Journal of Chromatography B, 879 (2011) 1870–1874

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Three phases hollow fiber LPME combined with HPLC-UV for extraction, preconcentration and determination of valerenic acid in *Valeriana officinalis*

Mohamad Mirzaei*, Hossein Dinpanah

Department of Chemistry, Shahid Bahonar University of Kerman, 76175-133 Kerman, Iran

ARTICLE INFO

Article history:
Received 19 November 2010
Accepted 2 May 2011
Available online 7 May 2011

Keywords:
Hollow-fiber membrane
Liquid phase microextraction
Valerenic acid
HPLC/UV
Valerian root

ABSTRACT

In the present work, the applicability of hollow fiber-based liquid phase microextraction (HF-LPME) was evaluated for the extraction and preconcentration of valerenic acid prior to its determination by reversed-phase HPLC/UV. The target drug was extracted from 5.0 mL of aqueous solution with pH 3.5 into an organic extracting solvent (dihexyl ether) impregnated in the pores of a hollow fiber and finally back extracted into 10 μL of aqueous solution with pH 9.5 located inside the lumen of the hollow fiber. In order to obtain high extraction efficiency, the parameters affecting the HF-LPME, including pH of the donor and acceptor phases, type of organic phase, ionic strength, the volume ratio of donor to acceptor phase, stirring rate and extraction time were studied and optimized. Under the optimized conditions, enrichment factor up to 446 was achieved and the relative standard deviation (RSD) of the method was 4.36% ($n = 9$). The linear range was 7.5–850 μg L^{-1} with correlation coefficient ($r^2 = 0.999$), detection limits was 2.5 μg L^{-1} and the LOQ was 7.5 μg L^{-1}. The proposed method was evaluated by extraction and determination of valerenic acid in some Iranian wild species of Valerianaceae.

1. Introduction

Valerianaceae contains about 40 genera and 400 species of almost cosmopolitan distribution, mostly at high elevations. Six species of *Valeriana officinalis* and one species of Centranthus DC grows in Iran [1]. The roots and rhizomes of valerian contain several compounds with demonstrable pharmacological activity. These include the essential oil and their sesquiterpenes (valerenic acid and its derivatives) and epoxy iridoid esters (valepotriates). In the last two decades, the essential oil and valerenic acid derivatives have become more important, not only from a pharmacological point of view, but also for the quality control and standardization of valerian phytomedicine [2]. Several clinical studies have presented some biological activities of valerian root extracts and introduced the plant as anti-HIV, sleep aid, antidepressants, anticoronaryspastic, antihypertensive and antibronchospastic [3–6]. Recently analytical technique based on reversed-phase high performance liquid chromatography (RP-HPLC) has been used for determining valerenic acid [8]. Because of low concentration of VA in some samples, pretreatment and pre-concentration step is generally required for determination of trace VA in the drugs. Liquid–liquid extraction (LLE), solid–phase extraction (SPE), supercritical fluid extraction (SFE) and thin layer chromatography (TLC) are widely used as sample preparation techniques for biological analysis [9,10]. The main drawbacks of LLE include being labour-intensive and the use of large volumes of organic solvent, which often leads to the formation of emulsions. SPE is less time-consuming than LLE but requires column conditioning and elution with organic solvents. SFE is faster, more efficient, has a wider range of selectivity, and produces less toxic waste. Such analyses are often expensive, in terms of time and uncertainty in the result or in the level of operator intervention. TLC possesses adequate resolutions for identifying many drugs, but it suffers from inability to quantify these drugs accurately and time consuming technique with inadequate sensitivity.

