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Determination of valproic acid in human serum and pharmaceutical preparations by headspace liquid-phase microextraction gas chromatography-flame ionization detection without prior derivatization

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Abstract

An efficient and fast extraction technique for the enrichment of valproic acid from human blood serum samples using the headspace liquid phase microextraction (HS-LPME) combined with gas chromatography (GC) analysis has been developed. The extraction was conducted by suspending a 2 μL drop of organic solvent in a 1 mL serum sample; following 20 min of extraction, withdrawing organic solvent into a syringe and injection into a GC with a flame ionization detector (FID), without any further pre-treatment. Four organic solvents, 1-decanole, benzyl alcohol, 1-octanol and *n*-dodecane, were studied as extractants, and *n*-dodecane was found to be the most sensitive solvent for valproic acid. The results revealed that HS-LPME is suitable for the successful extraction of valproic acid from human blood serum samples. Parameters like extraction time, ionic strength, pH, organic solvent volume, and temperature of the sample were studied and optimized to obtain the best extraction results. An enrichment factor of 27-fold was achieved in 20 min. The procedure resulted in a relative standard deviation of <13.2% ($n = 7$) and a linear calibration range from 2 to 20 μg mL^{-1} ($r > 0.98$), and the limit of detection was 0.8 μg mL^{-1} in serum blank samples. Overall, LPME proved to be a fast, sensitive and simple tool for the preconcentration of valproic acid from real samples. The proposed method was also applied to the analysis of valproate in pharmaceutical preparations.

Keywords: Headspace liquid phase microextraction; Valproic acid; Gas chromatography; Human serum analysis; Pharmaceutical preparations

1. Introduction

Valporic acid (VPA; 2-propylpentanoic acid) is a C8-branched carboxylic acid and an anti-epileptic drug widely used for the treatment of seizure disorders [1]. Monitoring of VPA levels in patient plasma is essential when there are changes in VPA dose, concomitant medication or clinical condition of patient [2].

Many analytical approaches have been used in determination of VPA, based on high-performance liquid chromatography (HPLC) [3–9], capillary electrophoresis [10,11] in combination with mass spectrometry (MS) [12–18], ultraviolet detection (UV) or fluorescence detection, usually after derivatization with a suitable chromophore or fluorophore [19–21], and also potentiometry ion selective sensors [22,23]. Although HPLC methods are frequently applied for this analysis but due to volatile nature of VPA, GC-MS is often preferred, offering unrivalled high resolution [24–27]. In most of these processes there is a need for prior derivatization to convert analyte into a suitable component for detection. Chromatography of analyte without prior derivatization would simplify the method, and as a result, the analysis time would be reduced. Underivatized VPA was analyzed using GC with flame ionization detection [28].

To achieve the necessary levels of sensitivity, enrichment and clean-up steps are needed before the chromatographic analysis. Extraction with solvent is most commonly used for isolation of VPA of serum solution prior to chromatographic analysis [3–12,19–21,29]. Evaporation and clean-up of extractive solvent prior to injection to GC column is essential to concentrate and eliminate background interferences. Thus, sensitivity would have been reduced due to loss of analyte through these tedious, labor-intensive, and time-consuming processes. Furthermore, there is preference for using solvent-less or solvent-free methods.

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Solid-phase microextraction (SPME), developed by Belardi and Pawliszyn [30] includes simultaneous extraction and preconcentration of analytes from aqueous samples or the headspace of the samples. The technique is simple, fast, solvent-free, easily automated and usually more feasibly combined with GC or HPLC [31–33]. However, there are still some drawbacks in this method, including limited life of the fiber, degradation of fibers with increased usage, bleeding of the SPME coating into the GC injector and sample carry-over [34,35]. In addition, SPME fibers are relatively expensive, and only a limited selection of stationary phases is available for the fibers. The free concentration of VPA in human plasma was determined by equilibrium dialysis followed by SPME and capillary gas chromatography by Krogh et al. [29].

