Title: Multivariate optimization of solvent bar microextraction combined with HPLC-UV for determination of Trace amounts of vincristine in biological fluids

Authors: Mahsa Kiani, Mahnaz Qomi, Farshad Hashemian, Mehdi Rajabi

PII: S1570-0232(17)30794-8
DOI: https://doi.org/10.1016/j.jchromb.2017.10.054
Reference: CHROMB 20884

To appear in: *Journal of Chromatography B*

Received date: 28-4-2017
Revised date: 25-9-2017
Accepted date: 28-10-2017

Please cite this article as: Mahsa Kiani, Mahnaz Qomi, Farshad Hashemian, Mehdi Rajabi, Multivariate optimization of solvent bar microextraction combined with HPLC-UV for determination of Trace amounts of vincristine in biological fluids, Journal of Chromatography B https://doi.org/10.1016/j.jchromb.2017.10.054

Multivariate optimization of solvent bar microextraction combined with HPLC-UV for determination of Trace amounts of vincristine in biological fluids

Mahsa Kiani [1], Mahnaz Qomi[2*], Farshad Hashemian[3], Mehdi Rajabi [4]

[1,2]Active pharmaceutical ingredients research center, Pharmaceutical Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran(APRIC)*

[3,4]Clinical pharmacy department, Pharmaceutical Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran

[*] Corresponding author: Dr. Mahnaz Qomi, Active pharmaceutical ingredients research center, Pharmaceutical Sciences Research Center,
Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran(APRIC), E-mail: Qomi@iaups.ac.ir, Tel.: +982122006221, Fax: +9821 22600099.

Highlights

- ✓ Trace amounts of vincristine were determined by SBME-HPLC-UV method.
- ✓ A multivariate optimization approach was applied to optimize extraction conditions.
- ✓ SBME method was simple, fast, and high efficient providing low LOD and high PF.
- ✓ The feasibility of the method was confirmed by analyzing plasma and urine samples.

Abstract

In the current work, an efficient method named solvent bar microextraction-high performance liquid chromatography-UV detection (HPLC-UV) was developed for preconcentration and determining the trace amount of vincristine (VCR) in biological samples such as plasma and urine. Briefly, VCR was extracted from an aqueous sample with pH 10.7 (donor phase) into 1-octanol as the supported liquid membrane (SLM) which is inserted into the pores of the hollow fiber and followed by back extraction into an aqueous receiving phase (pH = 3.1). Studying the factors

affecting the extraction performance in order to achieve a high extraction efficiency, requires the design of experiments (DOE) approach. In this regards, diverse factors' effects including the pH value of donor and acceptor phases, extraction time, extraction temperature, stirring rate and salt content of the donor phase were considered. The optimum experimental condition was as following: pH of the source phase, 10.7; pH of the receiving phase, 3.1; stirring rate, 1000 rpm; extraction temperature, 51 ^{o}C; extraction time, 60 min and 11.3% w/v NaCl in the sample solution. Under the optimal; extraction condition, a favorable preconcentration factor equal to 98.5 was achieved. The linearity range was obtained in the domain of 0.05-5 mg L^{-1}. The limits of detection and quantification were 0.015 and 0.05 mg L^{-1}. Within-day and between-day RSDs of the proposed SBME method were 4.1% and 12.5%, respectively. Finally, the applicability of the implemented SBME method was evaluated by the extraction and quantification of VCR from biological samples such as urine and plasma and satisfactory results were obtained.

Keywords: Solvent bar microextraction; Vincristine; Biological fluids; Preconcentration; Design of experiment.

Abbreviations: DOE, Design of the experiment; HF, Hollow fiber; LPME, Liquid phase microextraction; SBME, Solvent bar microextraction; SLM, Supported liquid membrane; SDME, Single-drop microextraction; DLLME, Dispersive liquid-liquid microextraction; SPE, Solid-phase microextraction; VCR, Vincristine.

1. Introduction

Vincristine (VCR, Fig. 1) is a chemotherapy medication of derived from *Catharanthus roseus* and belongs to vinca alkaloids family [1]. This biologically active compound is applied for treating a

number of cancers including acute myeloid leukemia, acute lymphocytic leukemia, Hodgkin's disease, neuroblastoma, small cell lung cancer, Wilms' tumor and rhabdomyosarcoma [1-4]. Common unfavorable effects of VCR treatment are as following: change in sensation, constipation, and difficulty in walking, hair loss, and headaches. Moreover, serious side effects such as lung damage, neuropathic pain, or low blood white cells may be observed during consumption of VCR [3]. Accordingly, it is favorable to monitor VCR concentration in the biological samples such as plasma and urine.

Various analytical techniques like liquid chromatography in combination with electrochemical detection [5] or ultraviolet detection [6], liquid chromatography-mass spectrometry (LC-MS) [7,8] and LC-MS/MS [9] have been utilized for the separation and determination of VCR in various samples. Liquid-liquid extraction (LLE) [10] and solid-phase extraction (SPE) [7-9] are the two widely used methods for extracting CVR from biological samples before a chromatographic determination. Nevertheless, SPE and LLE methods are quite labor intensive, time-consuming and in most cases consumes large quantities of expensive and non-environmentally friendly solvents [11]. In contrast, microextraction methods, such as liquid-phase microextraction (LPME) and solid-phase microextraction (SPME), solve the mentioned drawbacks because these methods integrate sampling, preconcentration, extraction and introducing the sample into one step [12,13].

LPME as an alternative LLE method is an interesting technique that uses a few microliter of an organic solvent for extracting the target analytes of interest. This method provides higher enrichment factor, efficient clean-up and consumes the very low amount of the extraction solvent compared to LLE. LPME is can be classified into three main modes including hollow fiber liquid-phase microextraction (HF-LPME), single-drop microextraction (SDME) [14] and dispersive liquid-liquid microextraction (DLLME) [15]. HF-LPME technique was proposed by Pedersen-

Bjergaard and his coworker [16] in 1999. Solvent bar microextraction (SBME) is a variation of HF-LPME method based on employing porous hollow fiber in a free moving format and has been developed by Jiang and Lee [17] In this method, the extraction solvent is inserted into a short porous hollow fiber segment (both ends are sealed), then it is situated in a stirring aqueous donor phase [18]. The free movement of the solvent bar (two-sided sealed hollow fiber) in the solution greatly enhances transferring the target analytes from the aqueous donor phase into the organic solvent and receiving phase, considerably [18]. Compared to HF-LPME, faster extraction rates with higher preconcentration factors are achievable owing to the stirring of the organic phase in the solvent bar as well as the aqueous phase.