Liquid phase microextraction (LPME) technique has been used in recent years as an eco-friendly and very efficient alternative in sample preparation for chromatography and electrophoresis [11,12]. To improve the stability and reliability of liquid–phase microextraction (LPME), Pedersen-Bjergaard and Rasmussen introduced hollow-fiber-based liquid–phase microextraction (HF-LPME) in 1999 [13]. In this microextraction technique, a water-immiscible organic solvent was immobilized in the pores of a porous hollow fiber by capillary forces, and formed a supported liquid membrane (SLM). The lumen of the hollow fiber was filled with a microliter amount of an acceptor phase (AP). The analytes were extracted from the donor phase (DP) into the organic solvent, and then captured into the AP. After extraction, the AP was directly injected into HPLC, GC, capillary electrophoresis (CE), or MS systems for final chemical analysis. Compared with polymeric membranes, the liquid membrane provides high mass-transfer rates and selectivity

* Corresponding author. Tel.: +98 341 3222033; fax: +98 341 3222033.
E-mail address: m_mirzaei@mail.uk.ac.ir (M. Mirzaei).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.05.005

[14,15]. In recent years, HF-LPME by both two-phase [16,17] and three-phase mode [18–20] have been applied for various sample preparation procedures, because of the major advantages such as high enrichment, clean-up and low solvent consumption.

In this work, HF-LPME in combination with HPLC/UV was applied for extraction and preconcentration of VA in biological samples. The enrichment factor (EF) was studied as a function of the nature of the immobilized organic phase, compositions of donor phase (DP) and acceptor phase (AP), extraction time and stirring rate. These parameters were optimized and the system was finally applied to extract of valerenic acid in some Iranian wild species of Valerianaceae.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical-reagents. Valerenic acid ($C_{15}H_{22}O_2$) was purchased from Sigma Chemical Company (USA). All solvents (dihexyl ether, *n*-octanol, cyclopentanol, cyclohexanol, dodecyletheron and acetone) and also sodium hydroxide and hydrogen chloride were obtained from Merck Chemical Company (Darmstadt, Germany). Phosphate and acetate buffers were prepared from phosphoric acid and glacial acetic acid and their appropriate salts, respectively (Merck Chemical Co.)

2.2. Apparatus

Chromatographic analyses were performed by Agilent (Wilmington, DE, USA) 1100 series HPLC system equipped with Agilent G1312A Bin pump and an Agilent G1314 VW detector. An Eclipse XDB-C_{18} column (250 mm × 4.6 mm, particle size 5 μm) was used as the analytical column. All the HF-LPME experiments were performed using Accurel Q3/2 polypropylene hollow fiber membrane (600 μm I.D., 200 μm wall thickness, 0.2 μm pore size) from Membrana (Wuppertal, Germany). The fiber was cut into small segments with the length of 6 cm. One end of each resulting hollow fiber was heat-sealed using a soldering iron. A 25 μL syringe model 702 NR from Hamilton (Bonaduz, Switzerland) was employed to introduce the AP solution into the lumen of the hollow fiber, to suspend the hollow fiber and also to inject the extracted analyte at the end of the extraction into the HPLC loop. The outer diameter of the needle was 800 μm and thus had to be inserted into the hollow fiber by applying some force.

2.3. Three-phase LPME procedure

Extraction and injection processes were performed in the following steps: (1) 5 mL of the aqueous sample solution was transferred into a 10 mL glass vial containing a 10 mm × 4 mm magnetic stirring bar; (2) the vial was placed on a magnetic stirrer model ZMS 74 from ZAG Chimi Chemical Company (Tehran, Iran); (3) a carefully measured portion of 10 μL of the acceptor phase was injected into the hollow fiber; (4) the fiber was submerged in the organic solution for 10 s and then into the reagent water for 5 s for washing the extra organic solution from the surface of the fiber; (5) the fiber was bent into a U-shape and together with a small part of the supporting syringe needle was submerged in the sample solution; (6) the vial was covered with Para Film and stirred for a prescribed time period; (7) at the end of the extraction time, the hollow fiber was removed from the sample solution, and the acceptoring phase was withdrawn into the syringe; (8) finally, 10 μL of the acceptor phase was injected into the HPLC. In initial experiments, the volumes of DP and AP solutions were 5 mL and 10 μL, respectively. Also to obtain suitable signals in the optimization experiment, relatively high concentration of aqueous solution of VA (40 μg L^{-1}) was used. The DP was stirred at a rate of 1000 rpm for a period of 30 min. Diagrammatic sketch of extraction platform was shown in Fig. 1.

