Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 836 (2006) 92–97
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Fast simultaneous determination of urinary 1-hydroxypyrene and 3-hydroxybenzo[*a*]pyrene by liquid chromatography–tandem mass spectrometry

Ruifang Fan [a,b,c], Yulian Dong [a], Wenbing Zhang [a,*], Yu Wang [a,c], Zhiqiang Yu [a], Guoying Sheng [a], Jiamo Fu [a]

[a] *Guangdong Key Laboratory of Environmental Protection & Resource Utilization, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, PR China*
[b] *College of Life Science, South China Normal University, Guangzhou, 510631, PR China*
[c] *Graduate School of the Chinese Academy of Sciences, Beijing 100049, PR China*

Received 6 October 2005; accepted 20 March 2006
Available online 17 April 2006

Abstract

A fast analysis method using liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry was developed for the simultaneous determination of the 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo[*a*]pyrene (3-OHBaP) in urine. Mass transitions were monitored at *m/z* 219.3–200.0 for 1-OHP and *m/z* 269.2–252.2 for 3-OHBaP. Only 10 min was needed for the analysis. The recovery was 60% for 3-OHBaP and 91% for 1-OHP, respectively. And the method detection limits were 0.49 μg/L for 1-OHP and 1.03 μg/L for 3-OHBaP. The inter- and intra-day relative standard deviations were in the range of 2.8–8.9% for 1-OHP and 9.7–20.8% for 3-OHBaP, respectively. The developed method was successfully used to measure urinary PAH metabolites of student volunteers in a high school.

Keywords: Polycyclic aromatic hydrocarbons; Metabolites; 1-Hydroxypyrene; 3-Hydroxybenzo[*a*]pyrene; Liquid chromatography–tandem mass spectrometry; Urine

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a class of compounds some of which (e.g., benzo[*a*]pyrene (BaP)) are known to be carcinogenic and/or co-carcinogenic. Humans are exposed to PAHs from various sources, including the work place, environmental, medicinal, and dietary sources, smoking and others, through lung, gastrointestinal, and skin absorption [1]. The assessment of human exposure to PAHs can be carried out not only by monitoring PAHs in the environment such as the atmosphere, rivers, and sediments, but also by biomonitoring PAHs, especially 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo[*a*]pyrene (3-OHBaP) metabolites in human liver, urine, etc. Since PAHs require metabolic activation to express their carcinogenicity, the determination of their phenol metabolites is more useful to assess PAH activation and provides a more accurate profile of an exposed individual's response to PAHs [2,3].

In 1987, Jongeneelen et al. [4] used solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) with fluorescence detection (FC) to determine urinary 1-hydroxypyrene, the main metabolite of pyrene. Since then, 1-OHP has been widely used as a reliable indicator of exposure to PAHs [5,6]. However, because many urinary PAH metabolites show carcinogenic properties, a sensitive and practical method is needed to determine more PAH metabolites, especially those with high carcinogenic properties. The use of a BaP metabolite as another indicator of exposure to carcinogenic PAHs appears to be more appropriate [7,8]. It would be significant to measure 1-OHP and 3-OHBaP simultaneously, because the concentrations of 3-OHBaP in urine are extremely low and difficult to monitor [8],

Grimmer et al. [9] reported a procedure for the simultaneous quantification of a variety of monohydroxy- and dihydroxy-

* Corresponding author. Tel.: +86 20 85291501; fax: +86 20 85290192.
E-mail address: zhwb@gig.ac.cn (W. Zhang).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.03.044

PAHs in urine by GC/MS. Smith et al. [10,11] further developed a screening method for up to 18 urinary monohydroxy-PAHs using solid-phase micro-extraction followed by GC-HRMS. Although GC/HRMS is highly sensitive, time-consuming sample pretreatment and derivatization steps are required prior to the analysis.

In 1992, Boos [12] developed an automated coupled-column (copper phthalocyanine modified porous glass pre-column) HPLC method for the determination of 1-OHP in urine. Some other PAH metabolites, such as 1-OHP, 1-, 4-, and 9-hydroxyphenanthrenes, 3-hydroxybenz[*a*]anthracene, and 1-, 3-, and 5-hydroxychrysenes were also monitored using similar methods [5,6]. Simon et al. [7] and Toriba et al. [8] used a column-switching HPLC method to detect traces of 3-OHBaP and to determine monohydroxybenzo[*a*]pyrene isomers and 1-OHP simultaneously in human urine. This method allowed clean up of the samples online, and they needed no pretreatment.