In recent years, microextraction into a solvent drop has been shown to be an alternative sample preparation method to the conventional liquid–liquid extraction (LLE) [36,37] and has attracted increasing attention. Liquid-phase microextraction (LPME) is a solvent-minimizing sample pretreatment (1–3 μL), which is quick, requires simple laboratory equipments, easy for automation, and inexpensive with minimal exposure to toxic organic solvents. One advantage of LPME is the integration of extraction, concentration and sample introduction into a single step [38–40]. Today, head-space LPME has been successfully used for the extraction of volatile to semi-volatile compounds, without any interference from the sample matrix.

The purpose of this work was to develop a simple, rapid, and reliable method for the quantitative determination of VPA in human blood serum. Extraction and determination of VPA in serum was carried out by HS-LPME combined with a capillary gas chromatography-flame ionization detector. For the purpose of the present studies, different parameters affecting the extraction process were studied and optimized. The optimized method is proposed as an efficient alternative to more expensive, time consuming conventional methods. The performance of the proposed method was also compared to that of SPME and also applied to the analysis of valproate in pharmaceutical preparations.

2. Experimental

2.1. Reagents

Sodium valproate was prepared from Roozdaroo Co., Iran. The stock solution of this compound was prepared at a concentration of 1 mg mL^{-1} in methanol. This stock standard solution was diluted with double distilled water weekly to prepare a standard solution with a concentration of 50 μg mL^{-1}. The model solutions containing the required amount of analyte (0.1–20 μg mL^{-1}) were prepared daily by diluting mixed standard solutions with double distilled water to study extraction performance under different conditions. Stock and working standards were stored at 4 °C. 1-Decanole, benzyl alcohol, 1-octanol and *n*-dodecane containing a fixed concentration (10 μg mL^{-1}) of butanoic acid as an IS from Merck (Darmstadt, Germany) were used as extraction solvents. All other chemicals and reagents were of analytical grade from Merck (Darmstadt, Germany).

2.2. Instrumentation

A 10 μL Hamilton gas-tight syringe model 1701 (Hamilton, Bonaduz, Switzerland), with a bevel needle tip (length: 5.1 cm, ID: 0.013 cm, bevel 22 °C) was used to perform HS-LPME experiments. A magnetic stirrer model MR 3001 K (Heidolph Instruments, Schwabach, Germany) and 2.5 mm × 8 mm stirring bar were used to stir the solution. A laboratory-made two compartment recirculating glass cell in conjunction with circulating water baths (Frigomix B. Braun UM-S) were used for adjusting the temperature of sample solutions with an accuracy of ±0.1 °C.

GC analysis of VPA was carried out using a Philips model PU4410 gas chromatograph (Philips Scientific, England) fitted with a split/splitless injector and flame ionization detector. Helium was used as the carrier gas with a flow rate of 1.8 mL/min. The components were separated using a BP-10 14% Cyanopropylphenyldimethylsiloxane fused-silica capillary column (25 m × 0.22 mm I.D., 2.5 μm film) from SGE Scientific (Sydney, Australia). The injection port and detector were operated at 250 and 280 °C, respectively. All injections were made in split mode (split ratio, 20:1). The flow rates of the detector gases were 300 mL min^{-1} of air and 30 mL min^{-1} of hydrogen. The oven temperature was programmed from 80 °C (for 2 min) to 140 °C at a rate of 15 °C min^{-1} and held at 140 °C for 1 min; then, the temperature was raised rapidly with a rate of 40 °C min^{-1} to a final temperature of 280 °C that was held for 8 min.

2.3. Sample preparation

The blood samples were taken from human (without epilepsy) and transferred in serum separator tubes. The blood samples were centrifuged at 2500 rpm for 20 min to obtain serum and then serum was stored at −20 °C prior to analysis. Working serum samples were prepared by combining 200 μL of blank serum and 800 μL of acidified water and spiked with an appropriate amount of the sodium valproate in a 2 mL glass vial with a PTFE-silicon septum. After addition of sodium chloride and a magnetic stirring bar, the vial was hermetically closed by a teflon/silicon septum and tightly sealed with an aluminum cap to prevent sample loss due to evaporation. The vial was thermostated using a heated circulating water bath at 45 °C, for 10 min before HS-LPME, to reach equilibrium.