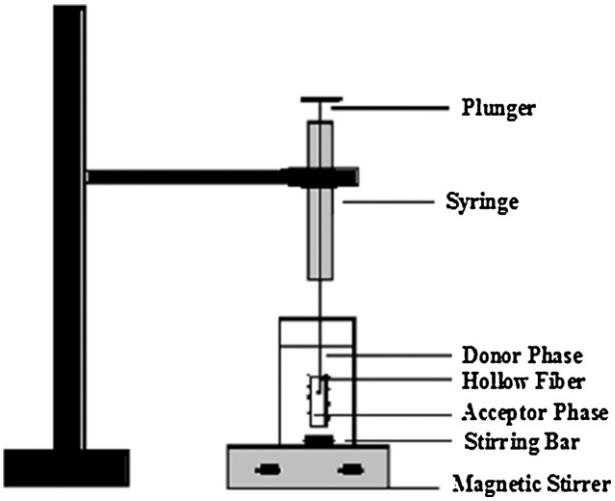

Fig. 1. Diagrammatic sketch of extraction platform.

2.4. Real sample preparation

0.2 g of freshly powdered valerian in a test tube with 5 mL dichloromethane for 1 min. Allow the mixture to stand for 5 min and then filter. Wash the filter with 2 mL of methanol, and evaporate the combined filtrate and washing to dryness on a water bath. Dissolve the residue in 0.2 mL of methanol and transfer the solution into a small sample vial.

3. Results and discussion

3.1. Selection of the hollow fiber and organic solvent

The composition of fiber should be inert and compatible with the extraction solvent. Other characteristics of the fiber such as its length, diameter, and pore size should also be considered. The results of Halvorsen experiments indicated a faster equilibrium time for an extraction using the smaller I.D. fiber [21]. Usually, polypropylene fiber was used in LPME procedures, because it is highly compatible with a broad range of organic solvents. In addition, with a pore size of approximately 0.2 μm, polypropylene strongly immobilizes organic solvents [22]. The mechanical stability of the hollow fiber was excellent. Therefore polypropylene hollow fiber was used in this work.

Choosing the most suitable organic solvent is very important for achieving a good selectivity of the target compounds. In addition choose of solvent should be based on the comparison of selectivity, extraction efficiency, and the level of toxicity, an also the polarity of the organic phase should be similar to that of the polypropylene fiber so that it can be easily immobilized within the pores of the fiber. This function greatly affects the performance of hollow fiber LPME since extraction occurs on the surface of the immobilized solvent [23,24]. In this work, organic solvents such as dihexyl ether, *n*-octanol, cyclopentanol, cyclohexanol, dodecylether, have been examined and the results (Fig. 2) showed dihexyl ether extraction recovery is higher than the others.

3.2. pH of donor and acceptor phase

The extraction involves pH adjustment of the sample solution to a pH where the analyses are uncharged, because uncharged

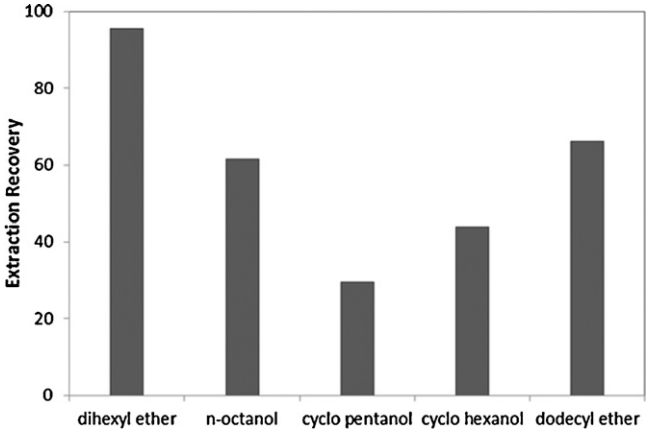

Fig. 2. Effect of organic solvent on the extraction recovery of 40 μg L^{-1} of VA. Extraction condition: sample solution (DP): 5 mL, stirring rate: 1000 rpm, extraction time: 30 min, donor phase pH 2.5, acceptor phase pH 9.5, volume of AP phase = 10.0 μL.