In recent years, high-performance liquid chromatography mass spectrometry (HPLC/MS) was advanced in biochemical research and environmental sample analysis. Some researchers have characterized the urinary PAH metabolites by HPLC/MS [13–16]. Galceran and Moyano [13,14] first studied the mass spectrometric characteristics of PAH metabolites in both electrospray and atmospheric pressure chemical ionization (ESI and APCI) modes. The results showed that APCI was more sensitive to determine urinary PAH metabolites. Letzel et al. [15] applied HPLC/APCI-MS to obtain fragmentation information of oxidized aromatic hydrocarbons by ozone, and B[*a*]P-diones were first identified in human urine. However, few researches were carried out to quantitatively determine the metabolites of PAHs by HPLC/MS [17–18], especially by HPLC/MS/MS. Multiple reaction monitoring (MRM), a method used in tandem mass spectrometry, is a very powerful technique for quantitative analysis in biochemical researches [19–21] due to its high sensitivity, selectivity, and specificity. Because of high selectivity provided by the tandem mass spectrometer, extensive sample clean-up procedures are not required. Furthermore, to our knowledge, simultaneous measurements of 1-OHP and 3-OHBaP by HPLC or HPLC/MS are sparse.

In this paper, a fast analysis method using HPLC/MS/MS was developed to measure urinary 1-OHP and 3-OHBaP simultaneously, after enzymatic hydrolysis of PAH metabolites by SPE. The analysis is fast and can be completed in only 10 min. The use of MRM mode for quantification of 1-OHP and 3-OHBaP can reduce matrix effects significantly. The method was applied successfully for detecting urinary PAH metabolites of volunteers non-occupationally exposed to PAHs from a high school in Guangzhou city, China.

2. Experimental

2.1. Chemicals and materials

3-Hydroxybenzo[*a*]pyrene and 1-hydroxypyrene were obtained from Aldrich (purity 98%, USA). β-Glucuronidase/arylsulphatase (124400β-glucuronidase units/ml, 36010 sulfatase units/ml) was purchased from Sigma (USA). The methanol was of HPLC grade (Merck, USA). Creatinine was obtained from Fluka (Buchs, Switzerland). Ammonium acetate (HPLC grade) was purchased from Tedia Company (USA). All the other reagents were of analytical grade and used without further purification. The SPE visiprep^{TM} cartridges (C-18 ENVI, 500 mg and 3 ml) were purchased from Supeclo (USA).

2.2. Sample collection

The urine samples were collected randomly from students of a high school in an urban area of Guangzhou city, China and stored in polyethylene bottles. All students are 16–18 years old and live in homes located in Guangzhou city. All selected individuals were asked to complete a questionnaire providing their age, sex, weight, height, dietetic habits, transport vehicle, and cigarette smoking habits.

The collected urine samples were immediately taken to the laboratory and the creatinine concentration of each urine sample was determined by the Jaffée colorimetric method [22] in order to eliminate the difference of urinary concentration. Then all the samples were stored at −20 °C until further analysis.

2.3. Enzymatic hydrolysis of the urine sample

Ten milliliters of urine was transferred to a conical flask. The pH of the solution was adjusted to 5.0 with 0.2 M HCl, and 2.5 mL of 0.5 M acetate buffer (pH 5.0) was added. After addition of 20 μL of β-glucuronidase/arylsulphatase the conical flask was covered with a tinfoil and then placed in a shaker bath overnight at 37 °C to completely hydrolyze the conjugated PAH metabolites.

2.4. Solid-phase extraction

The hydrolyzed urine samples were loaded after cartridges had been pre-conditioned with 5 mL of methanol and 5 mL of water. The cartridge was sequentially washed with 10 mL of water and 30% methanol. After the cartridges were dried completely, the trapped metabolites were eluted with 4 mL of methanol. The eluate was evaporated to dryness under nitrogen gas and re-dissolved in 1 mL of methanol. The solution was filtered through a 0.2 μm filter, and then stored at −20 °C until HPLC/MS/MS analysis.

