Journal of Chromatography B, 899 (2012) 66–71

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Low-density solvent-based dispersive liquid–liquid microextraction followed by high performance liquid chromatography for determination of warfarin in human plasma

Hoda Ghambari, Mohammadreza Hadjmohammadi*

Department of Chemistry, University of Mazandaran, Babolsar 47416-95447, Iran

ARTICLE INFO

Article history:
Received 31 January 2012
Accepted 28 April 2012
Available online 6 May 2012

Keywords:
Low-density solvent
Dispersive liquid–liquid microextraction
Warfarin
HPLC
Sample preparation
Plasma sample analysis

ABSTRACT

Extraction and determination of warfarin, a widely used anticoagulant drug, in human plasma were performed using a new generation of dispersive liquid–liquid microextraction (DLLME) and high performance liquid chromatography (HPLC). The extraction procedure is based on extraction solvents lighter than water and performing of extraction in a specially designed extraction cell. Some important parameters, including kind and volume of extraction and disperser solvents, pH of the sample solution, salt concentration in the sample solution and extraction time were investigated and optimized. Under the optimized conditions (150 μL 1-octanol as extraction solvent, 150 μL methanol as disperser solvent, pH_{sample} = 2.3, extraction time of 2 min, without salt addition), limit of detection (LOD) of 5 ng mL^{-1} and extraction recovery of 91.0% were obtained. The calibration curve was linear within the range of 15–3000 ng mL^{-1} with the square of correlation coefficient (R^2) of 0.998. Repeatability and reproducibility of method based on five replicate extraction and determination were 2.8% and 6.5%, respectively. The proposed method was applied successfully for the determination of warfarin in plasma sample from a patient under treatment with this drug, and was demonstrated to be sensitive, efficient, and convenient.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Warfarin is a coumarin derivative widely used as an oral anticoagulant drug in the prevention and treatment of venous and arterial thromboembolic disorders [1,2]. Due to the property of causing fatal hemorrhaging it is also applied as a rodenticide [3]. Warfarin exerts anticoagulant effect by decreasing the synthesis of vitamin K-dependent coagulation factors [4]. The therapeutic window of warfarin is very narrow. Exceeding the therapeutic window of this drug causes unwanted bleedings [5]. Knowledge of the plasma concentration of warfarin is valuable for clinical decisions and allows for effective treatment of severe intoxication. Plasma concentration can also be helpful in distinguishing noncompliance from genuine anticoagulant resistance. Therefore, various methods for example high-performance liquid chromatography (HPLC) with ultraviolet detector [1,5–9], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [10,11], micellar electrokinetic chromatography–electrospray ionization-mass spectrometry (MEKC–ESI-MS) [12] and capillary zone electrophoresis (CZE) [13] have been performed for the determination of warfarin in the biological samples. Due to the low warfarin concentrations and complex matrices, biological samples are not directly analyzed using these approaches. These approaches used liquid–liquid extraction (LLE) or solid-phase extraction (SPE) technique for sample preparation step. LLE offers high reproducibility and high sample capacity, but it is a tedious and time-consuming procedure, which can produce emulsions and requires large amounts of toxic and expensive organic solvents for analyte extraction [14]. Although consumption of organic solvents is relatively low in SPE, it requires lengthy processing (i.e., conditioning, washing, eluting and drying) [15]. These shortcomings have led to the development of new cost-effective methods with special emphasis on their speed, consumption of negligible volume of organic solvent and the ability to detect analytes at very low concentrations. In 2006, a novel microextraction technique termed as dispersive liquid–liquid microextraction (DLLME) was developed by Assadi and co-workers [16]. In this technique, an appropriate mixture of a high-density solvent (extraction solvent) and a water miscible polar solvent (disperser solvent) is rapidly injected into the aqueous sample by a syringe to form a cloudy solution. Chlorinated solvents (e.g., carbon tetrachloride, 1,1,2,2-tetrachloroethane and chlorobenzene) can be used as extraction solvent, whereas acetone, methanol, and acetonitrile are useful as

* Corresponding author. Tel.: +98 1125342350; fax: +98 1125342350.
E-mail addresses: hadjmr@umz.ac.ir, ghambari_h@yahoo.com (M. Hadjmohammadi).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.04.035

disperser solvent. The analytes in the sample are extracted into the fine droplets of the extraction solvent. After extraction, phase separation is performed by centrifugation and the enriched analytes in the sedimented phase are determined by chromatography or spectrometry methods. DLLME is a rapid, simple and low cost method with high recovery and enrichment factor. It has been widely applied for the analysis of different compounds such as phthalate esters [17], organochlorine pesticides [18], triazine herbicides [19] and organosulfur pesticides [20] in environmental samples.