In the current study, an efficient SBME method in conjugation with HPLC-UV technique was proposed for preconcentrating and determining vincristine at trace amounts in the biological fluids. To the best of our knowledge, this is the first report on microextraction of VCR from biological samples. To study the effect of diverse factors on the extraction performance and to achieve high extraction recovery, the design of experiments method (DOE) was performed. In this regards, the effect of diverse factors including the donor and acceptor phases pH, extraction time, extraction temperature, stirring rate and the salt content of donor phase was considered. Finally, the applicability of the SBME method was explored by extracting and determining VCR in human plasma and urine samples.

2. Experimental

2.1. Chemicals and Reagents

Vincristine (VCR) standard solution was kindly donated by Drug and Food Administration (Tehran, Iran). 1-Octanol, NaCl, NaOH, and KH_2PO_4 were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany). All of the chemicals used were of analytical-grade reagents. HPLC-grade methanol was purchased from Caledon Merck (Ontario, Canada). Ultrapure water was obtained by employing an Aqua Max-Ultra deionized water purification instrument (Youngling, Dongan-gu, South Korea). The Accurel Q3/2 polypropylene hollow fiber membranes with 600 µm I.D., 200 µm wall thickness, and 0.2 µm pore size were purchased from Membrana (Wuppertal, Germany).

2.2. Instrumentation

A Younglin YL9100 HPLC system (Seoul, South Korea) containing a Quaternary 9110 HPLC pump (South Korea), a 4-channel mixing valve with a 10 µL sample loop, YL 9101 vacuum degasser, and a YL 9120 UV-Vis detector was employed in order to determine VCR drug. Younglin AutoChro 3000 software was used for chromatographic data acquisition. A C18 HPLC column (250 mm × 4.6 mm, 5 µm) (Technochrome, Mainz, Germany) was utilized for separation of VCR. The mobile phase consisted of water-diethyl amine at a ratio of 59:1 (pH = 7.5, component A) and methanol (component B). Separation and determination of VCR were performed in isocratic mode by using 30% of component A and 70% of component B at the flow rate equal to 1 mL min^{-1}. The wavelength used for UV-Vis detector was 297 nm. A 100 µL Hamilton microsyringe (Bonaduz, Switzerland) was utilized for the extraction process and injection to HPLC-UV instrument. A Mtops MS300HS hotplate and stirrer (Seoul, Korea) was used for stirring of the solutions during extraction. Hettich centrifuge model EBA 20 (Oxford, England) was

employed in the case of real sample analysis. Measurement of pH was performed by employing an AZ 86502 pH meter (Taiwan) equipped with a glass calomel electrode.

2.3. Standard solutions and real samples

A 1000 mg L^{-1} stock solution of VCR kindly donated by Drug and Food Administration (Tehran, Iran) and stored at 4 ^{0}C prior to use. The working standard solutions were prepared freshly by step-by-step diluting the stock solution. Drug-free plasma was obtained from Iranian Blood Transfusion Organization (Tehran, Iran) and a urine sample was collected from a healthy volunteer. The collected samples were maintained at -20ºC prior to use. These two biological samples were prepared and pretreated according to the following process; for urine samples: The urine sample was diluted at the ratio of 1:4 with HPLC-grade water and subsequently centrifuged at 4000 rpm for 3 min and its pH adjusted to 10.7 by the adding NaOH/K_2HPO_4. For plasma samples: (1) To precipitate the proteins, $HClO_4$ (35%) was added to the plasma sample under stirring, (2) the obtained solution was shaken with vortex for one minute and then it was centrifuged (3000 rpm) for 6 min, (3) the plasma sample was diluted at the ratio of 1:4 by using deionized water, (2) the pH of diluted plasma was adjusted to 10.7 by adding NaOH/K_2HPO_4.

2.4. Extraction procedure

The extraction process was carried out in the following steps: (1) hollow fiber (HF) membranes were carefully and manually cut into 4.0 cm length segments and then they were ultrasonicated in acetone as a cleaning solvent for 10 min to discard the possible contamination and impurities and subsequently dried completely. (2) An aliquot of 10 mL sample solution of 1 mg L^{-1} of VCR (pH 10.7, 11.3% w/v NaCl) was placed in an 11 mL vial with a 4 mm × 14 mm magnetic stir bar. (3)

Afterward, 30 µL aqueous solution with pH = 3.1 (acceptor phase) was withdrawn into a 100 µL microsyringe and then its needle was inserted into the lumen of HF and the aqueous acceptor phase was injected into the fiber. (4) The HF was placed into 1-octanol (extraction solvent) for 15 s and subsequently, the excess amount of solvent was removed from the fiber surface carefully by washing its outside by using ultrapure water for 10 s. (5) Afterward, the needle was carefully pulled out from the fiber, and the open ends of HF were sealed with two pieces of aluminum at 2 mm from the ends of HF. (6) The solvent bar was subsequently, placed in the solution and was stirred at 1000 rpm for 60 min at 51 ºC. (7) Thereafter, the solvent bar was taken out and the ends of HF were unsealed, and the analyte-enriched acceptor phase was carefully withdrawn into the syringe. In the end, 10 µL of the acceptor solution was injected into HPLC-UV instrument for subsequent analysis.

3. Results and discussion

In this study, a new and efficient SBME method is combined with HPLC-UV for extractive preconcentration and determination of trace amount vincristine in human plasma and urine samples. In this regards, for obtaining the best extraction conditions the effect of diverse factors including source phase pH, acceptor phase pH, NaCl content in the donor phase, extraction solvent type, extraction time, temperature and stirring speed were investigated.

3.1. Organic solvent type

The supported liquid membrane (SLM) composition has a very vital role in microextraction based on the solvent bar and a suitable organic solvent ensures the favorable preconcentration factor and high extraction recovery. Accordingly, the extraction solvent was selected according to the

following criteria: (1) Extraction solvent should be strongly maintained in the pores of hollow fiber, thus the organic solvent polarity should be very close to the polarity of polypropylene hollow fiber; (2) the nature of SLM solvent has be conformed to the nature of the target analyte in order to favor their transfer [19]; (3) this solvent should be immiscible with water to prevent the mixing of donor and receiving phases; and (4) the extraction solvent should has a relatively non-volatile nature. Based on the above considerations, the effect of different extracting solvent such as dodecane, 1-octanol, *n*-hexane and isobutyl methyl ketone was investigated. The results exhibited that *n*-octanol can act as the best extraction solvent owing to its immiscibility with an aqueous solution and the acceptor phase and its appropriate polarity to extract VCR. Moreover, 1-octanol has a high-boiling-point that avoids its loss during the extraction process[26-35].