2.5. HPLC/MS/MS apparatus and conditions

All samples were analyzed using an Agilent 1100 high-performance liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) coupled with an API 4000 triple quadrupole mass spectrometry (Applied Biosystems, Foster City, CA, USA). The liquid chromatography was equipped with a HP G1379A degasser unit, a HP G1311A quaternary pump, a HP G1313A auto sampler, and a HP G1314A VWD detector.

A Kromasil C-18 reversed phase analytical column (100 mm × 4.6 mm and 3.5 μm particle diameter, Eka Chemicals AB, Bohus, Sweden) was used for the chromatographic separation. Methanol (A) and 10 mM ammonium acetate in

Table 1
Optimized parameter values of the tandem mass spectrometry used for the determination of 1-OHP and 3-OHBaP

Parameter	Optimum value
Ionization mode	Positive ion detection
Dwell time per transition (ms)	200
Vaporizer temperature (TEM) (°C)	450 °C
Ion source gas 1(GS1) (psi)	71
Declustering potential (DP) (V)	65 (3-OHBaP) 44 (1-OHP)
Entrance potential (EP) (V)	9
Collision gas (CAD) (psi)	6
Curtain gas (CUR) (psi)	25
Nebulizer current (NC) (μA)	5
Collision energy (CE) (V)	54
Collision cell exit potential CXP (V)	13
Precursor/product (*m/z*)	219.3/200.0 (1-OHP) 269.2/252.2 (3-OHBaP)

water (B, pH adjusted to 3.0 by acetic acid) (90:10, v: v) were used as mobile phase, and the flow rate was set at 0.6 mL/min. Standard solutions were prepared in methanol. And the HPLC effluent was introduced to an APCI interface without splitting. APCI mass data were acquired in the positive ion mode, and the details about the APCI parameters are shown in Table 1. The quantification of 1-OHP and 3-OHBaP was performed in the MRM mode. Data processing was carried out with the Analyst Software 1.3.2 (ABI-SCIEX, Toronto, Canada).

2.6. Instrument detection limit (IDL) and method detection limit (MDL)

The instrument detection limit (IDL) was defined by a signal-to-noise (S/N) ratio of 5:1. The method detection limit (MDL) was determined using a urine sample spiked with standards that equalled five times the IDL concentration. The spiked sample was analyzed six times, and the MDL was determined to be 3.36 times the standard deviation [23,24].

3. Results and discussion

3.1. Optimization of enzymatic hydrolysis and SPE conditions

10, 20, and 30 μL of β-glucuronidase/arylsulphatase were added to relatively high concentration samples to determine the optimal volume of β-glucuronidase/arylsulphatase, and 20 μL was found to be adequate to de-conjugate all the PAH hydroxide conjugates. To make certain that all PAH metabolite glucuronides were hydrolyzed completely, the enzymatic hydrolysis was performed overnight at 37 °C [2,25].

The sensitivity of the method has also been improved by separating the PAH metabolites and matrix compounds with solid-phase extraction. Thirty percent of methanol was found to be optimum solvent for washing the substrate without affecting the recovery. The experimental results also indicated that 4 mL of methanol was enough to elute the target compounds by SPE, and concentrating by using nitrogen gas yielded higher recoveries than by using a rotary evaporator.

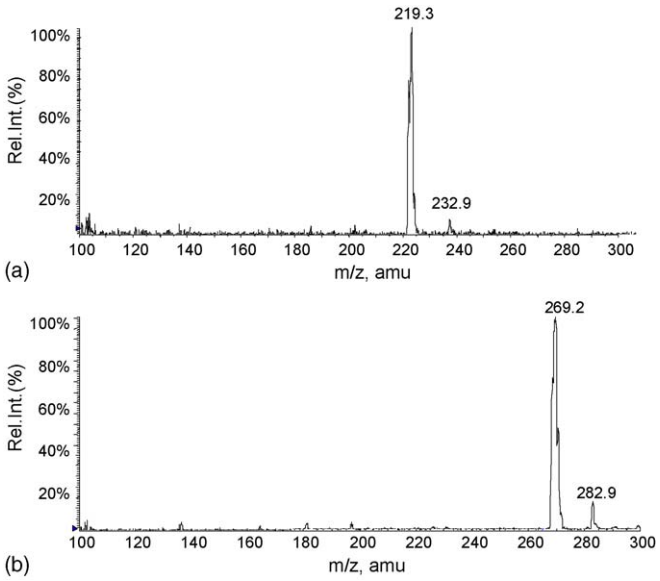

Fig. 1. Q_1 mass spectra of 1-OHP (a) and 3-OHBaP (b).