The main disadvantage of the common DLLME technique is the use of chlorinated solvents as extraction solvent that are potentially toxic to humans and the environment. In addition, because the extraction solvent is incompatible with liquid chromatography (LC), DLLME extract cannot be injected directly to LC system for analysis. Therefore, evaporation of the organic extraction solvent to dryness and reconstitution of analytes in a suitable solvent prior to LC is required. This is an effective but laborious approach and prone to loss of analytes during evaporation. On the other hand, in the determination of some important compounds, for example organochlorine pesticides using DLLME–GC-electron capture detector, chlorinated extraction solvents have a very high solvent peak which covers some analytes peaks. There are significant improvements in DLLME in recent years. Various kinds of design were developed using less toxic non-chloro solvent to replace chlorinated extraction solvent [21–23]. Water-immiscible extraction solvent was dispersed into the aqueous sample solution under the assistance of ultrasound to form an emulsion, without using disperser solvent [24,25]. In other studies, acetone, methanol, and acetonitrile, usually served as disperser solvents in DLLME, were used as chemical demulsifiers to break up the dispersed system into two separate phases [26,27]. In this work, a different DLLME technique, low-density solvent-based dispersive liquid–liquid microextraction (LDS-DLLME), followed by HPLC-UV was applied for extraction and determination of warfarin in the plasma sample. This technique was introduced by Farajzadeh et al. in 2009 [28]. By designing a special extraction cell, DLLME was performed using extraction solvents lighter than water. The method was simple and easy to use, and additional steps required in above mentioned techniques, such as ultrasonication or injection of chemical demulsifiers were not necessary. The effect of various experimental parameters on the extraction of warfarin was studied and the applicability of the proposed method was tested for the determination of warfarin in the patient plasma sample.

2. Experimental

2.1. Materials and solutions

Sodium warfarin (≥98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The chemical structure of this drug is shown in Fig. 1. Methanol and acetone (HPLC-grade), sodium hydroxide, hydrochloric acid, trichloroacetic acid, phosphoric acid, sodium chloride, 1-octanol, 2-ethyl-1-hexanol, *n*-hexane and toluene were obtained from Merck (Darmstadt, Germany). Acetonitrile (HPLC-grade) was purchased from Fluka (Buchs, Switzerland). Water used was double distilled deionized.

Stock solution of warfarin (500.0 $\mu g\,mL^{-1}$) was prepared in methanol and stored in the dark at 4 °C. The working solutions were prepared daily by an appropriate dilution of the stock solution with double distilled deionized water.

2.2. Instrumentation

Chromatographic measurements were carried out using a HPLC system equipped with a series 10 LC pump, UV detector model LC-95 set at 320 nm and model 7125i manual injector with a 20 μL sample loop (Perkin-Elmer, Norwalk, CT, USA). Column used was C_{18} (250 mm × 4.6 mm, 10 μm particle size) from waters (Milford, MA, USA). A mixture of methanol and 0.5% (w/v) phosphoric acid (65:35, v/v) with a flow rate of 1.0 $mL\,min^{-1}$ was used as mobile phase at room temperature. Measurement of solutions pH was done by a 3030 Jenway pH meter (Leeds, UK). A Hettich Rotanta centrifuge model D-7200 Tuttlingen (Kirchlengern, Germany) was used for centrifugation in the extraction process.

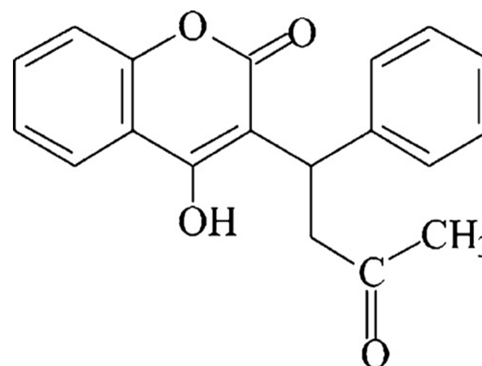

Fig. 1. Chemical structure of warfarin.