molecules have a good tendency for going to organic membrane. The acceptor solution has a pH where the analytes are charged preventing them from back diffusion into the organic solvent. For practical application, pH should be different from the pK_a values of the analytes by at the least 3 units [25–27]. The effect of pH upon valerenic acid extractability with HF-LPME was also investigated at the range of 1.5–4. Best peak area was observed at pH 2.5 (Fig. 3a). The effect of the acceptor phase pH on extraction recovery was studied at the range of 8.5–11. The results (Fig. 3b) showed the maximum extraction recovery observed at pH 9.5.

3.3. Effect of stirring speed

Sample agitation played an important role in enhancing extraction efficiency. Stirring the sample solution could enhance the mass transfer in the aqueous phase and consequently, reduce the extraction time to attain a thermodynamic equilibrium. Thus, the equilibrium between the aqueous and organic phases could be achieved more rapidly by stirring the aqueous sample [15]. Here, samples with a volume of 5.0 mL were continuously agitated at different stirring rates (500–1000 rpm) using the magnetic stirrer. It was observed (Fig. 4) that extraction recovery increased with the agitation rate of the sample up to 1000 rpm. Therefore, a stirring speed of 1000 rpm was chosen for further studies.

3.4. Effect of extraction time

HF-LPME is similar to LLE and SPME in that it is based on the analyte's partitioning between the aqueous sample and the organic solvent. Consequently, when using HF-LPME, it is not practical to match extraction time with extraction equilibrium in that the potential for solvent loss due to dissolution increases with time. Moreover, equilibrium exposure times are not necessary for analytical methods when extraction time, mixing rate, and sample volumes remain constant [28]. Extractions were conducted for 15–60 min at a stirring rate of 1000 rpm. Whereas for valerenic acid, extraction recovery increased until 30 min, and after that, the signal slightly decreased (Fig. 5). The decreased of peak area can be due to organic solvent which was extracted back into the sample along with the loss of organic solvent. Considering sensitivity and analysis time, an extraction time of 30 min was selected as the optimal condition.

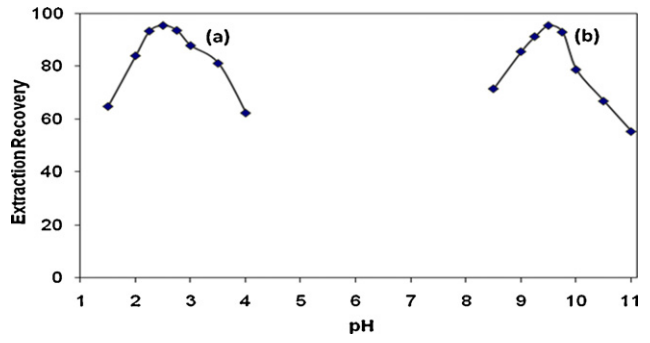

Fig. 3. Effect of donor phase pH on the extraction recovery (a), and effect of acceptor phase pH on extraction recovery (b), all other condition as in Fig. 1.

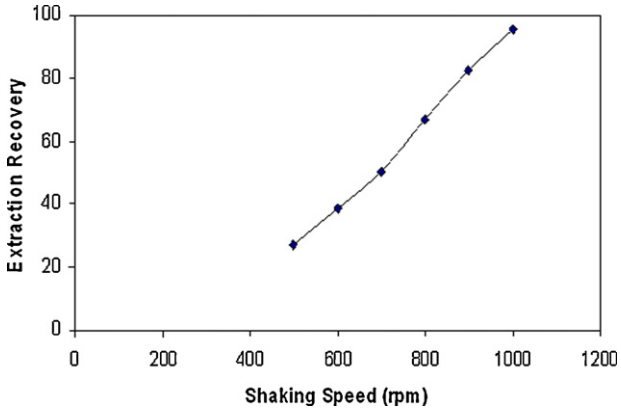

Fig. 4. Effect of shaking speed on the extraction recovery, all other condition as in Fig. 1.

