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Miniaturized hollow fiber assisted liquid-phase microextraction with in situ derivatization and gas chromatography–mass spectrometry for analysis of bisphenol A in human urine sample

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ABSTRACT

A new method that involves miniaturized hollow fiber assisted liquid-phase microextraction (HF-LPME) with in situ derivatization and gas chromatography–mass spectrometry (GC–MS) is described for the determination of trace amounts of bisphenol A (BPA) in human urine samples. The detection limit and the quantification limit of BPA in human urine sample are 0.02 and 0.1 ng ml^{-1} (ppb), respectively. The calibration curve for BPA is linear with a correlation coefficient of >0.999 in the range of 0.1–50 ng ml^{-1}. The average recoveries of BPA in human urine samples spiked with 1 and 5 ng ml^{-1} BPA are 101.0 (R.S.D.: 6.7%) and 98.8 (R.S.D.: 1.8%), respectively, with correction using the added surrogate standard, bisphenol A-^{13}C$_{12}$. This simple, accurate, sensitive and selective analytical method can be applicable to the determination of trace amounts of BPA in human urine samples.

1. Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl)propane, is a chemical contained in polycarbonate plastics and epoxy resins and is present in various products used in industry. In 1993, Krishnan et al. [1] documented that BPA was released from polycarbonate flasks during autoclaving and exhibited estrogenic activity. The estrogenic activity of BPA has been extensively evaluated in a variety of assays [2–5]. Recently, the elution of BPA from polycarbonate plastics and the contamination of food samples with BPA have been reported [6–8]. As healthy humans may be exposed to BPA via a variety of daily activities, exposure assessment of BPA in human is an important issue.

The excretion of orally ingested BPA as glucuronide derivative in human urine has been reported [9]. Thus, it is thought that the human exposure can be evaluated by measuring BPA in urine sample. However, as BPA concentration in human urine is very low, high-sensitivity and high-accuracy analytical methods are required.

Sample preparation techniques, such as solid-phase extraction (SPE) [10,11] and molecularly imprinted solid-phase extraction (MISPE) [12,13], have been developed for the determination of BPA in urine sample. However, the manual version of SPE or MISPE is tedious and time consuming. Although automated SPE [14] and online SPE [15] have been reported, a specific device is necessary.

Recently, solventless and solvent-minimized polymer sorption techniques, such as stir bar sorptive extraction (SBSE) [16,17], have been successfully used for the determination of BPA in human biological samples. In addition, liquid-phase microextraction (LPME), solvent microextraction (SME), and the single-drop microextraction (SDME) technique have been developed [18–20]. The main advantages of these techniques are good cost performance and a wide application range that includes polyaromatic hydrocarbons [21–23], polychlorinated biphenyls (PCBs) [24,25], pesticides [23,25–28] and organotin compounds [29]. In 1999, Pedersen-Bjergaard and Rasmussen reported the hollow fiber assisted liquid-phase microextraction (HF-LPME) method [30] in which LPME can be accomplished without making single drop by using a hollow fiber as the protective film. This method is an improvement of the existing LPME method and can be used to analyze the biological samples [31]. So, HF-LPME has been applied successfully to the determination of alkylphenols (APs), chlorophenols (CPs) and BPA in water samples [32].

Many analytical methods for the determination of BPA in human biological samples have been reported. These include

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liquid chromatography (LC) with fluorescence detection (FD) [33,34], electrochemical detection (ED) [10,35,36], mass spectrometry (MS) [10,37] or tandem mass spectrometry (MS-MS) [15,38]. However, oftentimes, these LC methods have low resolution and are influenced by the sample matrix. In addition, ion suppression might pose a problem in MS or MS-MS analysis. On the other hand, gas chromatography–mass spectrometry (GC–MS) was initially used for the determination of phenol compounds including BPA, even though derivatization was required [6–8,11,14]. The derivatization leads to sharper peaks and hence to better separation and higher sensitivity for the phenols. However, the derivatization procedure is tedious and time consuming. In order to avoid this problem, in situ derivatization was developed. We have reported the determination of BPA in river water sample by LPME with in situ derivatization [39]. The in situ derivatization simultaneously performs derivatization and sample preparation steps, thereby improving recovery and sensitivity of analysis.