2.4. HS-LPME procedure

Before every extraction, the syringe was rinsed 10–15 times with the organic solvent to avoid the formation of air bubbles and the carryover of compounds between extractions. Then, a 2 μL volume of organic solvent containing 10 μg mL^{-1} of butanoic acid (IS) was drawn into the microsyringe. The needle tip was wiped with a tissue, and the syringe was suspended over the vial using a metal stand (clamp). Then, the syringe needle was inserted through the silicone septum, with the end of the needle

about 1 cm above the surface of the solution. The plunger was totally depressed in order to generate a 2 μL solvent drop on the needle tip. Meanwhile, the solution was constantly stirred to promote the diffusion of VPA from the serum into the solvent drop. After a certain studied period of extraction, the drop was retracted into the needle, and the plunger was drawn back to 2 μL. The syringe was then taken out of the vial and the solvent injected into the GC.

3. Results and discussion

In order to obtain the optimized extraction conditions and best extraction efficiency, we used the ratio of peak area of analyte and that of internal standard as the GC response to evaluate the extraction efficiency under different conditions. Three replicate extractions and determinations were performed for each level. To optimize the method, all the extractions were initially carried out in aqueous solutions, then applied to on human serum, both blank and spiked with valproate, and finally to tablet and syrup samples.

3.1. Solvent selection

It is essential to select a suitable organic solvent for HS-LPME. The following factors should be considered. Firstly, the solvent should have a high boiling point and low vapor pressure so that it can stand under higher extraction temperature without apparent loss. Secondly, according to the theory of "like attracts like", the extraction organic solvent should have a high affinity for the analytes in sample. Finally, the solvent should be compatible with GC and have a satisfactory chromatographic resolution from the analyte. Four solvents, 1-decanole, benzyl alcohol, 1-octanol and *n*-dodecane were tested to select the best one for the extraction of VPA in water samples with this technique. Butanoic acid was used as internal standard to correct for variation in injection volumes. Peak area ratio of analytes to IS was used as the analytical signal. Preliminary experiments showed that *n*-dodecane gives the best extraction efficiency. Fig. 1 shows a chromatogram of the sample solution of VPA after HS-LPME using *n*-dodecane as extraction solvent.

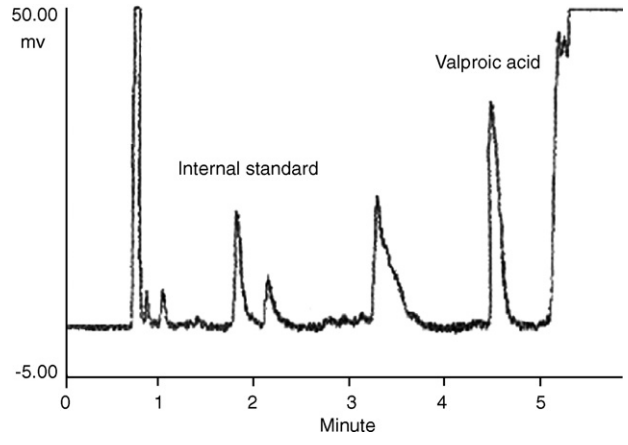

Fig. 1. Chromatogram of the aqueous solution spiked with 7 μg mL^{-1} of VPA after HS-LPME using *n*-dodecane as extraction solvent.

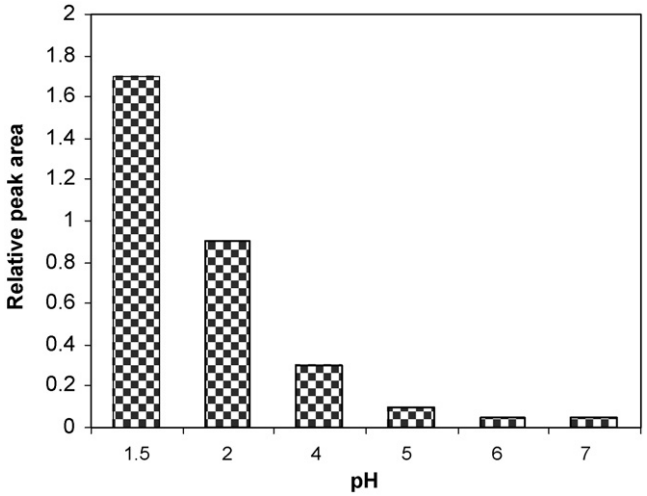

Fig. 2. Influence of pH on the HS-LPME efficiency. Experimental conditions; concentration of spiked VPA; 4 μg mL^{-1}, microdrop volume; 2 μL, concentration of NaCl, 4 M; sample volume, 1 mL; solution temperature, 20 °C; extraction time, 10 min; stirring speed, 350 rpm.