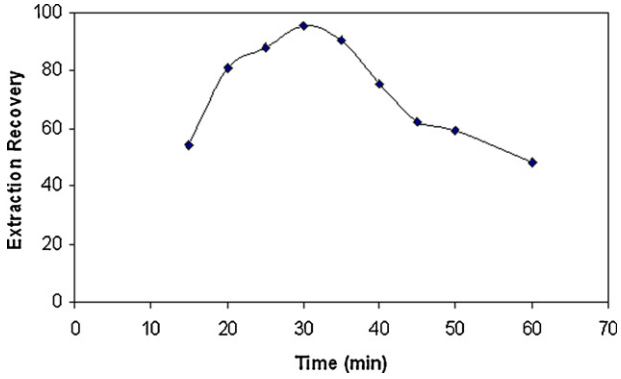

Fig. 5. Effect of extraction time on extraction recovery area, all other condition as in Fig. 1.

3.5. Effect of volume ratio of donor to acceptor phase

The influence of DP volume was further studied from 1 to 20 mL, with a constant AP volume of 10 μL and extraction time of 30 min. The peak area of VA increased rapidly from the volume of 1–5 mL

Table 1
Analytical characteristics of the proposed method.

Parameter	Analytical feature
Linear range (ng mL^{-1})	7.5–850
r^2	0.999
Limit of detection (ng mL^{-1})	2.5
Repeatability (R.S.D.%) (n = 9)	4.3
Enrichment factor (EF)	446
Sample volume (mL)	5.0

Conditions: 40 μg L^{-1} of VA: stirring rate: 1000 rpm, extraction time: 30 min, donor phase pH 2.5, acceptor phase pH 9.5, volume of AP phase 10.0 μL.

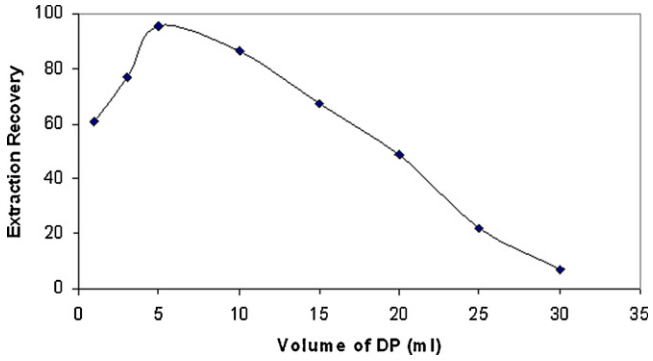

Fig. 6. Effect of DP volume on the extraction recovery, all other condition as in Fig. 1.

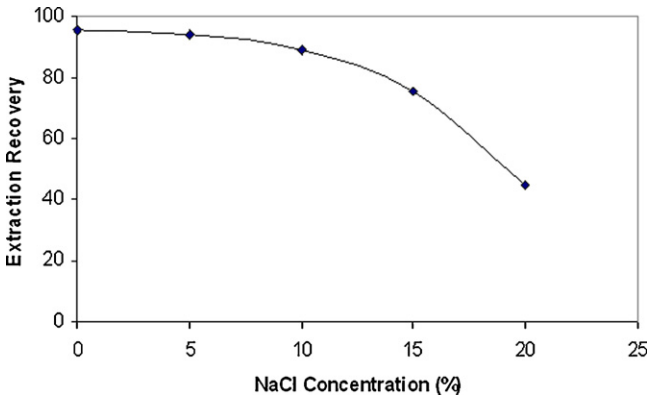

Fig. 7. Effect of salt on the extraction recovery, all other condition as in Fig. 1.

and then decreased (Fig. 6). The extraction recovery of target analyte was dependent on both the recovery and the donor–acceptor phase ratio. The results showed higher extraction recovery was obtained at about 5 mL of DP into a10 μL AP. Therefore, subsequent experiments were conducted with 5 mL DP and 10 μL AP.