The aim of this study was to develop an analytical method for the trace analysis of BPA in human urine sample, which employs miniaturized HF-LPME with in situ derivatization and GC–MS. The HF-LPME method was performed in conventional 2 ml vials for miniaturization.

2. Experimental

2.1. Materials and reagents

BPA of environmental analytical grade and acetic acid anhydride as derivatization reagent for trace analysis were purchased from Kanto Chemical Inc. (Tokyo, Japan). Bisphenol A-$^{13}C_{12}$ (BPA-$^{13}C_{12}$) was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Chemical structures of the compounds are shown in Fig. 1. *E. coli* β-glucuronidase (25000 units/0.4 ml, 62500 units ml^{-1}) and *H. pomatia* sulfatase (3540 units ml^{-1}) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Prior to use, the β-glucuronidase was added to 0.1 M ammonium acetate to make a total concentration of 10000 units ml^{-1}. Other reagents and solvents were of analytical grade and purchased from Wako Pure Chemical Inc. (Osaka, Japan). The water purification system was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Standard solutions

A stock solution (1.0 mg ml^{-1}) of BPA was prepared as required by the addition of methanol. Standard solutions for the calibration curve were prepared as required by the addition of purified water. The miniaturized HF-LPME with in situ derivatization method was applied to making the calibration curve. The calibration was performed with a surrogate standard.

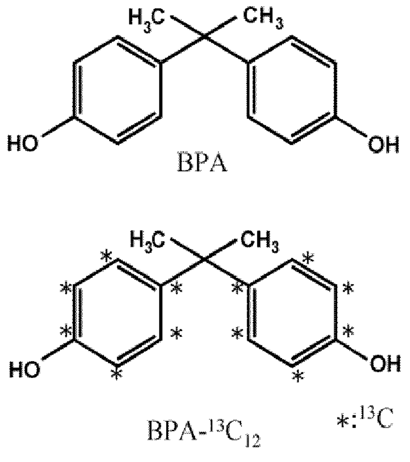

Fig. 1. Chemical structures of BPA and BPA-$^{13}C_{12}$.

2.3. Instrumentation

A 10 μl microsyringe (SGE 10F-HP-0.63) for miniaturized HF-LPME was purchased from SGE Japan (Kanagawa, Japan). The microsyringe needle had a cone tip, with 42 mm length and 0.63 mm o.d. Before extraction, the 10 μl microsyringe was rinsed with acetone followed by toluene 10 times to avoid carryover and air bubble formation. Accurel Q 3/2 polypropylene hollow fiber membrane with 600 μm i.d., 200 μm wall thickness and 0.2 μm pore size was purchased by Membrana (Wuppertal, Germany). The hollow fiber was cut manually and carefully into 1.1 cm lengths. The hollow fiber segments were cleaned in acetone prior to use. For the extraction, 2 ml vial was purchased from Agilent Technologies (Palo Alto, CA, USA). The 8 mm stir bar was purchased from Bel-Art Products (Pequannock, NJ, USA). GC–MS was performed using an Agilent 6890 gas chromatograph with a 5973 mass-selective detector (Agilent Technologies).

2.4. Analytical conditions

Injection was performed in the pulsed splitless mode. The injection pulse pressure was set to 25 psi for 1 min. The temperature of the inlet was at 250 °C. The separations were conducted on a DB-5MS fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies). The oven temperature was programmed to increase from 100 to 280 °C (held for 4 min) at 10 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1}. The mass spectrometer was operated in the selected ion monitoring (SIM) mode with electron ionization (EI). The ionization voltage was set to 70 eV. For SIM, three ions were monitored *m*/*z* 213 and 228 for the acyl derivative of BPA and *m*/*z* 225 for the acyl derivative of BPA-$^{13}C_{12}$ (The underlined number is the *m*/*z* of the ion used for quantification.).

2.5. Human urine sample

Human urine samples were collected from three healthy volunteers (22–27-year-old) and stored at −20 °C prior to use.