2.3. Extraction procedure

For each experiment, 11 mL of aqueous standard or plasma sample solution (pH = 2.3) was poured into a specially designed glass cell (Fig. 2) containing a magnetic stirring bar (14 mm × 4 mm). A mixture of methanol (150 μL) as disperser solvent and 1-octanol (150 μL) as extraction solvent was rapidly injected into the solution by a 500 μL syringe while solution was being stirred at 1000 rpm. After 2 min, the formed cloudy solution was centrifuged for 5 min at 3500 rpm. The collected phase in the narrow neck of cell (141 ± 2 μL) was removed using a 200 μL microsyringe and 30 μL of this phase was injected into the HPLC system for quantification.

2.4. Plasma sample

Blood plasma from healthy donors and from a patient under warfarin treatment were obtained from the Yahyaanejaad Hospital (Babol, Iran). Sampling was done 3 h after taking the drug by the patient. In order to eliminate the protein binding of the drug in plasma (greater than 99%) [29], the pretreatment as outlined in the work of Polson et al. was performed [30]. For this purpose, 5 mL of aqueous trichloroacetic acid (10%, w/v) was added to 2.5 mL of the plasma and the resulting mixture was strongly vortexed for 20 s. The mixture was refrigerated for 20 min at ∼4 °C, and then centrifuged for 10 min at 3000 rpm. The supernatant was transferred into a 25 mL volumetric flask and diluted to the mark with double distilled deionized water. pH of the final solution was adjusted to 2.3 and the extraction procedure was done under the optimized conditions.

3. Results and discussion

3.1. Optimization of LDS-DLLME

The effect of experimental variables such as kind of extraction and disperser solvents as well as their volumes, pH of the sample solution, salt concentration in the sample solution and extraction time on the extraction efficiency were investigated and optimized. For this purpose, LDS-DLLME was carried out using 11 mL of aqueous solution containing 0.1 $\mu g\,mL^{-1}$ of warfarin.

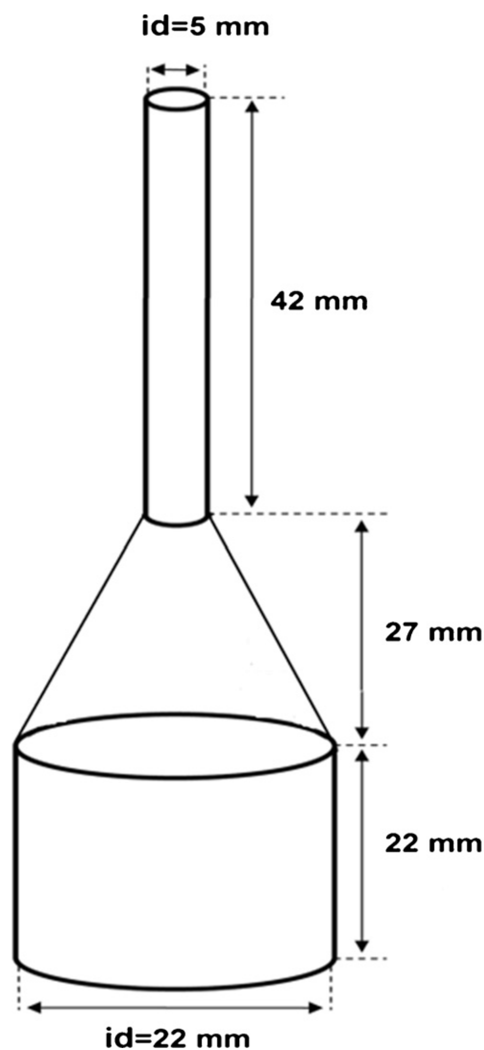

Fig. 2. Schematic figure of the extraction cell.

Extraction recovery was used to evaluate the extraction efficiency under different conditions and each experiment was repeated at least three times.

3.1.1. Selection of extraction and disperser solvents

The selection of appropriate extraction and disperser solvents is of great importance in LDS-DLLME technique in order to obtain efficient extraction. In most cases, reports regarding DLLME have used chlorinated solvents such as carbon tetrachloride, 1,1,2,2-tetrachloroethane, chlorobenzene, etc., which are heavier than water. In this study, the solvents lighter than water were tested due to their safety compared to chlorinated solvents. Tested extraction solvents also have low solubility in water, extraction capability of analyte and good chromatographic behavior. Toluene (density, 0.87 g mL^{-1}), 1-octanol (density, 0.83 g mL^{-1}), *n*-hexane (density, 0.66 g mL^{-1}) and 2-ethyl-1-hexanol (density, 0.83 g mL^{-1}) [31] were examined as extraction solvent. Disperser solvent should be miscible with both sample solution and extraction solvent. Therefore, acetonitrile, acetone and methanol, which have this ability, were applied for this purpose. The experimental procedure was done by injecting each one of the combinations of 40 μL of disperser solvent and 100 μL of extraction solvent into the sample solution. All combinations of extraction solvents and disperser solvents were tested and results were shown in Fig. 3. The combination of 1-octanol as extraction solvent and methanol as disperser solvent showed the highest extraction recovery for warfarin. Therefore,