3.6. *Effect of ionic strength*

The effect of salt addition on the extraction recovery of HF-LPME was evaluated by increasing NaCl concentration from 0% to

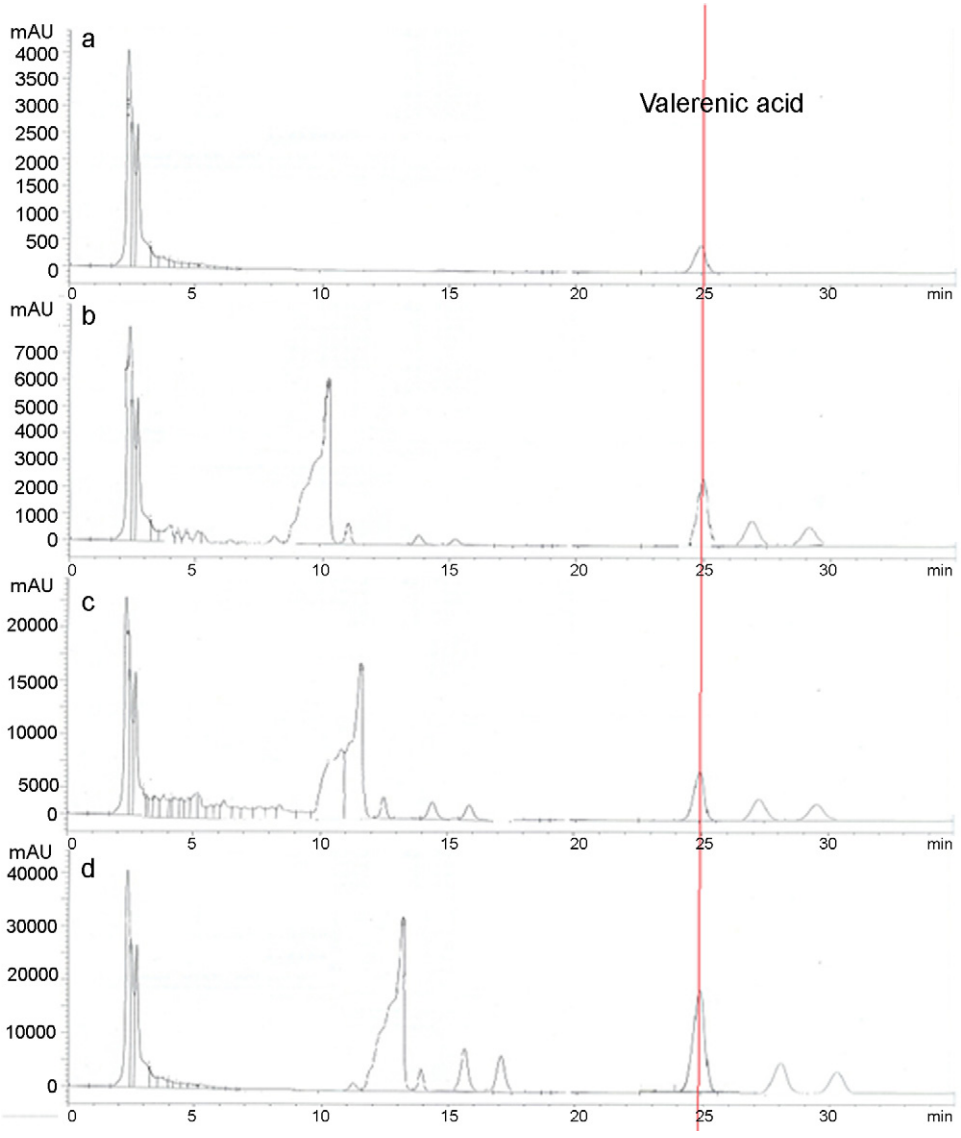

Fig. 8. Chromatograms of reference (standard solution 15 ppb) (a) and real samples, sample 1: (valerian, Kerman) (b), sample 2: (valerian, Tehran) (c), sample 3: (valerian, Mazandran) (d).