3.2. pH effect

Valproic acid with a pK_a of 5 exists in neutral (un-ionized) form at low pH, and is completely ionized at pH higher than 4; thus, it has more tendency to get dissolved in water and efficiency of extraction would be reduced. Different amounts of HCl and NaOH were added to sample solution to investigate the effect of pH on the extraction. As Fig. 2 shows, the best results were obtained at pH 1.5; therefore, pH was adjusted to 1.5 in further analysis. At this pH, VPA will be completely protonated (Fig. 3).

3.3. Extraction time

The time for reaching equilibrium determines the maximum amount of analyte that can be extracted by the solvent drop, which controls the sensitivity of the method. Therefore, extraction time is a major parameter affecting the extraction efficiency.

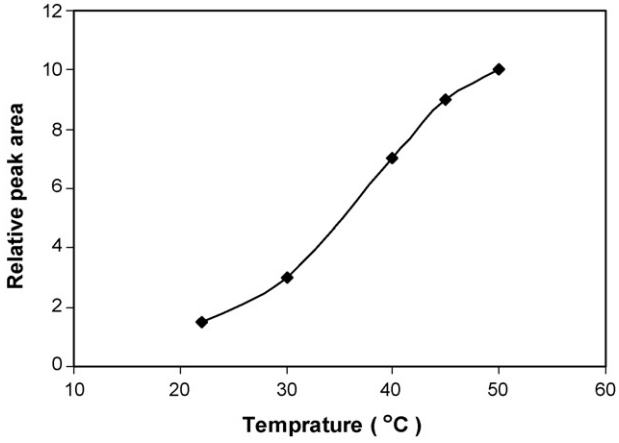

Fig. 3. Effect of sample solution temperature on the HS-LPME efficiency. Experimental conditions; concentration of spiked VPA, 2 μg mL^{-1}; concentration of NaCl, 4 M; sample volume, 1 ml; pH = 1.5; microdrop volume, 2 μL; extraction time, 20 min; stirring speed, 350 rpm.

The optimum extraction time was determined by varying the exposure time of the microdrop in the headspace of a sample solution from 1–30 min. The amount of analyte transferred into the microdrop reaches its maximum value when equilibrium between the extracting microdrop, headspace and the sample solution is established. Although equilibrium in the extraction of VPA was not reached even after 30 min; longer times were not studied because of loss of extraction solvent volume at longer times. Therefore, an extraction time of 20 min was chosen for present work.

3.4. Extraction temperature

Temperature is a major parameter affecting the extraction efficiency. Fig. 3 presents the effect of solution temperature on the extraction ability of VPA, obtained by plotting the peak area as a function of temperature. As can be seen, the extraction ability increases with increasing temperature from 20 to 45 °C. This can be explained by increasing the Henry's constant and vapor pressure of analyte with temperature. Therefore, the concentration of analyte in the headspace is increased. In order to decrease the volume of microdrop (and the consequent problems and damages), it is recommended not to work at high temperatures. Thus, the sample temperature was held at 45 °C for further analysis.

3.5. Ionic strength

Addition of a certain amount of a salt can decrease the solubility of analytes in aqueous samples and enhance the distribution constant of compounds between the aqueous phase and headspace. The salting-out effect was assessed by the addition of NaCl to the extracting solution in a range of 0–6 M. Under the same experimentally condition, the extraction efficiency increased by increasing concentration of NaCl, reaches a maximum in the presence of 5 M NaCl and remains constant, thereafter. The result indicates that addition of salt has dramatic effect on extraction of VPA; thus, we carried out further analysis at 5 M NaCl. This can be explained by the engagement of more water molecules in the hydration spheres around the ionic salt, reduces the concentration of water available to dissolved analyte molecules. Consequently, solubility of the analytes in the aqueous sample are reduced and distribution constant of compounds between aqueous phase and headspace is enhanced [41].