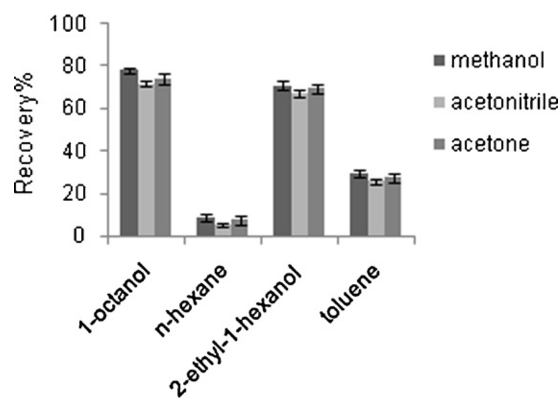

Fig. 3. Effect of extraction and disperser solvents kind on the extraction recovery of warfarin. Extraction conditions: sample volume, 11.0 mL; sample pH, 2.3; extraction solvent volume, 100 μL; disperser solvent volume, 40 μL; stirring speed, 1000 rpm; extraction time, 2 min; centrifuging time, 5 min and centrifuging speed, 3500 rpm.

1-octanol and methanol were selected as the extraction and disperser solvent, respectively.

3.1.2. Effect of extraction solvent volume

In order to study the effect of extraction solvent volume on the extraction efficiency of warfarin, different volumes of 1-octanol (30–200 μL) containing 40 μL of methanol were subjected to the same LDS-DLLME procedure. Fig. 4 shows the variations of extraction recovery and enrichment factor versus volume of the extraction solvent. According to this figure, by increasing volume of 1-octanol, the extraction recovery increased till 150 μL and then remained nearly constant. By increasing the volume of 1-octanol from 30 to 200 μL, volume of the collected phase increased from 19 to 192 μL and the enrichment factor decreased. The decrease

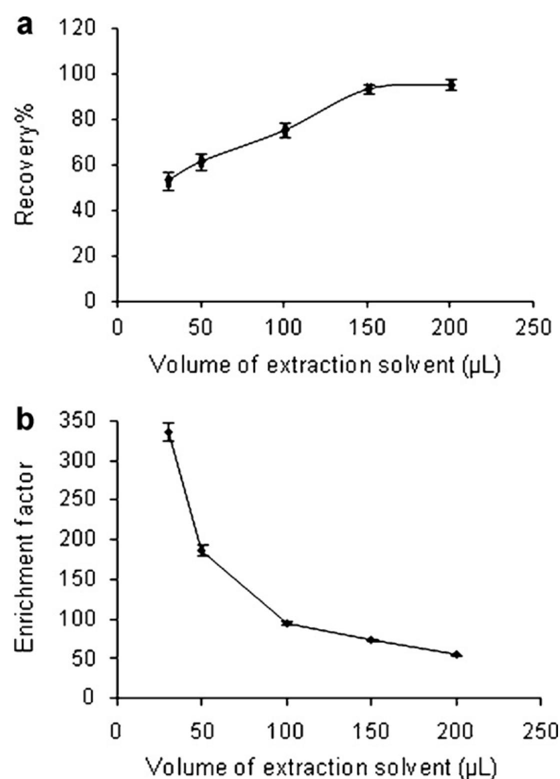

Fig. 4. Effect of the volume of extraction solvent on (a) the extraction recovery and (b) enrichment factor of warfarin. Extraction conditions: extraction solvent, 1-octanol; disperser solvent, methanol. Other conditions as in Fig. 3.