3.2. Optimization strategy

In this step, the effect of various factors such as pH of donor and receiving phases, NaCl concentration in donor phase, extraction temperature, extraction time and agitation rate were considered. To obtain desirable extraction conditions, experimental design approach in via response surface methodology was applied. Application of multivariate optimization approach such as response surface methodology allows the analyst to select the best extraction conditions and to investigate the possible interaction between the affecting factors. The low, middle and high values of these parameters are tabulated in Table 1. Accordingly, 27 experiments were designed with Minitab software and performed randomly to eliminate the main effects of extraneous or nuisance variables. Peak area of VCR was chosen as the experimental response and the obtained results are depicted in Table 2. Fig. 2 and 3 depict some RSM along with two-dimensional contour plots obtained from the design, respectively. The presence of curvature in theses plots exhibits the

presence of interactions between the main factors and also confirms that these variables are optimized in the studied region of design. Each individual plot in these figures demonstrates the way by which each factor affects the peak area values.

As illustrated in Fig. 4, pH value of donor and acceptor phases has the highest effect on extraction performance of the SBME method. VCR is a weakly basic compound (pK_a = 8.66, 10.85) and so the pH of donor solution should be adjusted in alkaline range to ensure that VCR molecule present in their charge less form which leads to an increase in their transfer from the aqueous solution into 1-octanol as SLM. In contrast, the pH of acceptor solution should be adjusted in the acidic region to ensure that VCR molecule have a positive charge which leads to its transfer from the organic layer into the receiving phase and prevents the possible back diffusion of VCR to the donor phase. Accordingly, the best performance of extraction was obtained when pH value of donor and acceptor solutions was adjusted at 10.7 and 3.1, respectively.

It is expected that extraction efficiency of VCR should increase as the agitation speed increases owing to the enhancement of mass transfer rate from the donor solution into the organic layer and subsequently into the acceptor phase. As shown in Fig. 2, 3 and 4, the extraction performance of VCR was improved by increasing the stirring speed up to 1000 rpm. In the lower stirring rate, the mass transfer rate of the target analyte is not sufficient to extract the VCR molecules effectively [20]. Moreover, as it is illustrated in Fig. 4, the highest desirability value is obtained under this condition. Extraction temperature is another factor that affects the extraction efficiency by accelerating the mass transfer rate of analytes from donor phase into an organic phase and subsequently into the acceptor phase. As the results reveal (Fig. 2, 3, 4), the extraction performance was increased when the temperature increased up to 51 ºC and beyond this value, a decrease in

extraction efficiency was observed which can be related to increasing the solubility of 1-octanol in the sample solution and its evaporation.

VCR has a Log *P* equal to 2.8, and so the addition of a salt such as NaCl can improve the extractability of this analyte via salting-in effect. As illustrated in Fig. 4, the extraction performance is enhanced by increasing the NaCl concentration up to 11.3% w/v and then a decrease was observed. This observation can be clarified as follows [21]. At first (up to 11.3% w/v NaCl), the salting-out effect is the predominant phenomenon and enhances the extraction efficiency of the target analyte by decreasing it's and further, enhances the distribution of the analytes from donor phase into the organic phase [21]. By increasing the NaCl concentration higher than 11.3% w/v, the electrostatic interaction process between VCR molecules and salt ions, occurring with the salting-out effect simultaneously, plays a more dominant effect on the extraction efficiency. This process barricades the transfer of VCR into1-octanol and thereby reduces the extraction recovery. Moreover, at the high concentration, NaCl enhances the viscosity of the donor aqueous solution and may cause to change the physical properties of the Nernst diffusion film, so diffusion into the organic phase is limited [18,22].

SBME is an equilibrium process rather than an exhaustive extraction like the other microextraction methods. The analyte is distributed between the donor and acceptor solutions until the equilibrium is established [18,11]. The obtained results exhibited that extraction efficiency enhances with the increase extraction time. It can be seen, the analytical signals increase quickly within 60 min of extraction time.

The highest extraction efficiency and desirability value (D = 0.9949) was obtained by performing the extraction under the following conditions: pH of donor phase, 10.7; pH of acceptor phase, 3.1;

an extraction time of 60 min; an extraction temperature of 51 °C; an stirring rate of 1000 rpm and 11.3% w/v of NaCl in the donor phase.

3.3. Analytical characteristics of the method

Analytical characteristics of the developed SBME-HPLC-UV method such as linear dynamic range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision (RSD%) and enrichment factor was established under the optimized extraction conditions. The linearity was achieved in the domain of 0.05-5 mg L^{-1} in a water sample with an r^2 value equal to than 0.9989. The enrichment factor (EF) was computed according to the following equation [23,24]:

$$EF = \frac{Slope_2}{Slope_1} \qquad (1)$$

where, $Slop_2$ and $Slop_1$ represent the slope of calibration curve after and before preconcentration, respectively. In this study, and EF factor as large as 98.5 was obtained. Within-day RSDs (n = 3 sample, at 1.0 mg L^{-1} level of VCR) and between-day RSDs (n = 3 day, at 1.0 mg L^{-1} of VCR) of the method were also evaluated. LOD and LOQ were obtained at the signal to noise ratio of 3 and 10, respectively. The obtained results are summarized in Table 3.

3.4. Determination of vincristine biological fluids

The accuracy and feasibility of the developed SBME-HPLC-UV method were explored by extraction and determination of VCR in biological samples such as plasma and urine. Accordingly, real plasma and urine samples at 1.0 mg L^{-1} level of VCR were analyzed under the optimal extraction conditions. The obtained results are tabulated in Table 4 and the determined concentrations are in a fine settlement with the spiking amounts. The chromatograms of the real

urine and plasma samples analysis are exhibited in Fig. 5 that shows the peak of VCR without any interference peak. The real plasma and urine samples were taken from a patient.

3.5. Comparison of the current method with the other reported methods

The analytical efficiency of the developed SBME-HPLC-UV method is compared with the other reported methods in Table 5. As indicated in this table, SBME-HPLC-UV method is sensitive and has wider linear dynamic ranges. Besides, LOD and RSD values of the SBME method are comparable to those of the other methods. Hence, the analytical characteristics of the developed method are acceptable.