3.6. Microdrop volume

From a theoretical point of view, an increase in microdrop volume will result in increased extraction efficiency; however, a large injection volume will result in bond broadening in the chromatographic analysis, therefore it is important to optimize the solvent volume. It was found a change in microdrop volume from 1 to 3 μL dose not have any effect on HSME efficiency compared with other parameters. The microdrop volume of 2 μL was used for further studies.

3.7. Quantitative analysis

The spiked water and serum blank samples were employed to investigate the linearity, repeatability, enrichment factor and limit of detection under the optimal experimentally conditions. Table 1 shows all the quantitative results of this method. In order to assess the repeatability, relative peak areas for seven replicate analyses were studied by extracting spiked water and serum samples (5 μg mL^{-1}) and expressed as relative standard deviation (RSD%). The RSD for VPA was lower than 13.2% (inter-day analyses). The linearity of the method was tested over the ranges 0.2–10 μg mL^{-1} in aqueous samples and 2–20 mg L^{-1} in serum sample of VPA. For each level, three replicate extractions were carried out. The HS-LPME procedure showed a satisfactory linear behavior in the tested range, with correlation coefficients 0.99 and 0.98, respectively. In order to determine the enrichment factors, three replicate extractions were performed at optimal conditions from aqueous solutions containing 0.5 μg mL^{-1} of analyte. The enrichment factor, calculated as the ratio of the final concentration of the analyte in the organic solvent and its concentration in the original solution, was found to be 27 for VPA.

The limits of detection for VPA in serum blank when using the optimized conditions were determined 0.8 μg mL^{-1} at a signal-to-noise ratio (S/N) of 3. The optimized HS-LPME procedure was compared with previously published SPME method [29]. Comparing the results for extraction and determination of VPA shows that the proposed method has comparable detection limit and dynamic linear range with SPME (Table 1).

3.8. Applications

The applicability of this extraction method to the real samples was investigated for human serum sample and pharmaceutical preparations. HS-LPME of non-spiked serum sample provided a chromatogram with only a few peaks from the matrix, supporting an effective sample clean-up (Fig. 4a). Thus, the serum samples were spiked with 5 μg mL^{-1} of VPA, and seven replicate analyses were performed for each serum sample using HS-LPME followed by GC-FID at optimal conditions. A sample of spiked chromatogram is shown in Fig. 4b. With regard to calibration

Table 1
Precision, enrichment factors (EF) for the analysis of VPA with HS-LPME–GC–FID, and comparison of dynamic linear range (DLR) and limits of detection (LOD) in serum sample with literature data of SPME [29]

Drug	RSD (%) (n = 7)	EF (fold)	DLR (mg L^{-1})		LOD (μg mL^{-1})	
			HS-LPME	SPME	HS-LPME	SPME
Valproic acid	13.2	27	2–20	2–20	0.8	1