2.6. Sample preparation

One milliliter of human urine sample spiked with surrogate standards was pipetted into the 2 ml vial. Then, 1.0 M ammonium acetate (100 μl, pH 6.8) was added. After β-glucuronidase (10 μl, 10,000 units ml^{-1}) and sulfatase (10 μl, 3540 units ml^{-1}) were added, the sample was gently mixed. Quantitative glucuronidase and sulfatase hydrolysis to release free BPA was accomplished by incubating at 37 °C for 3 h. After enzymatic de-conjugation, 1.0 M sodium hydroxide solution (100 μl) for pH adjustment, acetic acid anhydride (20 μl) as the derivatization reagent, and a stir bar (8 mm) were added to the vial. The needle tip was inserted into a 1.1-cm-long hollow fiber segment. Then, the hollow fiber was immersed in toluene for about 10 s to impregnate its pores with toluene. Toluene in the syringe was injected carefully into the hollow fiber, after which the needle tip together with the hollow fiber was immersed in sample solution. Extraction was performed at room temperature for 15 min while stirring at 500 rpm. The schematic of miniaturized HF-LPME is shown in Fig. 2. After the extraction, 2 μl of extract was carefully withdrawn into the syringe and injected into the GC–MS system.

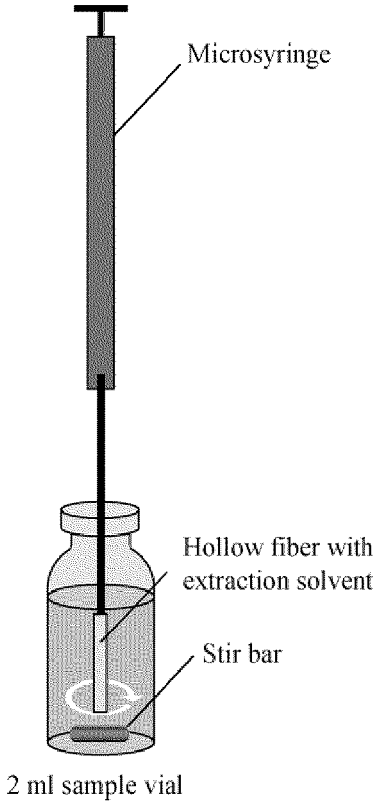

Fig. 2. Schematic of HF-LPME method.

3. Results and discussion

3.1. Derivatization

In EI-MS analysis of the acyl derivative of BPA in the scan mode, *m*/*z* 213 and 228 were observed as the major and minor signals, respectively. On the other hand, in EI-MS analysis of the acyl derivative of BPA-$^{13}C_{12}$ in the scan mode, *m*/*z* 225 and 240 were observed as the major and minor signals, respectively. In this study, human urine sample was subjected to miniaturized HF-LPME with in situ derivatization and GC–MS. As these ion peaks were completely separated from those of other compounds, these ions were used as monitoring ions. The mass spectrometer was operated in the SIM mode.

3.2. Optimization of conditions for miniaturized HF-LPME with in situ derivatization conditions

One important parameter affecting miniaturized HF-LPME was the extraction solvent. In our previous paper, to determine the optimum extraction solvent, hexane, pentafluorohexane, and toluene were tested [39]. When toluene was used, strong peak response of the acyl derivative of BPA was obtained. Based on that result, toluene was used as the extraction solvent in this study.

Another important parameter affecting miniaturized HF-LPME was the extraction time. To optimize the extraction time, a 5 ng ml^{-1} standard solution of BPA was used. The extraction time profile of the acyl derivative of BPA in 1 ml standard solutions using miniaturized HF-LPME with in situ derivatization was determined by GC–MS, and is shown in Fig. 3. Maximum extraction of the acyl derivative of BPA was achieved after approximately 15 min. This condition was therefore used for the determination of BPA. In our previous study, the extraction time profile of the acyl derivative of BPA in 10 ml standard solutions using single-drop LPME with in situ derivatization was determined by GC–MS [39], and maximum extraction of the acyl derivative of BPA was achieved after approximately 90 min. The miniaturized HF-LPME used in the present study succeeded in shortening the time to reach maximum extraction. Two reasons are considered for the phenomena. One is the difference in interfacial area. Interfacial area was increased because a hollow fiber was used. The other reason is the difference in phase ratio (β), where $\beta = V_{sample}/V_{extract}$ ($V_{extract}$ is volume of extract and V_{sample}, volume of sample). In this study, extraction time was shortened because sample volume was decreased.