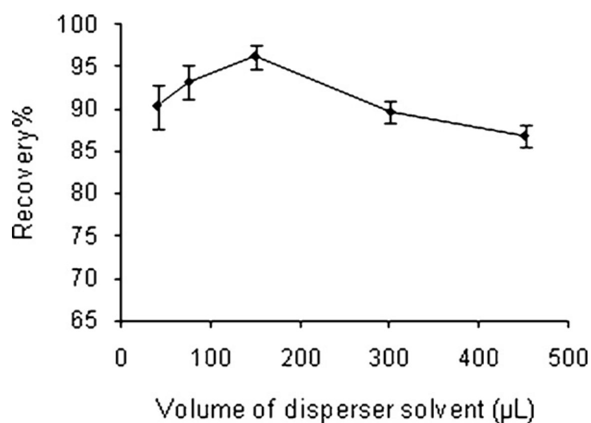

Fig. 5. Effect of the volume of disperser solvent (methanol) on the extraction recovery of warfarin. Extraction conditions: extraction solvent (1-octanol) volume, 150 μL. Other conditions as in Fig. 4.

in enrichment factor can be attributed to dilution of extracted warfarin in the extraction solvent at higher volumes. However, at 150 μL as volume of the extraction solvent, a high recovery, and acceptable enrichment factor was obtained. Hence, this volume was chosen as the optimum volume of extraction solvent.

3.1.3. Effect of disperser solvent volume

To obtain optimum volume of methanol, various volumes of methanol (40–450 μL) were tested with 150 μL of 1-octanol. It was observed that the extraction recovery was increased by increasing the volume of methanol up to 150 μL and then decreased (Fig. 5). At low volumes of methanol the cloudy state is not formed well, and the extraction recovery is low. At high volumes of methanol, the solubility of warfarin in water increases, thereby the extraction recovery decreases. Thus, 150 μL of methanol was chosen as the optimum volume.

3.1.4. Effect of sample pH

Warfarin is an acidic drug (pK_a = 5.0) [11]. To obtain high extraction efficiency for acidic compounds, the sample solution should be acidified to deionize the analytes and consequently increase their tendency to extract into the organic solvent. For this purpose, hydrochloric acid was used to adjust acidity (pH = 1.3–5.0). Results in Fig. 6 show that the highest extraction recovery was obtained at pH = 2.3. In a more acidic solution, a decrease in the extraction recovery was observed. This decrease can be attributed to

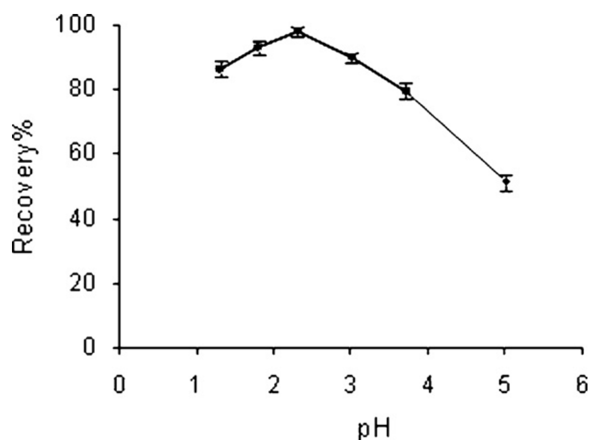

Fig. 6. Effect of sample pH on the extraction recovery of warfarin. Extraction conditions: disperser solvent (methanol) volume, 150 μL. Other conditions as in Fig. 5.

Table 1
Figures of merit of the proposed method for the analysis of warfarin in pretreated human plasma.

LOD (ng mL^{-1})	5
Linear range (ng mL^{-1})	15–3000
R^2	0.998
EF ± S.D.[a]	72.9 ± 2.2
ER (%) ± S.D.[b]	91.0 ± 3.8
RSD (%) (intra-day, *n* = 5)	2.8
RSD (%) (inter-day, *n* = 5)	6.5

[a] Mean enrichment factor ± standard deviation (*n* = 3).
[b] Mean extraction recovery ± standard deviation (*n* = 3).

protonation of the carbonyl groups of warfarin. Therefore, pH = 2.3 was used for subsequent experiments.

3.1.5. Effect of extraction time

In LDS-DLLME, extraction time is defined as the interval between injection of mixture of disperser solvent and extraction solvent, and the beginning of sample centrifugation. The effect of extraction time was investigated in the range of 0–30 min. Results showed that the extraction recovery was increased by increasing the extraction time up to 2 min and then remained constant. It is noted that the surface area between extraction solvent and sample solution is infinitely large. Therefore, the mass transfer from sample solution to extraction solvent is fast. As a result, equilibrium state is achieved quickly which cause the extraction time to be short. This is an important advantage of LDS-DLLME technique. The time of 2 min was chosen as the optimum extraction time.

3.1.6. Salt addition effect

Some researchers have reported that the addition of salt to the sample solution has been beneficial for the extraction efficiency in microextraction techniques [19,32]. So, in this study, the effect of salt addition on the extraction recovery was examined by adding different amounts of NaCl into the sample solution in the range of 0.0–3.0 M. Results showed that salt addition has no considerable effect on the extraction efficiency. Thus, the further studies were performed in the absence of salt.