Table 2
Comparison of the proposed method with other developed methods to determine valerenic acid in aqueous solutions.

Ref.	Instrumentation	LOD ($\mu g\,L^{-1}$)	Dynamic range ($\mu g\,L^{-1}$)	RSD (%)
Present work	HF-LPME-HPLC/UV	2.5	7.5–850	4.3 ($n=9$)
[7]	SFE-HPLC/UV	1000	–	2.0 ($n=3$)
[8]	LLE-CE	5.8	–	3.0 ($n=3$)
[9]	LLE-SPE-LC/MS/MS	0.1	0.1–10	5.0 ($n=6$)
[1]	LLE-TLC/UV	2000	2000–51,000	–
[11]	LLE-HPTLC	500	500–2500	1.5 ($n=6$)

20% (w/v) in samples. For valerenic acid the extraction recovery remained nearly constant with the increase of NaCl concentration from 0% to 5% and decreased with increasing the concentration of NaCl (Fig. 7). These results could be explained from triple aspects. Firstly, the dissolution of NaCl in water might change the physical properties of the Nernst diffusion film and reduce the rate of diffusion of the target analytes into the extraction solvent. Secondly, the addition of salt could lead to an increase in the ionic strength of the solution and then decrease the solubility of the target analytes in the aqueous phase and enhance their partitioning into the organic phase. Thirdly, the addition of salt could also affect the phase ratio. The first and the third factors would lead to a decrease on the extraction efficiency, while the second one causes an increase. This result has also been seen by other authors [29]. To simplify the manipulation, sample direct analysis without the salt addition was employed in this work.

3.7. Figures of merit

Under the optimized conditions the calibration curve was linear in the range of 7.5–850 $\mu g\,L^{-1}$ for valerenic acid with the correlation coefficient ($r^2 = 0.999$), LOD was 2.5 $\mu g\,L^{-1}$ for analyte which was calculated by Knoll method ($C_{LOD} = K_{LOD} h_n (C_s/h_s)$) [30]. It seen that the RSD values are smaller than 4.36% for nine replicate runs. The enrichment factor was 446. Overall, the detection limits which were achieved by the proposed method are better or comparable to others published extraction techniques for valerenic acid compounds [9,10] (Table 1)

3.8. Comparison of the applied method with other reported methods

The present method was compared with the other methods in terms of validation and precision. As can be seen, the method is quite comparable to those mentioned in Table 2.

3.9. Application of HF-LPME for real samples

In order to assess the applicability of the extraction system to the analysis of the VA in real samples, three samples 1 (Valerian, Kerman, Iran), sample 2 (Valerian, Tehran, Iran) and sample 3 (Valerian, Mazandaran, Iran) were selected and valerenic acid was extracted and analyzed using the proposed method under optimum conditions (Fig. 8). To investigate the accuracy of this method, definite value of standard was added to sample which was mentioned above. Recovery was in the range of 87–89% and showed the results of three replicate analyses of each real sample obtained by the proposed method are in good accord with the spiking amounts (Table 3).

Table 3
Concentration ($\mu g\,L^{-1}$) of valerenic acid detected in real samples.

Compound	Sample 1	Sample 2	Sample 3
Valerenic acid	105.3 ± 5.21	450.25 ± 4.43	978.3 ± 4.22
VA added	250	250	250
Found	321.25 ± 5.21	671.5 ± 5.02	1201.72 ± 5.12
Recovery	87%	88.5%	89.4%

Condition as in Table 1.