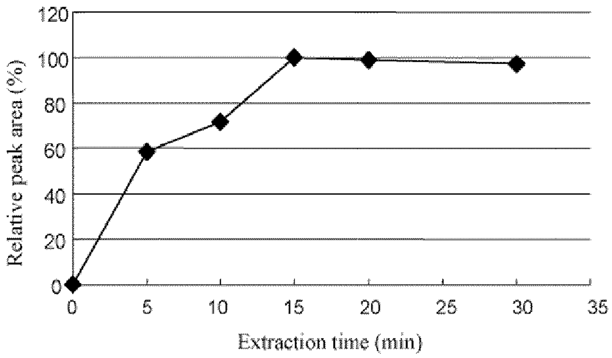

Fig. 3. Extraction time profile of BPA.

3.3. Comparison of analytical figures of merit

The calculated detection limits (LODs) of BPA in human urine sample with in situ derivatization and that without derivatization were 0.02 and 2 ng ml^{-1}, respectively, by miniaturized HF-LPME and GC–MS when the ratio of the compound's signal to the background signal (S/N) was 3. In addition, the calculated limits of quantification (LOQs) when S/N > 10 were 0.1 and 10 ng ml^{-1} for BPA in human urine sample with in situ derivatization and that without derivatization, respectively. The peak area ratios with respect to each surrogate standard were plotted and the response was found to be linear over the calibration range with correlation coefficients (*r*) higher than 0.999 for miniaturized HF-LPME with in situ derivatization. The validation results are summarized in Table 1.

The recovery and precision of the method were assessed by replicate analysis ($n = 6$) of human urine samples spiked at 1 and 5 ng ml^{-1} levels with the surrogate standard. Non-spiked and spiked urine samples were subjected to miniaturized HF-LPME with in situ derivatization and GC–MS. The recoveries were calculated by subtracting the results for the non-spiked urine samples from those for the spiked urine samples vs. spiked water samples. Isotope dilution mass spectrometry (IDMS) was used for quantification. The average recoveries of BPA in human urine samples spiked at 1 and 5 ng ml^{-1} levels are 101.0 (R.S.D.: 6.7%) and 98.8 (R.S.D.:

Table 1
Figures of merit of HF-LPME with in situ derivatization method

Compound	LOD[a] (ng ml^{-1})	LOQ[b] (ng ml^{-1})	Range (ng ml^{-1})	Correlation coefficient (*r*)
BPA	0.02	0.1	0.1–50	0.999

[a] LOD: limit of detection (S/N = 3).
[b] LOQ: limit of quantification (S/N > 10).

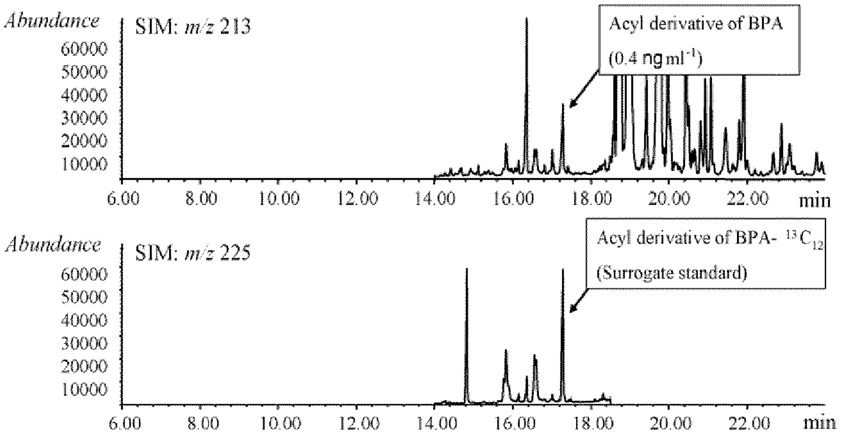

Fig. 4. Chromatograms of acyl derivatives of BPA and surrogate standard in human urine samples (C).