4. Conclusion

In the current study, for the first time, a simple and high efficient solvent bar microextraction method was applied for extractive preconcentration of trace amounts of vincristine from human biological fluids and its subsequent determination by HPLC-UV. To obtain the best extraction performance the effect of factors affecting the extraction recovery were optimized using experimental methodology via response surface methodology. Under the opted extraction condition, a high enrichment factor (98.5) and extensive sample clean-up was obtained which is vital for detection of vincristine at physiologically relevant concentrations. A wide linear dynamic range, low detection limit, low intra and inter-day RSDs and acceptable recovery were obtained too. Besides, the risk of cross-contamination and carry-over problems was solved by using a new hollow fiber segment for each extraction and discarding the fiber after each extraction.

References

[1] M.S. Schmidt, R. Huang, R.J. Classon, D.J. Murry, Determination of vincristine in infant plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectroscopy. J. Pharm. Biomed. Anal. 41 (2006) 540-543.

[2] W.R. Crom, A.M. Glynn-Barnhart, J.H. Rodman, M.E. Teresi, R.E. Kavanagh, M.L. Christensen, M.V. Relling, W.E. Evans, Pharmacokinetics of anticancer drugs in children. Clin. Pharmacokinet. 12 (1987) 168-213.

[3] "Vincristine Sulfate". The American Society of Health-System Pharmacists. Retrieved Jan 2, 2015.

[4] V.S. Sethi, J.C. Kimball, Pharmacokinetics of vincristine sulfate in children, Cancer Chemother. Pharmacol. 6 (1981) 111-115.

[5] E. Groninger, P. Koopmans, W. Kamps, S. de Graaf, D. Uges, An automated HPLC method to determine intracellular vincristine concentrations in mononuclear cells of children with acute lymphoblastic leukemia, Ther. Drug Monit. 25 (2003) 441-446.

[6] H. Bloemhof, K.N. Van Dijk, S.S.N. De Graaf, D.E.M.M. Vendrig, D.R.A. Uges, Sensitive method for the determination of vincristine in human serum by high-performance liquid chromatography after on-line column-extraction. J. Chromatogr. B 572 (1991) 171-179.

[7] M.S. Schmidt, R. Huang, R.J. Classon, D.J. Murry, Determination of vincristine in infant plasma by liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy, J. Pharm. Biomed. Anal. 41 (2006) 540-543.

[8] P. Guo, X. Wang, F. Zhou, J.M. Gallo, Determination of vincristine in mouse plasma and brain tissues by liquid chromatography-electrospray mass spectrometry, J. Chromatogr. B 809 (2004) 273-278.

[9] R. Guilhaumou, C. Solas, A. Rome, M. Giocanti, N. Andre, B. Lacarelle, Validation of an electrospray ionization LC/MS/MS method for quantitative analysis of vincristine in human plasma samples, J. Chromatogr. B 878 (2010) 423-427.

[10] J. Ramírez, K. Ogan, M.J. Ratain, Determination of vinca alkaloids in human plasma by liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, Cancer Chemot. Pharm. 39 (1997) 286-290.

[11] H. Ebrahimzadeh, A.A. Asgharinezhad, H. Abedi, F. Kamarei, Optimization of carrier-mediated three-phase hollow fiber microextraction combined with HPLC-UV for determination of propylthiouracil in biological samples, Talanta 85 (2011) 1043.

[12] S. Risticevic, H. Lord, T. Gorecki, C.L. Arthur, J. Pawliszyn, Protocol for solid-phase microextraction method development, Nat. Protoc. 5 (2010) 122.

[13] F. Pena-Pereira, I. Lavilla, C. Bendicho, Liquid-phase microextraction techniques within the framework of green chemistry, TrAC-Trends Anal. Chem. 29 (2010) 617.

[14] H. Ebrahimzadeh, N. Mollazadeh, A.A. Asgharinezhad, N. Shekari, F. Mirbabaei, Multivariate optimization of surfactant-assisted directly suspended droplet microextraction combined with GC for the preconcentration and determination of tramadol in biological samples, J. Sep. Sci. 36 (2013) 3783-3790.

[15] K. Molaei, A.A. Asgharinezhad, H. Ebrahimzadeh, N. Shekari, N. Jalilian, Z. Dehghani, Surfactant-assisted dispersive liquid–liquid microextraction of nitrazepam and lorazepam from plasma and urine samples followed by high-performance liquid chromatography with UV analysis, J. Sep. Sci. 38 (2015) 3905-3913.

[16] S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid-liquid-liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis, Anal. Chem. 71 (1991) 2650-2656.

[17] X. Jiang, H.K. Lee, Solvent bar microextraction, Anal. Chem. 76 (2004) 5591-5596.

[18] H. Ebrahimzadeh, F. Mirbabaei, A.A. Asgharinezhad, N. Shekari, N. Mollazadeh, Optimization of solvent bar microextraction combined with gas chromatography for preconcentration and determination of methadone in human urine and plasma samples, J. Chromatogr. B 947 (2014) 75-82.

[19] C. Desoubries, F. Chapuis-Hugon, A. Bossée, V. Pichon, Three-phase hollow fiber liquid-phase microextraction of organophosphorous nerve agent degradation products from complex samples, J. Chromatogr. B 900 (2012) 48-58.

[20] Q. Xiao, B. Hu, Hollow fiber-liquid phase microextraction combined with gas chromatography for the determination of phenothiazine drugs in urine, J. Chromatogr. B 878 (2010) 1599-1604.

[21] L. Guo, H.K. Lee, Ionic liquid based three-phase liquid–liquid–liquid solvent bar microextraction for the determination of phenols in seawater samples, J. Chromatogr. A 1218 (2011) 4299-4306.

[22] P.H. Howard, W.M. Meylan, Handbook of Physical Properties of Organic Chemicals, CRC Press/Lewis Publishers, Boca Raton, FL, 1997.

[23] A.A. Asgharinezhad, H. Ebrahimzadeh, A simple and fast method based on mixed hemimicelles coated magnetite nanoparticles for simultaneous extraction of acidic and basic pollutants, Anal. Bioanal. Chem. 408 (2016) 473-486.

[24] A.A. Asgharinezhad, H. Ebrahimzadeh, Poly (2-aminobenzothiazole)-coated graphene oxide/magnetite nanoparticles composite as an efficient sorbent for determination of non-steroidal anti-inflammatory drugs in urine sample, J. Chromatogr. A 1435 (2016) 18-29.