3.2. Optimization of HPLC/MS/MS conditions

The APCI parameters were tuned according to the MS signal response of the target compound and the results indicated that the positive mode was more sensitive than the negative mode. The optimized values are shown in the Table 1. From the Q_1 mass spectrum we observe the molecular ions of 1-OHP and 3-OHBaP ($[M+H]^+$) at *m/z* 219.3 and 269.2, respectively (Fig. 1). The ions of *m/z* 232.9 and 282.9 [M + H + 14] may be resulted from the protonated pyrene-quinone and BaP-quinone, respectively [26]. The product ion mass spectra of 1-OHP and 3-OHBaP are shown in Fig. 2. It is obvious that the main product ions of 1-OHP were *m/z* 200.0 and 189.0

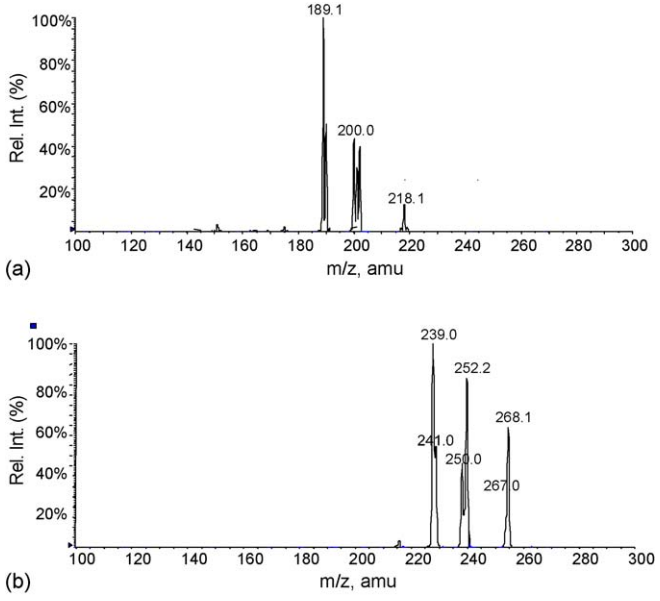

Fig. 2. The product ions mass spectra of 1-OHP (a) and 3-OHBaP (b).

whereas the main product ions of 3-OHBaP are *m/z* 252.2 and 239.0. The parent ion of 3-OHBaP ($[M+H]^+$, *m/z* 269.2) undergoes a loss of H_2 to give *m/z* 267.0 ($[M+H-2]^+$), further loss of CO to give *m/z* 239.0 ($[M+H-2-28]^+$) [16]. When the molecular ions of 1-OHP (*m/z* 219.3) and 3-OHBaP (*m/z* 269.2) were broken into product ions, the molecular ions may be unstable and are liable to be deprotonated and yield *m/z* 218.1 $[M+H-H]^+$ and *m/z* 268.1$[M+H-H]^+$, respectively. For *m/z* 200.0 and 250.0 corresponding to the product ions of 1-OHP and 3-OHBaP, respectively, they are possibly formed from loss of a water molecule. Although the fragment at *m/z* 189.0 for 1-OHP and the fragment at *m/z* 239.0 for 3-OHBaP are more intensive than other fragments, the S/N values indicate that transitions at *m/z* 219.3–200.0 for 1-OHP and *m/z* 269.2–252.2 for 3-OHBaP have the maximum sensitivity in the MRM mode, so they are used to quantify the two compounds.