3.2. Analytical performance

Under the optimum conditions, figures of merit of the proposed method including limit of detection (LOD), linear range, repeatability, reproducibility, extraction recovery and enrichment factor were evaluated for extraction and determination of warfarin in pretreated human plasma and results were summarized in Table 1. The LOD value (5 ng mL^{-1}) was calculated based on the signal to noise ratio of 3. Linear correlation was obtained between peak area of analyte and its concentration within the range of 15–3000 ng mL^{-1} with square of correlation coefficient (R^2) of 0.998. The enrichment factor (*EF*) was defined as the ratio of concentration of warfarin in the collected phase to its concentration in the pretreated plasma sample. The extraction recovery (*ER*) was calculated by the following equation:

$$ER = \frac{C_{col}V_{col}}{C_0 V_s} = EF\frac{V_{col}}{C_s}$$

where C_{col} and C_0 are the concentration of warfarin in the collected phase and the concentration of warfarin in the pretreated plasma sample, respectively. C_{col} of the extracted drug was obtained from calibration curve of standard solutions. V_{col} and V_s are the volume of collected phase and volume of the pretreated plasma sample, respectively.

The repeatability (intra-day) and reproducibility (inter-day) of the method were evaluated by carrying out five replicate extractions and determination of analyte at a concentration level of