[25] J. Chen, H. He, S. Li, Q. Shen, An HPLC method for the pharmacokinetic study of vincristine sulfate-loaded PLGA–PEG nanoparticle formulations after injection to rats, J. Chromatogr. B 879 (2011) 1967-1972.

[26] A Afshari, R.; Qomi, M., A Novel Application of Three Phase Hollow Fiber Based Liquid Phase Microextraction (HF-LPME) for the HPLC Determination of Tamsulosin from Biological Fluids. Current Pharmaceutical Analysis 2016, 12 (3), 258-265.

[27] Akhavan, S.; Qomi, M.; Charmahali, G.; Akhavan, S., Hollow IFber Based Liquid Phase Microextraction Combined High Performance Liquid Chromatograph for the Determination Trace Amounts of Opiclone in Biological Fluids. Biomedical and Pharmacology Journal 2015, 8 (1), 503-512.

[28] Boloori, V.; Qomi, M.; Piroozi, F.; Raofie, F., Development of a Simple and Efficient Method for Preconcentration and Determination of Trace Levels of

Fexofenadine in Plasma and Urine Samples. Journal of Applied Chemical Research 2016, 10 (3), 7-14.

[29] Charmahali, G.; Qomi, M.; Akhavan, S.; Chaharmahali, M.; Tafti, F. F., Determination of Trace Amounts of Risperidone in Human Urine Sample by Hollow Fiber Liquid-Phase Microextraction Combined with High Performance Liquid Chromatography.

[30] Darvish, M.; Qomi, M.; Akhgari, M.; Raoufi, P., Determination of trace amounts of methamphetamine in biological samples by hollow fiber liquid-phase microextraction followed by high performance liquid chromatography. Biosci Biotechnol Res Asia 2015, 12 (1), 587-597.

[31] Emadzadeh, S.; Qomi, M.; Saadat, M.; Piroozi, F., Three-phase Hollow fiber Liquid-phase Micro Extraction for Determination and Analysis of Terazosin in Biological Fluids Via High Performance Liquid Chromatography at Trace Levels. Current Analytical Chemistry 2016, 12 (5), 489-495.

[32] Minaii, M.; Qomi, M.; Hoseini, S. S.; Sadri, A., Preconcentration and Determination of Cyproheptadine by using Liquid Phase Microextraction and Solvent Bar in Biological Fluids in Trace Level. Biosci. Biotechnol. Res. Asia 2015, 12, 521-529.

[33] Miraee, S. N.; Qomi, M.; Shamshiri, F.; Raoufi, P., Hollow-Fiber liquid-phase Microextraction Followed by High Performance Liquid Chromatography for the Determination of Trace Amounts of Methylphenidate Hydrochloride in Biological Fluids. Biomedical & Pharmacology Journal 2014, 7 (2), 715-725.

[34] Piroozi, F.; Ghasemi, E.; Qomi, M.; Rezaee, R.; Hashemian, F., Hollow fiber liquid phase microextraction combined with high performance liquid chromatography for preconcentration and determination of cabergoline in biological samples. Journal of Liquid Chromatography & Related Technologies 2014, 37 (5), 760-771.

[35] Rezaee, R.; Qomi, M.; Piroozi, F., Hollow-fiber micro-extraction combined with HPLC for the determination of sitagliptin in urine samples. J. Serb. Chem. Soc. 2015, 80 (10), 1311-1320.

Figure caption:

Fig. 1: Structure of VCR and its physiochemical properties.

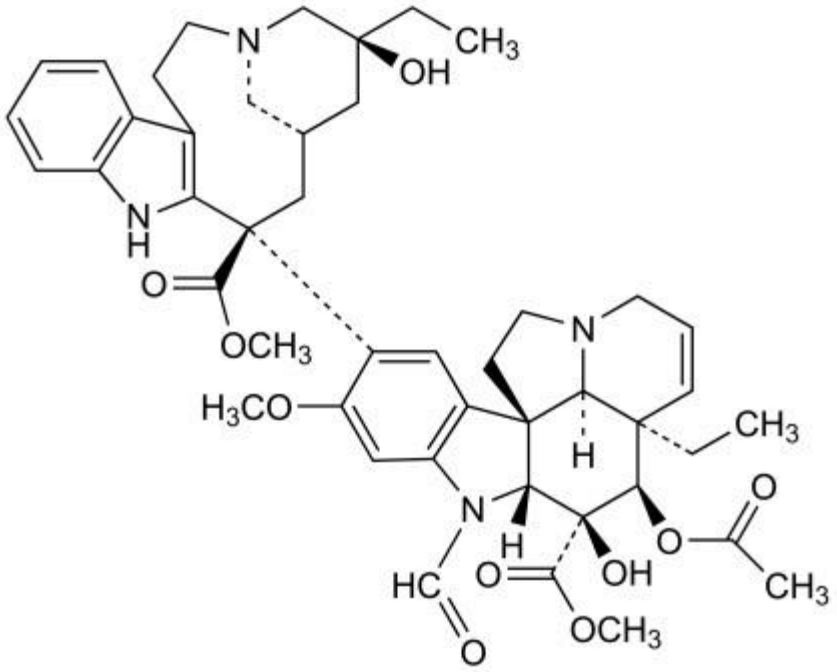
Fig. 2: Response surfaces obtained from experimental design.

Fig. 3: Two dimensional contour pots resulted from design of experiments approach**.**

Fig. 4: The optimization plot and desirability value obtained from design of experiment.

Fig. 5: The chromatograms of: (A) blank plasma sample (a) real plasma sample at 1 mg L^{-1} of VCR (b), (B) blank urine sample (a) real urine sample at 1 mg L^{-1} of VCR (b), after SBME under optimal conditions.

