amount of 1-OHP or/and β-NAP or/and 9-OHPe or the sample solutions were added each in turn. Then the mixture was diluted to the 10 mL with 0.1 mol L^{-1} Tris–HCl buffer solution of pH 7.0. After well shaked, the synchronous fluorescence spectra were obtained by scanning simultaneously at constant-wavelength difference of $\Delta\lambda$ = 15 nm for 1-OHP, β-NAP and 9-OHPe and their mixture, respectively. The synchronous fluorescence intensities were measured at the synchronous maxima of each compound (for 1-OHP λ_{max} = 369.6 nm, for β-NAP λ_{max} = 330.0 nm, for 9-OHPe λ_{max} = 358.0 nm, respectively).

3. Results and discussion

3.1. Spectral characteristics

The fluorescence spectra, the three-dimensional fluorescence spectra and the synchronous fluorescence spectra for 1-OHP, β-NAP, 9-OHPe and their mixture were obtained under the same experimental conditions. Fig. 1 showed the fluorescence emission maximum of 1-OHP (5.00×10^{-7} mol L^{-1} curve 1), β-NAP (2.00×10^{-6} mol L^{-1} curve 2), and 9-OHPe (2.00×10^{-6} mol L^{-1}

Download English Version:

https://daneshyari.com/en/article/1168665

Download Persian Version:

https://daneshyari.com/article/1168665

Daneshyari.com