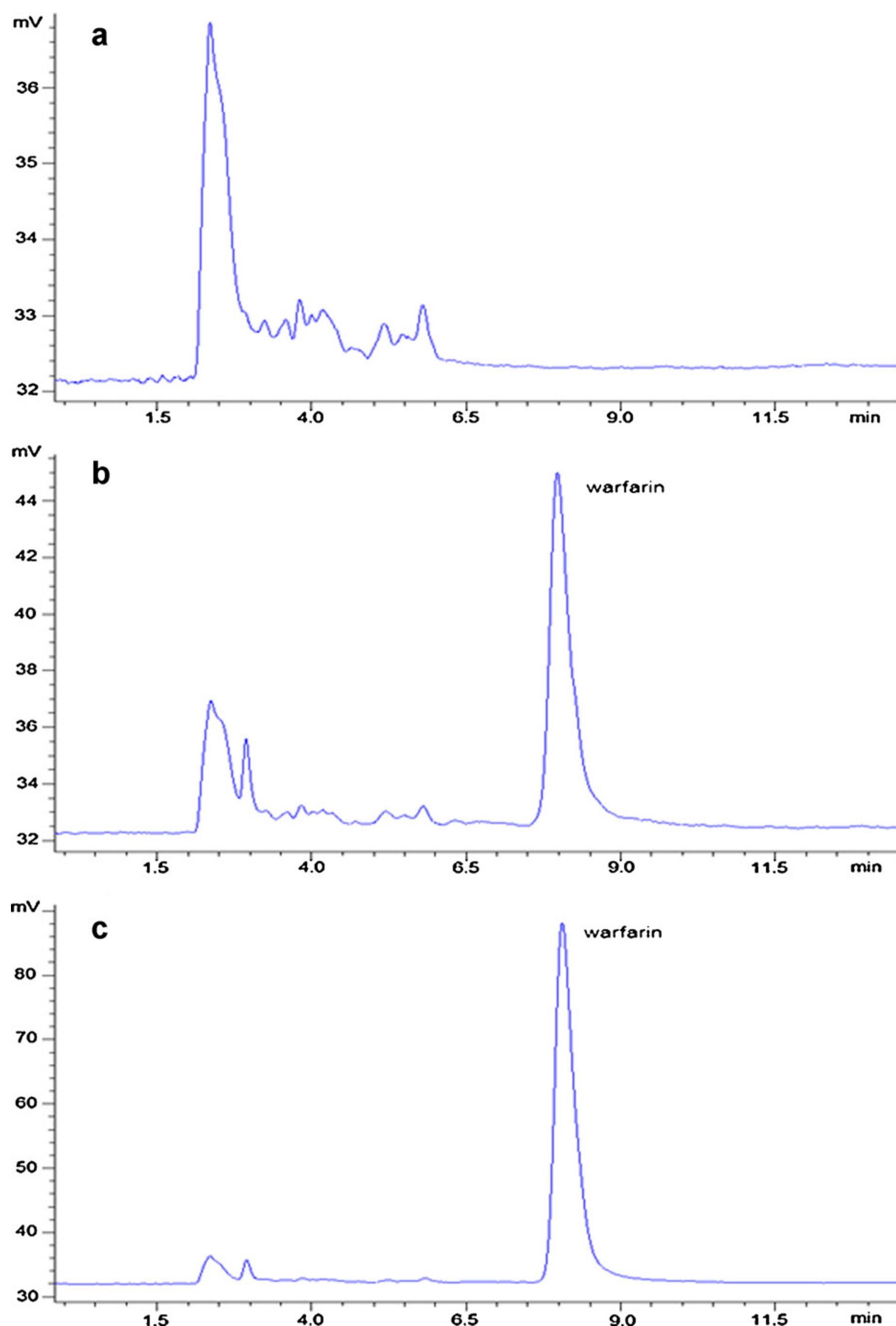

Fig. 7. HPLC chromatograms of (a) blank plasma sample, (b) patient plasma sample and (c) 300 ng mL^{-1} spiked patient plasma sample after extraction at optimum conditions. Mobile phase, methanol: 0.5% phosphoric acid (65:35, v/v); flow rate, 1.0 mL min^{-1}; column, C_{18} (250 mm × 4.6 mm, 10 μm); UV detection at 320 nm.

0.1 μg mL^{-1} during a day (intra-day) and five replicates in five subsequent days (inter-day). The values of intra-day relative standard deviation (RSD) and inter-day RSD were 2.8 and 6.5%, respectively.

3.3. Real sample analysis

Due to the importance of analysis of warfarin in plasma samples, the optimized method was applied to determine the concentration of this drug in the pretreated plasma sample from a patient under warfarin treatment, and the obtained results are summarized in Table 2. The chromatograms of LDS-DLLME extracts from blank plasma sample, patient plasma sample and spiked patient plasma sample are shown in Fig. 7(a–c). The chromatogram of blank plasma does not show any interfering peak at the retention time of warfarin. This indicates that warfarin can be determined without any interference in the sample.

3.4. Comparison of the applied method with other reported methods

Table 3 shows LOD, linear range and RSD of the present method (LDS-DLLME–HPLC-UV) and those of other methods reported in literature for analysis of warfarin. Compared with other reported methods (Table 3), in most cases the present method exhibits adequately low LOD, broad linear range (ratio of maximum linear range to the minimum linear range) and good repeatability and reproducibility, and low quantities of the solvent are consumed.

Table 2
Determination of warfarin in pretreated patient plasma sample.

C_{added} (ng mL^{-1})	C_{found} (ng mL^{-1})[a]	RSD (%) (n = 3)[b]	EF[c]	ER (%)[d]
–	83	3.0	–	–
100	177	3.9	70.4	90.0
300	369	3.6	69.9	88.1

[a] Mean concentration (n = 3).
[b] Intra-day RSD (%).
[c] Mean enrichment factor.
[d] Mean extraction recovery.

Table 3
Comparison of the present method with other reported methods for the determination of warfarin.

Method	LOD (ng mL^{-1})	Linear range (ng mL^{-1})	RSD (%) (intra-day)	RSD (%) (inter-day)	Real sample	Reference
LDS-DLLME–HPLC-UV[a]	5	15–3000	2.8	6.5	Plasma	This work
SPE-MEKC–ESI-MS[b]	100	250–5000	–	–	Plasma	[12]
LLE-HPLC–ESI-MS[c]	–	0.5–100	<8.5	<9.5	Serum	[33]
LLE–HPLC-UV[d]	–	120–3000	<6.89	<5.27	Plasma	[5]
Stripping voltammetry	0.3	15.4–123.3	–	–	Pharmaceutical Serum Urine	[34]
HF-LPME–HPLC-UV[e]	5	15–550	4.2	11.1	Plasma	[35]

[a] Low-density solvent-based dispersive liquid–liquid microextraction-high performance liquid chromatography-ultra violet detection.
[b] Solid-phase extraction-micellar electrokinetic chromatography–electrospray ionization-mass spectrometry.
[c] Liquid–liquid extraction-high performance liquid chromatography–electrospray ionization-mass spectrometry.
[d] Liquid–liquid extraction-high performance liquid chromatography-ultra violet detection.
[e] Hollow fiber liquid phase microextraction-high performance liquid chromatography-ultra violet detection.

4. Conclusions

In the present study, the LDS-DLLME technique followed by HPLC-UV was applied for extraction and determination of warfarin in aqueous solution and plasma sample. The presented method has high recovery, good repeatability and low limit of detection. Wide linear range of this method can be satisfactorily applied for warfarin in therapeutic drug monitoring. LDS-DLLME provides a simple, inexpensive, efficient and benign to the environment technique for extraction and preconcentration of warfarin from plasma samples.