Fig. 1



pKa = 8.66, 10.85
Log *P* = 2.8

Fig. 2

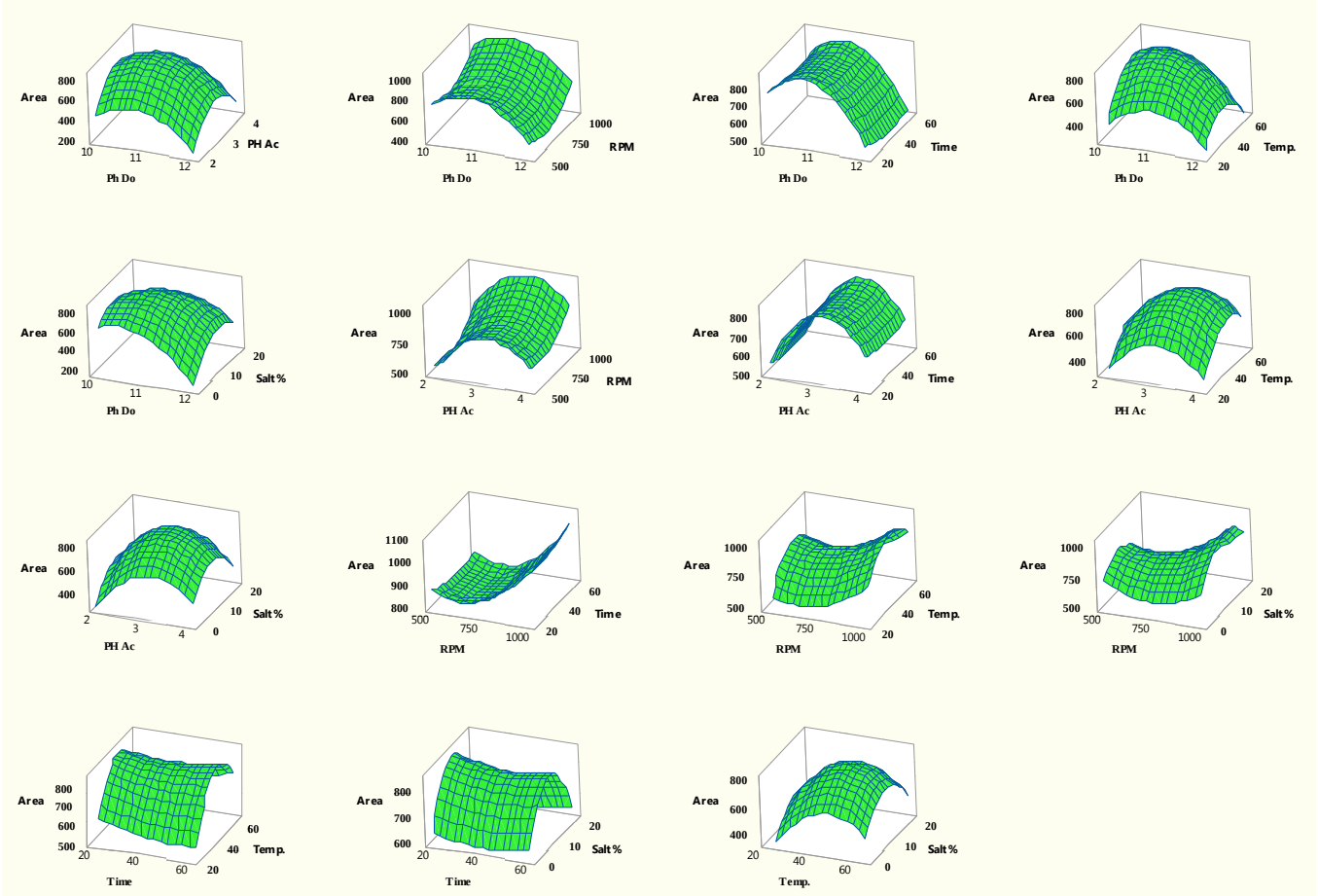


Fig. 3

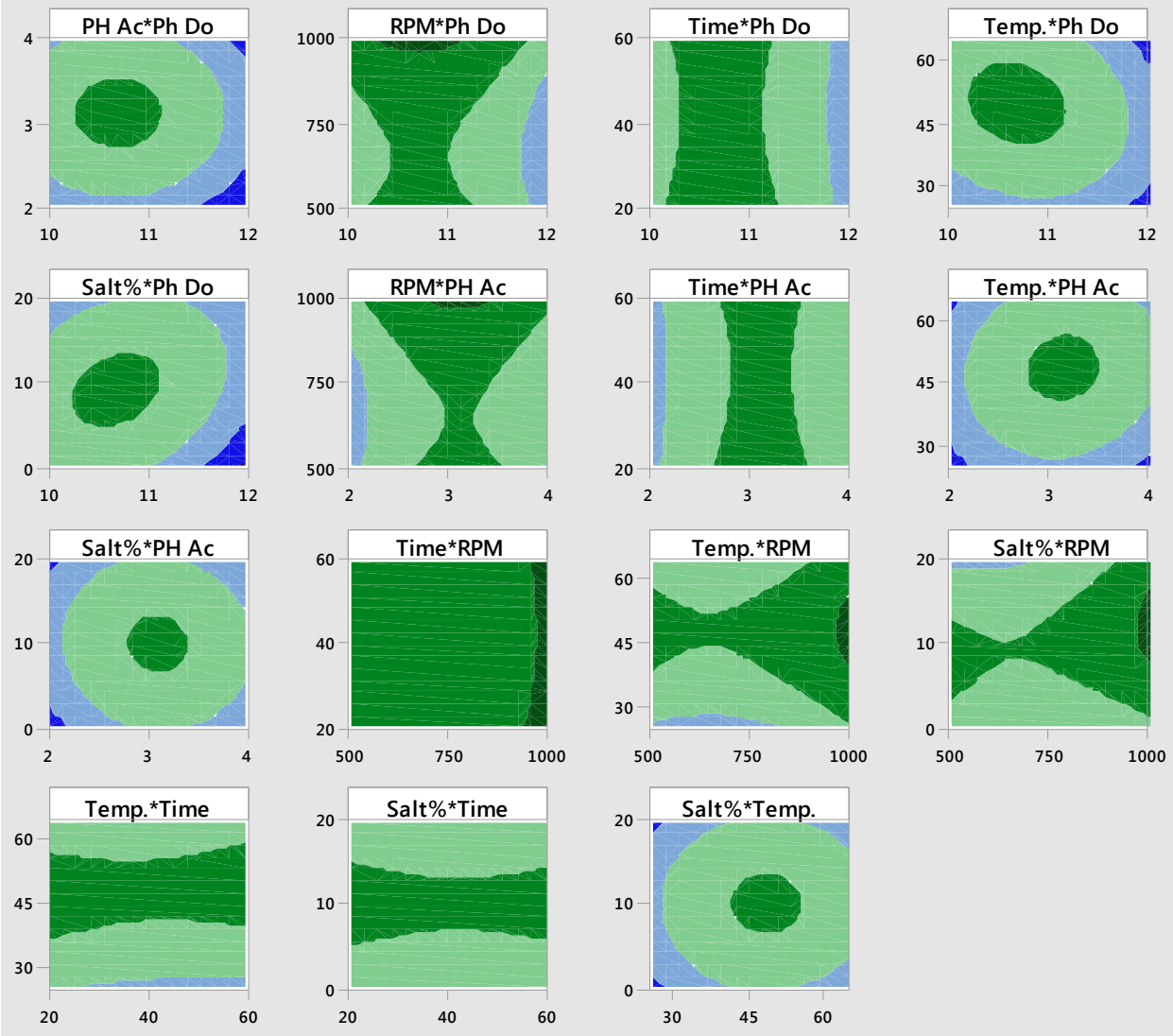