Table 2
Recoveries of BPA in human urine samples

Compound	Amount spiked			
	1 ng ml^{-1}		5 ng ml^{-1}	
	Recovery (%)	R.S.D. (%)[a]	Recovery (%)	R.S.D. (%)[a]
BPA	101.0	6.7	98.8	1.8

[a] The recoveries and precision were also examined by replicate analysis ($n = 6$) of human urine samples.

1.8%), respectively. The results are shown in Table 2. Therefore, the method enables the precise determination of standards and can be applied to the determination of trace amounts of BPA in human urine samples.

3.4. Determination of BPA in human urine samples

Three human urine samples were analyzed using the present method. BPA was detected in the samples within the range of 0.1–0.4 ng ml^{-1} by this method. The results are shown in Table 3. Typical chromatograms of the human urine samples are shown in Fig. 4. BPA concentrations in the samples were very low and could not be determined quantitatively by HF-LPME and GC–MS without derivatization but with enzymatic hydrolysis. However, the combination of miniaturized HF-LPME with in situ derivatization and GC–MS led to the successful determination of trace amounts of BPA in the human urine samples. We have previously reported the measurement of BPA in human urine sample by SBSE with in situ derivatization followed by thermal desorption (TD)-GC–MS [16]. Then, we compared miniaturized HF-LPME with SBSE. Although the sensitivities were the same, cost performance was excellent for miniaturized HF-LPME. SBSE required a polydimethylsiloxane (PDMS)-coated stir bar and a TD–GC–MS system, and the TD system had high-running cost because liquid nitrogen was used. By contrast, miniaturized HF-LPME required only a 10 μl microsyringe, a porous hollow fiber, and conventional GC–MS.

Table 3
Concentrations of BPA in human urine samples

Human urine sample	BPA (ng ml^{-1})	
	Without β-glucuronidase	With β-glucuronidase
A	N.D.[a]	0.1
B	N.D.	0.2
C	N.D.	0.4

[a] N.D. indicates not detected.

4. Conclusions

The determination of trace amounts of BPA in human urine samples using miniaturized HF-LPME with in situ derivatization and GC–MS was described. The proposed method has many practical advantages, including simplicity of extraction and high-sensitivity, and can be applied to biological samples. The detection limit and the quantitation limit of BPA in human urine samples were 0.02 and 0.1 ng ml^{-1}, respectively. In addition, the present method showed good linearity and high correlation coefficients using surrogate standards. The average recoveries were 98.8–101.0% with good precision ($n = 6$, R.S.D.: 1.8–6.7%) for human urine samples spiked at 1 and 5 ng ml^{-1} levels. This simple, accurate, and highly sensitive method is expected to have potential application in biological samples.