For HPLC-FC a gradient elution and a long time were often required to resolve the various components in the matrix. The MRM mode allows the selective detection of compounds without very good chromatographic separation, which means that it can reduce the matrix effects significantly. In our study different mobile phases consisting of water–methanol were used to improve the HPLC separation and enhance sensitivity in the MS. The best MS response was achieved using methanol (A): 10 mM ammonium acetate in water (B, pH adjusted to 3.0 by acetic acid) (90:10, v:v). The acetic acid was found to be appropriate in order to lower the pH to protonate the PAH metabolites and thus produce a good peak shape. The total run time was only 10 min and the retention times of 1-OHP and 3-OHBaP were 3.8 and 7.1 min, respectively. The typical MRM ion-chromatograms resulting from authentic urine samples show that this method provides a good peak shape and only a slight matrix effect (Fig. 3).

3.3. Calibration and method detection limits

Calibration was performed in the MRM mode under the described optimized conditions given in the Table 1. Calibration curves of this method were linear ($r^2 = 0.990$ for 1-OHP and $r^2 = 0.9994$ for 3-OHBaP) over a concentration range of 0.16–16 ng/μL for 1-OHP and 0.085–8.5 ng/μL for 3-OHBaP.

The IDL for 1-OHP and 3-OHBaP was 0.049 and 0.105 μg/L, respectively. The MDL was 0.49 μg/L for 1-OHP and 1.03 μg/L for 3-OHBaP. The detection limits in our method were comparable to or slightly lower than those in the literatures, e.g., in the reference 4 the detection limit of 3-OHBaP was 4 nmol/L (1 μg/L) and the MDL of 1-OHP was 0.6 μg/L in the reference [18].

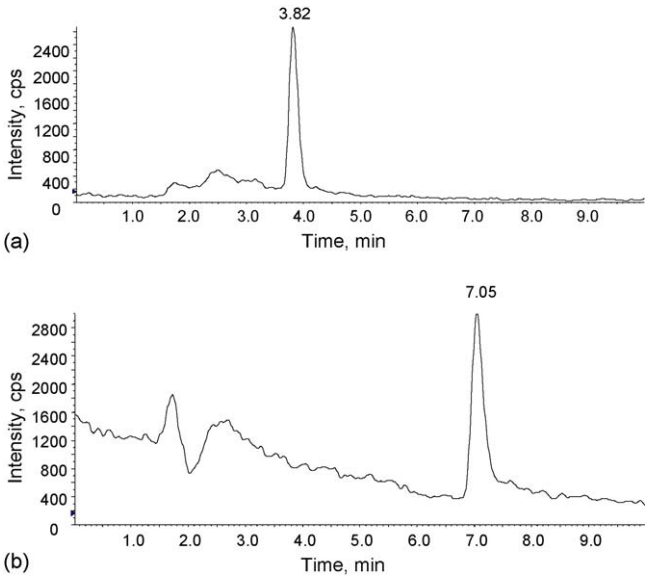

Fig. 3. Typical MRM ion-chromatograms resulting from authentic urine samples (a) 1-OHP (0.347 μmol/mol creatinine) and (b) 3-OHBaP (0.025 μmol/mol creatinine).

3.4. Recovery and relative standard deviation (R.S.D.)

To validate the method, urine from nonsmokers was pooled and spiked with different levels of 1-OHP and 3-OHBaP standards. Recovery experiments were performed by addition of the standards to a 10 mL urine sample before the enzymatic hydrolysis. The recovery was in the range of 60–91% (Table 2). Low recovery had also been observed for 3-OHBaP by other researchers. Toriba et al. [8] found that the recovery of 3-OHBaP ($n = 3$) was only 47% with the method of SPE. The lower recovery of 3-OHBaP is presumably due to the instability of the compound in the acetate buffer, though it is stable in methanol. Simon et al. [7] found that aqueous standard of 3-OHBaP was stable for 24 h at 4 °C if a methanol solution of Triton X100 R was added, then the recoveries of 3-OHBaP rose to 96%. Whiton et al also found that 3-OHBaP was not stable over time [27]. So further research is needed to improve the recovery.