Fig. 4

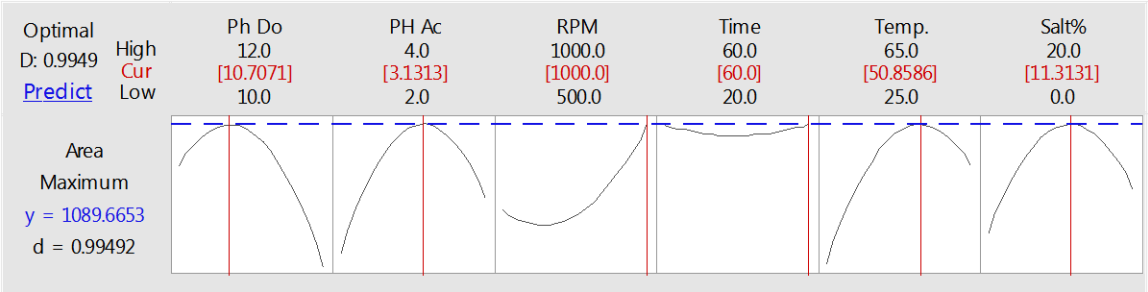
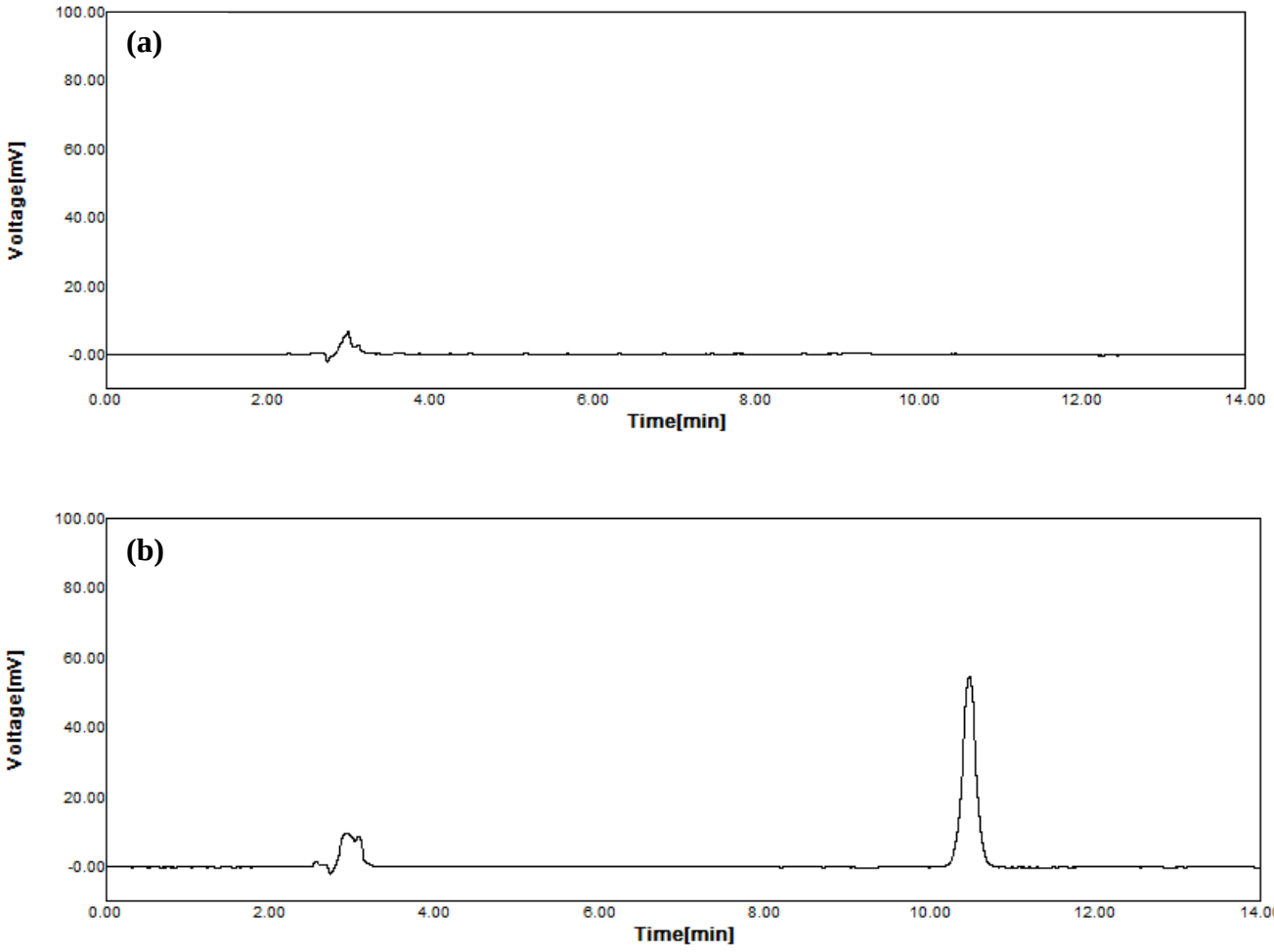


Fig. 5

(A)