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References

[1] A.V. Krishnan, P. Stathis, S.F. Petmuth, L. Tokes, D. Feldman, Endocrinology 132 (1993) 2279.
[2] G.G.J.M. Kuiper, J.G. Lemmen, B. Carlsson, J.C. Corton, S.H. Safe, P.T. van der Saag, B. van der Burg, J.-Å. Gustafsson, Endocrinology 139 (1998) 4252.
[3] C. Sonnenschein, A.M. Soto, J. Steroid Biochem. Mol. Biol. 65 (1998) 143.
[4] S.C. Nagel, F.S. Vom Saal, K.A. Thayer, M.G. Dhar, M. Boechler, W.V. Welshons, Environ. Health Perspect. 105 (1997) 70.
[5] K.W. Gaido, L.S. Leonard, S. Lovell, J.C. Gould, D. Babaï, C.J. Portier, D.P. McDonnell, Toxicol. Appl. Pharmacol. 143 (1997) 205.
[6] R.A. Rudel, S.J. Melly, P.W. Geno, G. Sun, J.G. Brody, Environ. Sci. Technol. 32 (1998) 861.
[7] H.M. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201.
[8] O.P. Heemken, H. Reincke, B. Stachel, N. Theobald, Chemosphere 45 (2001) 245.
[9] J.G. Teeguarden, J.M. Waechter Jr., H.J. Clewell III, T.R. Covington, H.A. Barton, Toxicol. Sci. 85 (2005) 823.
[10] J. Sajiki, K. Takahashi, J. Yonekubo, J. Chromatogr. B 736 (1999) 255.
[11] T. Tsukioka, J. Brock, S. Graiser, J. Nguyen, H. Nakazawa, T. Makino, Anal. Sci. 19 (2003) 151.
[12] J.H. Zhang, M. Jiang, L.J. Zou, D. Shi, S.R. Mei, Y.X. Zhu, Y. Shi, K. Sai, B. Lu, Anal. Bioanal. Chem. 385 (2006) 780.
[13] M. Kawaguchi, Y. Hayatsu, H. Nakata, Y. Ishii, R. Ito, K. Saito, H. Nakazawa, Anal. Chim. Acta 539 (2005) 83.
[14] Z. Kuklenyik, J. Ekong, C.D. Cutchins, L.L. Needham, A.M. Calafat, Anal. Chem. 75 (2003) 6820.
[15] X.Y. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, J. Chromatogr. B 831 (2006) 110.

[16] M. Kawaguchi, K. Inoue, M. Yoshimura, R. Ito, N. Sakui, N. Okanouchi, H. Nakazawa, J. Chromatogr. B 805 (2004) 41.
[17] M. Kawaguchi, N. Sakui, N. Okanouchi, R. Ito, K. Saito, S. Izumi, T. Makino, H. Nakazawa, J. Chromatogr. B 820 (2005) 49.
[18] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[19] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[20] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235.
[21] L. Zhao, H.K. Lee, Anal. Chem. 74 (2002) 2486.
[22] J.-F. Liu, G.-B. Jiang, Y.-G. Chi, Y.-Q. Cai, Q.-X. Zhou, J.-T. Hu, Anal. Chem. 75 (2003) 5870.
[23] C. Basheer, R. Balasubramanian, H.K. Lee, J. Chromatogr. A 1016 (2003) 11.
[24] C. Basheer, H.K. Lee, J.P. Obbard, J. Chromatogr. A 1022 (2004) 161.
[25] C. Basheer, J.P. Obbard, H.K. Lee, J. Chromatogr. A 1068 (2005) 221.
[26] L. Hou, H.K. Lee, J. Chromatogr. A 1038 (2004) 37.
[27] C.-H. Yan, H.-F. Wu, Rapid Commun. Mass Spectrom. 18 (2004) 3015.
[28] L. Hou, G. Shen, H.K. Lee, J. Chromatogr. A 985 (2003) 107.
[29] V. Colombini, C. Bancon-Montigny, L. Yang, P. Maxwell, R.E. Sturgeon, Z. Mester, Talanta 63 (2004) 555.
[30] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[31] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 22 (2003) 565.
[32] C. Basheer, H.K. Lee, J. Chromatogr. A 1057 (2004) 163.
[33] A. Matsumoto, N. Kunugita, K. Kitagawa, T. Isse, T. Oyama, G.L. Foureman, M. Morita, T. Kawamoto, Environ. Health Perspect. 111 (2003) 101.
[34] K. Schoringhumer, M. Cichna-Markl, J. Chromatogr. B 850 (2007) 361.
[35] K. Ouchi, S. Watanabe, J. Chromatogr. B 780 (2002) 365.
[36] Z.S. Liu, M.S. Wolff, J. Moline, J. Chromatogr. B 819 (2005) 155.
[37] M. Liu, Y. Hashi, F. Pan, J. Yao, G. Song, J.M. Lin, J. Chromatogr. A 1133 (2006) 142.
[38] W. Volkel, N. Bittner, W. Dekant, Drug Metab. Dispos. 33 (2005) 1748.
[39] M. Kawaguchi, R. Ito, N. Endo, N. Okanouchi, N. Sakui, K. Saito, H. Nakazawa, J. Chromatogr. A 1110 (2006) 1.