Table 3
Intra-day and inter-day precision ($n = 6$, $P < 0.05$)

	Inter-day (R.S.D., %)		Intra-day (R.S.D., %)	
	1-OHP	3-OHBaP	1-OHP	3-OHBaP
High concentration	2.8	4.3	9.7	20.8
Low concentration	3.3	8.9	13.1	20.8

Table 2
Recoveries of 1-OHP and 3-OHBaP ($P < 0.05$)

Compound	High concentration ($n = 6$)	R.S.D. (%)	Low concentration ($n = 6$)	R.S.D. (%)
1-OHP	91 ± 12.7% (85 ng)	3.3	88 ± 8.9% (4.25 ng)	2.8
3-OHBaP	60 ± 23.0% (160 ng)	8.9	66 ± 2.8% (8 ng)	2.8

Table 4
Analysis results of authentic urine samples from students in Guangzhou, China (unit: μmol/mol creatinine)

	Overall ($n=49$)			Group A ($n=23$)	Group B ($n=26$)
	<MDL	$\bar{x} \pm s$	Range	$\bar{x} \pm s$	$\bar{x} \pm s$
1-OHP	0	0.220 ± 0.136	0.065–0.663	0.253 ± 0.138	0.192 ± 0.129
3-OHBaP	22	0.007 ± 0.012	0–0.025	0.007 ± 0.011	0.006 ± 0.012

$\bar{x}$: mean; s: standard deviation; Group A: smokers and passive smokers; Group B: non-smokers.

3.5. Precision

To determine the precision and accuracy of the present system, known high and low concentrations of 1-OHP and 3-OHBaP were added to urine samples of non-smokers. The inter-day precision was determined by six-fold analysis of the urine sample. The spiked sample was analyzed once a day for six continuous days to evaluate the intra-day precision. The inter-day R.S.D. ranged between 2.8% (1-OHP) and 8.9% (3-OHBaP) and the intra-day R.S.D. ranged between 9.7% for 1-OHP and 20.8% for 3-OHBaP (Table 3). The intra-day R.S.D. of 3-OHBaP was relatively higher, indicating that the instability of 3-OHBaP in aqueous solution might be the major reason [7,27].

3.6. Examinations of non-exposed students

The method was applied to 49 urine samples collected from student volunteers in Guangzhou city. The average concentrations of 1-OHP and 3-OHBaP were 0.220 ± 0.136 μmol/mol creatinine and 0.007 ± 0.012 μmol/mol creatinine, respectively. For the smoker and passive smoker groups the average concentrations of urinary 1-OHP and 3-OHBaP were 0.253 ± 0.138 μmol/mol creatinine and 0.007 ± 0.011 μmol/mol creatinine, respectively, distinctively higher than that of the non-smoking group (0.192 ± 0.129 μmol/mol creatinine for 1-OHP and 0.006 ± 0.012 μmol/mol creatinine for 3-OHBaP (Table 4). Other researchers have also shown that the concentrations of PAH metabolites for smokers are higher than for non-smokers [28,29].

The urinary 1-OHP levels in non-occupationally exposed individuals in Canada and Germany were 0.08 μmol/mol creatinine [30] and 0.3 μg/L [31], respectively. The overall geometric mean urinary 1-OHP level in the USA was 0.039 μmol/mol creatinine [32]. In China, the 1-OHP concentration of the general population is 0.42–2.42 μmol/mol creatinine [33]. So the results obtained by our method are comparable to those reported before. Few data of 3-OHBaP for the non-exposed population are available in the literature.

4. Conclusions

HPLC-APCI-MS/MS was shown to be a sensitive and rapid method for the simultaneous determination of 1-OHP and 3-OHBaP in human urine. The analysis can be completed in only 10 min. Under optimized conditions, this method can detect 0.49 μg/L of 1-OHP and 1.03 μg/L of 3-OHBaP. The recovery of the method is 61–91%. The inter- and intra-day precisions are in the range of 2.8–8.9% and 9.7–20.8%, respectively. The analysis results of authentic urine samples show that this method is effective for assessing PAH exposure.

Acknowledgements

This work was supported financially by National Natural Science Foundation of China (NSFC-40590393) and a Special Scientific Research Start-up Fund for the Gainer of the Excellent Ph.D. Dissertation Award and the President Scholarship of Chinese Academy of Sciences (2005).