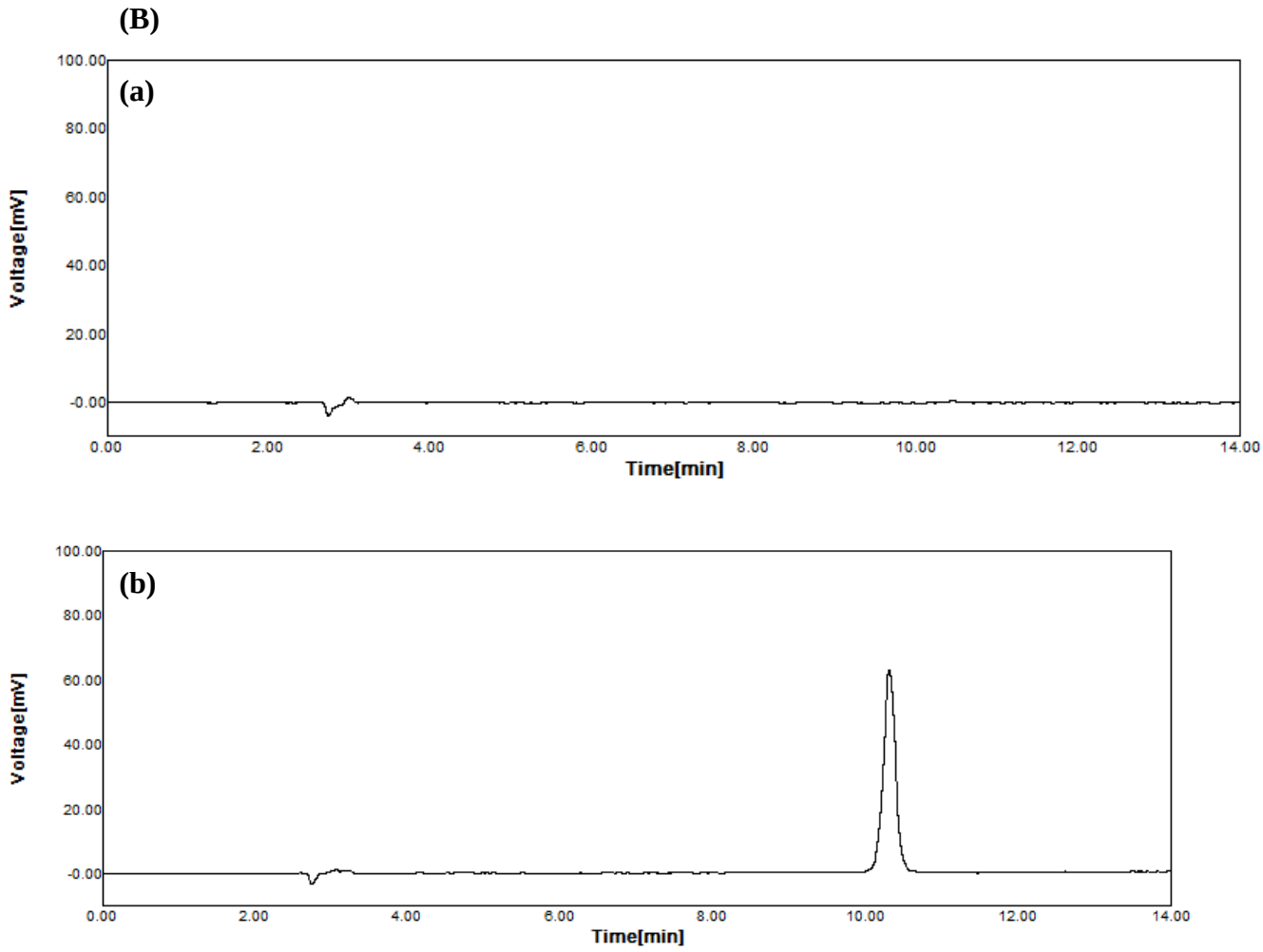


Table 1

Experimental variables and their levels in chemometric design.

Factor	Levels		
	Low (-1)	Center (0)	High (+1)
pH of donor phase	10	11	12
pH of acceptor phase	2	3	4
Stirring rate (rpm)	500	750	1000
Extraction time (min)	10	40	60
Extraction temperature (ºC)	25	45	65
NaCl concentration (%, w/v)	0	10	20

Table 2

The design experiments and their related responses

No	pH_d [a]	pH_a [b]	Stirring rate (rpm)	Extraction time (min)	Temperature (°C)	Salt concentration (%, w/v)	Peak area
1	10	2	500	20	25	0	225
2	10	2	500	20	45	10	280
3	10	2	500	20	65	20	153
4	10	3	750	40	25	0	390
5	10	3	750	40	45	10	801
6	10	3	750	40	65	20	195
7	10	4	1000	60	25	0	73
8	10	4	1000	60	45	10	920
9	10	4	1000	60	65	20	530
10	11	2	750	60	25	10	370
11	11	2	750	60	45	20	290
12	11	2	750	60	65	0	153
13	11	3	1000	20	25	10	785
14	11	3	1000	20	45	20	1095
15	11	3	1000	20	65	0	498
16	11	4	500	40	25	10	302
17	11	4	500	40	45	20	278
18	11	4	500	40	65	0	585
19	12	2	1000	40	25	20	179
20	12	2	1000	40	45	0	58
21	12	2	1000	40	65	10	196
22	12	3	500	60	25	20	242
23	12	3	500	60	45	0	150
24	12	3	500	60	65	5	410
25	12	4	750	20	25	10	45
26	12	4	750	20	45	0	111
27	12	4	750	20	65	5	85

[a] Donor phase pH

[a] Acceptor phase pH

Table 3

Analytical figures of merit of the developed SBME-HPLC-UV method

Regression equation	LDR[a]	r^2	LOD	LOQ[b]	EF[c]	RSD (%)[d]	RSD (%)[e]
$Y = 1776\ X^g + 29.33$	0.05-5	0.9989	0.015	0.05	98.5	4.1	12.5

[a] Linear dynamic range (mg L^{-1}). [b] Limit of quantification. [c] Enrichment factor is calculated as the ratio of the slopes of the calibration curves before and after extraction. [d] Intra-day relative standard deviation (n = 3 replicates). [e] Inter-day relative standard deviation (n = 3 days).

[g] VCR (analyte) concentration in mg L^{-1}.

Table 4
Determination of vincristine in human plasma and urine samples

Sample	C_{added} (mg L^{-1})	C_{found} (mg L^{-1})	RSD% (n = 3)	RR (%)[a]
Plasma	-	ND	-	-
	1.0	0.76	5.1	76
Urine	-	ND	-	-
	1.0	0.81	5.8	81

[a] Relative recovery. ND: Not detected.

Table 5
Comparison of the performance of the developed SBME method with the other reported methods

Extraction technique [a]	Average Recovery%	Linear range (mg L^{-1})	LOD (mg L^{-1})	RSD%	Ref.
SPE-LC-MS	57	0.01-2	0.013	4.5-11.7	[8]
SPE-HPLC-ECD	89.6	0.001-0.0508	-	4.8	[5]
LLE-HPLC-UV	83	0.05-5.0	0.015	< 9.0	[25]
SBME-HPLC-UV	81	0.05-5.0	0.015	< 5.8	Current study

[a] Electrochemical detection (ECD), mass spectrometry (MS), solid phase extraction (SPE), liquid-liquid extraction (LLE).