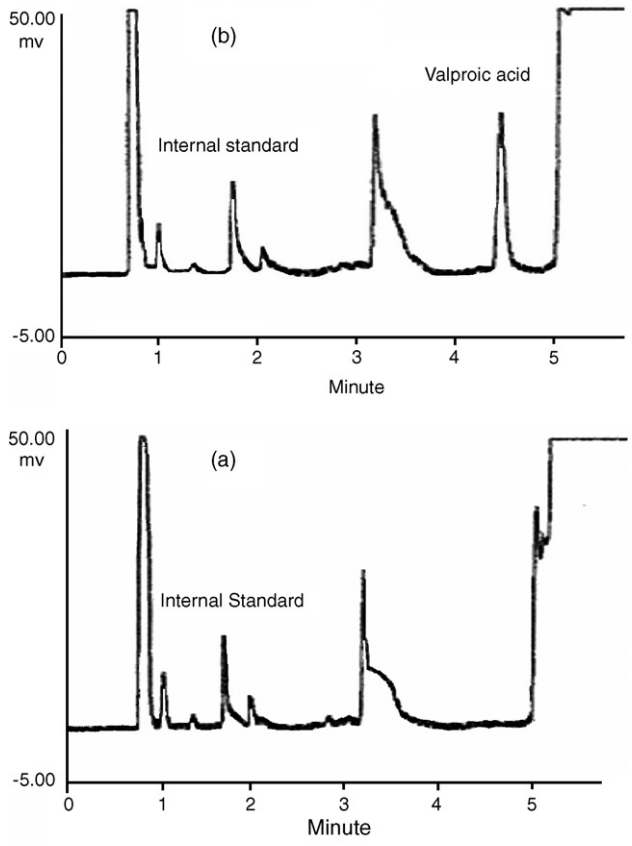

Fig. 4. Chromatogram of the (a) non-spiked and (b) spiked (with 12 μg mL^{-1} VPA) human serum sample by HS-LPME–GC–FID.

Table 2
HS-LPME and GC determination of valproate in human serum sample and pharmaceutical preparations

Sample	Concentration (μg mL^{-1})	Valproate (μg mL^{-1})		RSD (%)
		Added	Found	
Human serum	0	0	0.0 ± 0.1	
		5	4.6 ± 0.1	13.2 (n = 7)
Syrup	2	0	1.8 ± 0.2	4.4 (n = 3)
Tablet	2	0	1.7 ± 0.2	6.8 (n = 4)

graph, the mean of founded amount are shown in Table 2. The results obtained by the proposed method and amounts added are in satisfactory agreement. Finally, the proposed method was applied for analysis of antiepileptic tablet and syrup samples. The tablet sample was dissolved in water and both samples were diluted for preparing a solution containing 2 μg mL^{-1} of sodium valproate (SV). The concentrations of SV were found as 1.7 μg mL^{-1} and 1.8 μg mL^{-1} in tablet and syrup sample solutions, respectively, using the standard addition method. The analytical results are summarized in Table 2.

4. Conclusion

The potential of HS-LPME as a sample preparation technique prior to GC for VPA drug analysis in biological matrices has been demonstrated. The simplicity, low solvent consumption, ease of operation, good sensitivity and precision, high enrichment factors, and short analysis time are clear advantages of the proposed method for the determination of VPA in serum samples. Furthermore, there was no need for evaporation of solvent and derivatization of VPA prior to injection into the GC. Most importantly, sample clean-up that is an essential step to reduce of interferences from matrices was omitted in our proposed method. The capability of HS-LPME to extract VPA has been compared with the results obtained using the HS-SPME method, which showed comparable results for the studied compound. The experimental results demonstrated the potential of HS-LPME followed by GC-FID for the extraction and determination of valproate drug in pharmaceutical preparations; the method was shown to be highly reliable.