4. Conclusion

The present study exhibited an excellent performance of the HF-LPME technique for the extraction of antidepressant drugs from biological samples. Up to 446-fold enrichment factor and effective sample clean-up were obtained. Due to the simplicity and low cost of the extraction device, the hollow fiber can be discarded after each extraction to avoid carry over and cross contamination. This serves to maintain high reproducibility and repeatability. The whole operation is very convenient to handle because the receiving phase is contained and protected by the hollow fiber. Accordingly, it is concluded that HF-LPME is an effective method to enrich antidepressant drugs from the biological samples prior to HPLC analysis. The results indicated that hollow fiber microextraction method has an excellent clean-up high-preconcentration factor and can be served as a simple and sensitive method for monitoring of antidepressant drug in the biological samples.

References

[1] H. Ebrahimzadeh, T.S. Radjabian, T. Ekhteraei, Z.B. Sadr, V. Niknam, M. Zarrei, Asian J. Plant Sci. 7 (2008) 195.
[2] C. Bounthanh, L. Bergmann, J. Beck, M.H. Berruier, R. Anton, Planta Med. 41 (1981) 21.
[3] H. Wagner, K. Jurcic, Planta Med. 37 (1979) 84.
[4] R. Bos, H.J. Woerdenbag, H. Hendriks, H.F. Smit, H.V. Wikstrom, J.C. Scheffer, Flavour Frag. J. 12 (1997) 123.
[5] P.J. Houghton, J. Pharm. Pharmacol. 51 (1999) 505.
[6] C. Hobbs, Herbalgram 21 (1989) 19.
[7] C. Bicchi, A. Binello, P. Rubiolo, Phytochem. Anal. 7 (2000) 179.
[8] G.D. Anderson, G.W. Elmer, E.D. Kantor, I.E. Templeton, M.V. Vitiello, Phytother. Res. 19 (2005) 801.
[9] N. Singh, A.P. Gupta, B. Singh, V.K. Kaul, Chromatographia 3 (2006) 63.
[10] R. Bos, H.J. Woerdenbag, H. Hendriks, J.H. Zwaving, Phytochem. Anal. 7 (1996) 143.
[11] D.A. Lambropoulou, T.A. Albanis, J. Biochem. Biophys. Methods 70 (2007) 195.
[12] K. Demeestere, J. Dewulf, B. De Witte, H. Van Langenhove, J. Chromatogr. A 1153 (2007) 130.
[13] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[14] S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1184 (2008) 132.
[15] K.E. Rasmussen, S. Pedersen-Bjergaard, Trends Anal. Chem. 23 (2004) 1.
[16] S. King, J.S. Meyer, A.R.J. Andrews, J. Chromatogr. A 982 (2002) 201.
[17] L. Zhao, H.K. Lee, Anal. Chem. 74 (2002) 2486.
[18] L. Zhu, K.H. Ee, L. Zhao, H.K. Lee, J. Chromatogr. A 963 (2002) 335.
[19] A. Sarafraz Yazdi, Z. Es'haghi, J. Chromatogr. A 1094 (2005) 1.
[20] H.G. Ugland, M. Krogh, L. Reubsaet, J. Chromatogr. B 798 (2003) 127.
[21] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmusen, J. Chromatogr. B 760 (2001) 219.
[22] K.E. Rasmusen, S. Pedersen-Bjergaard, Trends Anal. Chem. 23 (2004) 1.
[23] E. Psillakis, D. Mantzavinos, N. Kalograkis, Anal. Chim. Acta 501 (2004) 3.
[24] S. Palmarsdottir, E. Throdarson, L.E. Edholm, J.A. Jonsson, Anal. Chem. 69 (1997) 1732.
[25] R.P. Frankewich, W. Hinze, Anal. Chem. 66 (1994) 944.
[26] A. Eiguren Fern ndez, Z. Sosa Fereera, J.J. Santana Rodriguez, Anal. Chim. Acta 358 (1998) 145.
[27] J. Xiong, B. Hu, J. Chromatogr. A 1193 (2008) 7.
[28] D.A. Lambropoulou, T.A. Albanis, J. Chromatogr. A 1072 (2005) 55.
[29] G. Shen, H.K. Lee, Anal. Chem. 74 (2002) 648.
[30] P.A. Martos, J. Pawliszyn, Anal. Chem. 69 (1997) 206.