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Sensitive determination of deferasirox in blood of patients with thalassemia using dispersive liquid-liquid microextraction based on solidification of floating organic drop followed by HPLC–UV

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ABSTRACT

Deferasirox is an oral iron chelator that has been on the market since 2005 and has been a suitable replacement for injectable chelators. It is important to check the amount of this drug in the blood of patients due to side effects. In this study, a new dispersive liquid-liquid microextraction based on solidification of floating drganic drop (DLLME – SFO) was applied to the extraction of deferasirox in the blood of patients with thalassemia prior to its analysis by high-performance liquid chromatography–ultraviolet detection (HPLC – UV). In this method, two long alcohols of the normal chain are mixed in a particular ratio, and then it is injected into the sample solution, which is on the magnetic stirrer. In this case, the mixture of the two alcohol changes to new double-solvent aggregate. This new double-solvent system is used as an extractant, which has a higher extraction power than any of its components alone. Under the optimum conditions, the calibration graph was linear in the rage of 0.2–200 μg L^{-1} with detection limit of 0.06 μg L^{-1}. Repeatability (intra–day) and reproducibility (inter–day) of method based on seven replicate measurements of 100.0 μg L^{-1} of deferasirox were 3.8 % and 5.7 %, respectively. The results showed that DLLME – SFO is a very simple, inexpensive, environmental friendly, sensitive and efficient analytical method for the determination of trace amount of drugs in biological samples and suitable results were obtained.

1. Introduction

Iron is one of the essential elements that help maintain the human body's metabolic and biological processes. However, the presence of excess iron deposits in some organs and damages the liver, myocardium, spleen and endocrine organs, causing heart failure, diabetes, hypothyroidism, hypogonadism and liver disease as cirrhosis or liver cancer [1]. Thalassemia is the most common monogenetic disorder globally, and more than 60,000 children are born with thalassemia, annually [2]. Regular blood transfusions will be necessary to treat anemia and reduce dysfunctional erythropoiesis, but unfortunately, transfusions can be problematic if the iron is overloaded [3]. Prolonged blood transfusions may cause excess iron in patients such as beta-thalassemia, sickle cell anemia (SCA), and aplastic anemia [4]. Therefore, iron chelating agents are needed to prevent iron overload. Deferoxamine is the first chelator to be used 30 years ago, but its injectable administration and short half-life have limited its use [5]. Also, in repeated subcutaneous injections, the patient gradually becomes tired of subcutaneous injection of the drug. The patient's tolerance for drug use decreases, which in turn causes a vicious cycle in increasing the iron load in the body. Deferiprone is another iron chelator prepared in the form of solid tablets and is prescribed three times a day. It has a narrow therapeutic window, and its safety risks may include agornocytosis and arthropathy [6]. The disadvantages of these chelators made researchers move to a better chelator. Deferasirox is an oral iron chelator that has been on the market since 2005 and has been a suitable replacement for injectable chelators and has been approved by the US Food and Drug Administration (FDA) and licensed by the European Medicines Agency (EMA) for this purpose [7]. This drug is currently prescribed in more than 85 countries as a first-line treatment for thalassemia and iron overload [8]. Based on the deferasirox pharmacokinetics, this drug is suitable for once-daily oral administration because it can provide chelating coverage

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in patients with transfusion anemia for 24 h [9]. Patients with thalassemia require lifelong use of chelators with iron overload. Therefore, these people may be exposed to the side effects of these drugs. In deferasirox's use, side effects such as gastrointestinal disorders, renal disorders (creatinine increase), and dose-dependent proteinuria have been observed, which are usually reversible upon discontinuation of the drug [10]. On the other hand, discontinuation of the drug leads to an increase in iron load. To optimize the dosage and minimize the risk of side effects, it is necessary to measure the concentration of deferasirox in plasma accurately.

Numerous instrumental techniques such as high performance liquid chromatography- ultraviolet detection (HPLC–UV) [6,11,12], liquid chromatography ion–trap mass spectrometry (LC–ITMS) [13], liquid chromatography-mass spectrometry/mass spectrometry (LC–MS/MS) [2,9,14], micellar electrokinetic chromatography [4], terbium-sensitized fluorescence [15] and cyclic voltammetry, chronoamperometry and electrochemical impedance techniques [16] have been used for the sensitive and selective determination of deferasirox in biological fluids or its dosage forms. For determination of deferasirox LC–MS is often used because of its sensitivity, but the cost of the technique itself is restricting its applicability. On the other hand, HPLC–UV appears as atechnique that could be found in all research laboratories.

The complexity of biological samples, due to the large number of different compounds, may hinder the method selectivity. However, in spite of the development of modern analytical instruments, a purification and enrichment step for the determination of low amounts of deferasirox is still required.

The sample preparation stage is the most essential and critical part of the analysis process, often associated with spending much time, high consumption of organic solvents, and inaccuracy. In order to overcome these problems, miniature extraction techniques based on liquid-liquid extraction have been developed today. One of the best of these techniques is dispersive liquid-liquid microextraction (DLLME), the conventional mode of which was first introduced as a triple solvent system consisting of an extracting solvent, a disperser solvent, and an aqueous phase. DLLME has been used for extraction and preconcentration of organic and inorganic species in different matrices [17–20]. The advantages and disadvantages of this technique are mentioned in the articles [21–25]. To be more compatible with the green chemistry, DLLME based on solidification of floating organic droplet (SFO) [26–28], DLLME based on the deep eutectic solvent (DES) [29–31] and supramolecular solvent microextraction [32–34] have emerged. These innovations are aimed at further reducing the consumption of organic solvents and using organic extraction solvents lighter than water with less toxicity, cheaper, and more environmentally friendly. In the DLLME–SFO method, long-chain alcohols are used as the extraction solvent, characterized by lower density than water, lower toxicity, cheaper, and an environmentally friendly and freezing point near room temperature. Although their extraction efficiencies are high in most cases, they do not have acceptable efficiencies in the extraction of some analytes.