References

[1] R. Denooz, Z. Douamba, C. Charlier, J. Chromatogr. B 877 (2009) 2344.
[2] C. Huang, J. Yang, Y. Du, L. Miao, Clin. Chim. Acta 393 (2008) 85.
[3] G. de Armas, M. Miró, J.M. Estela, V. Cerdà, Anal. Chim. Acta 467 (2002) 13.
[4] D. de Orsi, L. Gagliardi, L. Turchetto, D. Tonelli, J. Pharm. Biomed. Anal. 17 (1998) 891.
[5] S. Sun, M. Wang, L. Su, J. Li, H. Li, D. Gu, J. Pharm. Biomed. Anal. 42 (2006) 218.
[6] A. Osman, K. Arbring, T.L. Lindahl, J. Chromatogr. B 826 (2005) 75.
[7] I. Locatelli, V. Kmetec, A. Mrhar, I. Grabnar, J. Chromatogr. B 818 (2005) 191.
[8] P.R. Ring, J.M. Bostick, J. Pharm. Biomed. Anal. 22 (2000) 573.
[9] K.R. Henne, A. Gaedigk, G. Gupta, J.S. Leeder, A.E. Rettie, J. Chromatogr. B 710 (1998) 143.
[10] Z. Zuo, S. Kwan Wo, C.M.Y. Lo, L. Zhou, G. Cheng, J.H.S. You, J. Pharm. Biomed. Anal. 52 (2010) 305.
[11] W. Naidong, P.R. Ring, C. Midtlien, X. Jiang, J. Pharm. Biomed. Anal. 25 (2001) 219.
[12] J. Hou, J. Zheng, S.A. Shamsi, J. Chromatogr. A 1159 (2007) 208.
[13] W. Yau, E. Chan, J. Pharm. Biomed. Anal. 28 (2002) 107.
[14] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 22 (2003) 565.
[15] J. Lee, H.K. Lee, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 624 (2008) 253.
[16] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1.
[17] P. Liang, J. Xu, Q. Li, Anal. Chim. Acta 609 (2008) 53.
[18] C. Cortada, L. Vidal, R. Pastor, N. Santiago, A. Canals, Anal. Chim. Acta 649 (2009) 218.
[19] D. Nagaraju, S. Huang, J. Chromatogr. A 1161 (2007) 89.
[20] J. Xiong, B. Hu, J. Chromatogr. A 1193 (2008) 7.
[21] P. Hashemi, S. Beyranvand, R.S. Mansur, A.R. Ghiasvand, Anal. Chim. Acta 655 (2009) 60.
[22] M.A. Farajzadeh, D. Djozan, R.F. Bakhtiyari, Talanta 81 (2010) 1360.
[23] P. Zhang, Z. Shi, Q. Yu, Y. Feng, Talanta 83 (2011) 1711.
[24] A. Saleh, Y. Yamini, M. Faraji, M. Rezaee, M. Ghambarian, J. Chromatogr. A 1216 (2009) 6673.
[25] S. Wei, M. Leong, Y. Li, S. Huang, J. Chromatogr. A 1218 (2011) 9142.
[26] L. Guo, H.K. Lee, J. Chromatogr. A 1218 (2011) 5040.
[27] H. Chen, R. Chen, S. Li, J. Chromatogr. A 1217 (2010) 1244.
[28] M.A. Farajzadeh, S.E. Seyedi, M.S. Shalamzari, M. Bamorowat, J. Sep. Sci. 32 (2009) 3191.
[29] J.M. Steyn, H.M. Van Der Merwe, M.J. De Kock, J. Chromatogr. B: Biomed. Sci. Appl. 378 (1986) 254.
[30] C. Polson, P. Sarkar, B. Incledon, V. Raguvaran, R. Grant, J. Chromatogr. B 785 (2003) 263.
[31] http://www.merck-chemicals.com (accessed 09.12.11).
[32] A. Mohammadi, Y. Yamini, N. Alizadeh, J. Chromatogr. A 1063 (2005) 1.
[33] M. Jin, X. Chen, H. Chen, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 2641.
[34] M.M. Ghoneim, A. Tawfik, Anal. Chim. Acta 511 (2004) 63.
[35] M. Hadjmohammadi, H. Ghambari, J. Pharm. Biomed. Anal. 61 (2012) 44.