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References

[1] R.L. Macdonald, M. Kevien-Kelly, Epilepsia 36 (1995) 52.
[2] S.A. Konig, J. Knolle, S. Friedewald, W. Koelfen, E. Longin, T. Lenz, D. Hannak, Epilepsia 44 (2003) 708.
[3] R. Alric, M. Cociglio, J.P. Blayac, R. Puech, J. Chromatogr. 224 (1981) 289.
[4] J.P. Moody, S.M. Allan, Clin. Chim. Acta 127 (1983) 263.
[5] M. Nakamura, K. Kondo, R. Nishioka, S. Kawai, J. Chromatogr. 310 (1984) 450.
[6] F.A.L. van der Horst, G.G. Eikelboom, J.J.M. Holthuis, J. Chromatogr. 456 (1988) 191.
[7] E. Bousquest, S. Tirendi, N.A. Santagati, Salvo Marta, P. Moncada Paterno Castello, Pharmazie 46 (1991) 257.
[8] K. Kushida, T. Ishizaki, J. Chromatogr. 338 (1985) 131.
[9] T. Mino, M. Nakajima, H. Wakabayashi, S. Yamato, K. Shiada, Anal. Sci. 17 (2001) 999.
[10] V. Ioffe, T. Kalendarev, I. Rubinstein, G. Zupkovitz, J. Pharm. Biomed. Anal. 30 (2002) 391.
[11] V. Pucci, R. Mandrioli, M.A. Raggi, Electrophoresis 24 (2003) 2076.
[12] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, M. Santosh, J. Chidambara, B.R. Kumar, Rapid Commun. Mass Spectro. 19 (2005) 1970.
[13] M.R. Anari, R.W. Burton, S. Gopaul, F.S. Abbott, J. Chromatogr. B 742 (2000) 217.
[14] Z. Hussein, K. Patterson, J. Lamm, J. Cavanaugh, G. Granneman, Biopharm. Drug Dispos. 14 (1993) 389.
[15] H. Muro, T. Tatsuhara, T. Sugimoto, M. Woo, N. Nishida, K. Murakami, Y. Yamaguchi, J. Chromatogr. B 663 (1995) 83.
[16] E. Fisher, W. Wittfoht, H. Nau, Biomed. Chromatogr. 6 (1992) 24.
[17] T. Tatsuhara, H. Muro, Y. Matsuda, Y. Imai, J. Chromatogr. 399 (1987) 183.
[18] M. Liu, K. Brouwer, G. Pollack, Drug Metab. Dispos. 20 (1992) 810.
[19] C. Lucarelli, P. Villa, E. Lombaradi, P. Prandini, A. Brega, Chromatographia 33 (1992) 37.
[20] S. Hara, M. Kamura, K. Inoue, M. Fukuzawa, N. Ono, T. Kuroda, Biol. Pharm. Bull. 22 (1999) 975.
[21] S. Rompotis, M. Parissi-Poulou, E. Gikas, M. Kazanis, A. Vavayannis, I. Panderi, J. Liq. Chromatogr. Related Technol. 25 (2002) 2833.
[22] T. Katsu, K. Ido, A. Moriya, Y. Nakae, I. Sakata, Electroanalysis 12 (2000) 1282.

[23] S. Sabah, M. Aghamohammadi, N. Alizadeh, Sens. Actuators B 114 (2006) 489.
[24] E. Gaetani, C.F. Laureri, M. Vitto, J. Pharm. Biomed. Anal. 10 (1992) 193.
[25] D. Yu, J.D. Gordon, J. Zheng, S.K. Panesar, K.W. Riggs, D.W. Rurak, F.S. Abbott, J. Chromatogr. B 666 (1995) 269.
[26] J. Darius, F.B. Meyer, J. Chromatogr. B 656 (1994) 343.
[27] R. Libert, J. Draye, F. Vanhoof, A. Schanck, J. Soumillion, E. Dehoffmann, Biol. Mass Spectrom. 20 (1991) 75.
[28] A. Carlin, J. Simmons, J. Chromatogr. B 694 (1997) 115.
[29] M. Krogh, K. Johansen, F. Tonnesen, K.E. Rasmussen, J. Chromatogr. B 673 (1995) 299.
[30] R.P. Belardi, J. Pawliszyn, J. Water Pollut. Res. J. Can. 24 (1989) 179.
[31] J. Pawliszyn, Solid-Phase Microextraction: Theory and Practice, Wiley-VCH Inc., New York, 1997.
[32] R. Baciocchi, M. Attina, J. Chromatogr. A 911 (2001) 135.
[33] A. Mohammadi, Y. Yamini, N. Alizadeh, J. Chromatogr. A 1063 (2005) 1.
[34] J. Namiesnik, B. Zygmunt, A. Jastrzebska, J. Chromatogr. A 885 (2000) 405.
[35] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 938 (2001) 113.
[36] J. Cacho, V. Ferreira, P. Fernandez, Anal. Chim. Acta 264 (1992) 311.
[37] M. Guidotti, J. High Resolut. Chromatogr. 19 (1996) 469.
[38] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[39] A. Mohammadi, N. Alizadeh, J. Chromatogr. A 1107 (2006) 19.
[40] S. Shariati, Y. Yamini, N. Bahramifar, Anal. Chim. Acta 489 (2003) 21.
[41] A.A. Boyd-Boland, J. Pawliszyn, J. Chromatogr. A 704 (1995) 163.