References

[1] L.H. Keith (Ed.), Environmental Endocrine Disruptors, Wiley, New York, 1998, p. 206.
[2] J. Hollender, B. Koch, W. Dott, J. Chromatogr. B 739 (2000) 225.
[3] F.J. Jongeneelen, Sci. Total Environ. 199 (1997) 144.
[4] F.J. Jongeneelen, R.B.M. Anzion, P.T. Henderson, J. Chromatogr. 413 (1987) 227.
[5] J. Lintelmann, C. Hellemann, A. Kettrup, J. Chromatogr. B 660 (1994) 67.
[6] F.J. Jongeneelen, Ann. Occup. Hyg. 45 (2001) 3.
[7] P. Simon, M. Lafontaine, P. Delsaut, Y. Morele, T. Nicot, J. Chromatogr. B 748 (2000) 337.
[8] A. Toriba, H. Nakamura, T. Cheiyanukornkul, R. Kizu, T. Makino, H. Nakazawa, T. Yokio, K. Hayakawa, Anal. Biochem. 312 (2003) 14.
[9] G. Grimmer, J. Jacob, G. Detebarn, K.W. Naujack, Int. Arch. Occup. Environ. Health 69 (1997) 231.
[10] C.J. Smith, W.L. Huang, C.J. Walcott, W. Turner, J. Grainger, D.G. Patterson Jr., Anal. Bioanal. Chem. 372 (2002) 216.
[11] C.J. Smith, C.J. Walcott, W.L. Huang, V. Maggio, J. Grainger, D.G. Patterson Jr., J. Chromatogr. B 778 (2002) 157.
[12] K.S. Boos, J. Chromatogr. 600 (1992) 189.
[13] M.T. Galceran, E. Moyano, J. Chromatogr. A 731 (1996) 75.
[14] M.T. Galceran, E. Moyano, J. Chromatogr. A 683 (1994) 9.
[15] T. Letzel, U. Pöschl, R. Wissiack, E. Rosenberg, M. Grasserbauer, R. Niessner, Anal. Chem. 73 (2001) 1634.
[16] T. Letzel, E. Rosenberg, R. Wissiack, M. Grasserbauer, R. Niessner, J. Chromatogr. A 855 (1999) 501.
[17] S. Ferrari, F. Mandel, J.D. Berset, Chemosphere 47 (2002) 173.
[18] T.R.V. Wiele, K.M. Peru, W. Verstraete, S.D. Siciliano, J.V. Headley, J. Chromatogr. B 806 (2004) 245.
[19] N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, S. Manoj, M. Santosh, S. Wishu, M. Koteshwara, J. Chidambara, B. Gopinadh, B. Sumatha, J. Chromatogr. B 805 (2004) 13.
[20] F. Pommier, R. Frigola, J. Chromatogr. B 784 (2003) 301.
[21] M.L. Constanzer, C.M. Chavez, B.K. Matuszewski, J. Pharm. Biomed. Analysis 15 (1997) 1001.
[22] H.H. Taussky, J. Biochem. 208 (1954) 853.
[23] U.S.EPA, SW-B46, Test Methods for Evaluating Solid Waste, third ed., 1986.
[24] APHA, AWWA, WEF, Standard Methods, 19th ed., 1995.
[25] J. Gündel, J. Angerer, J. Chromatogr. B 738 (2000) 47.
[26] S.M.V. Leeuwen, H.H.U. Karst, Anal. Bioanal. Chem. 378 (2004) 917.

[27] R.S. Whiton, C.L. Witherspoon, T.J. Buckley, J. Chromatogr. B 665 (1995) 390.
[28] E. Siwinska, D. Mielzynska, A. Bubak, E. Smolik, Mutat. Res. 445 (1999) 147.
[29] K. Hara, T. Hanaoka, Y. Yamano, T. Itani, Sci. Total Environ. 199 (1997) 159.
[30] C. Viau, A. VyskoČil, L. Maetel, Sci. Total Environ. 163 (1995) 191.
[31] G. Grimmer, G. Dettbarn, K.W. Naujack, J. Jacob, Int. J. Environ. Anal. Chem. 43 (1991) 177.
[32] W.L. Huang, J. Grainger, D.G. Patterson Jr., W.E. Turner, S.P. Caudill, L.L. Needham, J.L. Pirkle, E.J. Sampson, Int. Arch. Occup. Environ. Health 77 (2004) 491.
[33] Z.H. Zhao, W.Y. Quan, D.H. Tian, Sci. Total Environ. 92 (1990) 145.