For the first time in the present research, a double-solvent system (DSS) was developed for DLLME. In this method, the first two long alcohols of the normal chain are mixed in a particular ratio, and then it is injected into the sample solution, which is on the magnetic stirrer. In this case, the mixture of the two alcohols is changed to new double-solvent aggregate and diffused in tiny droplets with a very high contact surface (without the need for a disperser solvent) in the sample solution. In this case, the extraction of analytes with different polarities is done through intermolecular hydrogen bonding or high-efficiency hydrophobic interaction. When the stirring stops, the DSS, which also contain analytes (without the need for centrifugation), slowly accumulate on the surface of the sample solution and float in a droplet. Separation of the organic and aqueous phases is possible by cooling the sample solution and solidifying the organic phase.

The DLLME–DSS method was evaluated to determine the level of deferasirox in blood of patients with thalassemia by HPLC–UV. High extraction recovery, lower solvent consumption, ease of operation, and low cost are some of the advantages of this method. The other advantages of the proposed method include the use of DSS as a non-toxic, powerful and cheap extractant which makes the method environmentally friendly. Compared with conventional DLLME, DLLME–DSS does not require a disperser solvent and centrifugation.

2. Experimental

2.1. Reagents and solutions

Deferasirox (purity ≥99 %) and mifepristone (purity ≥98 %) as an internal standard (IS) were kindly provided from Novartis Pharma AG (Basel, Switzerland). The deferasirox stock standard solution was prepared in methanol and double-distilled water (50:50, v/v) at the concentration level of 1000 mg L^{-1} and was stored in a freezer at −20 °C. More diluted working solution were prepared daily by diluting the stock standard solution. Stock solution of internal standard was prepared by dissolving an accurately weighed amount of mifepristone in methanol to obtain a final concentration of 1000 mg L^{-1}, then stored at −20 °C. The ultra-pure water (six times distillated) used was obtained from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). Methanol (for spectroscopy), acetonitrile (hyper grade for liquid chromatography), trimethylamine (analytical grade), 1-undecanol, 1-dodecanol, 1-decanol, orthophosphoric acid, hydrochloric acid and sodium chloride were obtained from Merck (Darmstadt, Germany).

2.2. Instrumentation

The analysis of deferasirox was carried out on a HPLC Knauer equipped with a quaternary pump, online degasser, detector Smartline-UV-2500 variable wavelength programmable (Berlin, Germany) and manual sample injector fitted with a 20 μL injection loop (model 7725i, Rheodyne, Cotati, CA, USA). Separation was achieved with H5-ODS C_{18} column (15 cm ×4.6 mm, with 5 μm particle size) from Anachem (Luton, UK), preceded by a Security Guard Cartridge C_{18} (Anachem, Luton, UK). The mobile phase consisted of solvent A/acetonitrilie/methanol (40:40:20, v/v). Solvent A consisted of trimethylamine (2 %), methanol (20 %) and water (78 %), adjusted for pH 5 by orthophosphoric acid. Amobile phase flow-rate of 1.0 mL min^{-1} was used in isocratic elution mode and the analyte was detected at 254 nm. The pH values were measured by Metrohm pH meter Model 692 (Herisau, Switzerland).

2.3. Sampling and sample preparation

Blood samples were taken from patients with thalassemia who were admitted and treated in the Dr. Mohammad Kermanshahi Hospital from Kermanshah, Iran. In this way 1 female and 3 male (aged 11–26 years) were randomly selected and 1.0 mL blood sample of each of them was taken and transferred to our research lab. For preparation and clean up of samples, 400 μL of whole blood was placed in EDTA-contained tube and one mL mixture of acetonitrile and $ZnSO_4$ (15 %, w/v) (2:3) was added to a test tube and vertex for 10 min. After holding the test tube at 5 °C for few minutes, it was centrifuged at 6000 rpm for 5 min. The obtained supernatant were transferred to another clean tube and was reached to a volume of 5 mL by distilled water to reduce the matrix effects. The resulting solution was then subjected to the DLLME–DSS process.

2.4. Extraction procedure

A 5.0 mL of a pretreated and diluted blood sample (spiked or not with deferasirox) containing 1.0 μg L^{-1} of mifepristone (internal standard) was placed in a 10-mL glass vial. The pH of the sample solutions were adjusted to an appropriate amount (pH = 5.5 by using phosphate buffer) and vial fixed on a magnetic stirrer. Fourty microliter of double extractant (1-undecanol/1-decanol; 2:5 v/v) was injected rapidly into the sample solution and the magnetic stirrer was turned on at 1000 rpm for 20 min. The double extractant dispersed in tiny droplets with a very high contact surface (without the need for a disperser solvent) in the sample solution. In this case, the extraction of analytes with different polarities is done through intermolecular hydrogen bonding or high-efficiency hydrophobic interaction. When the stirring stops, the extractant, which also contain analytes (without the need for centrifugation), slowly accumulate on the surface of the sample solution and float in a droplet. The glass vialis put in the freezer for a few minutes to solidify the double extractant, and the solid phase is transferred to aconic tube with a spatula. After melting at room temperature, diluted to 50 μL with methanol, and 30 μL of the solution was used for HPLC analysis.

3. Results and discussion

3.1. Selection of double extractants

Appropriate extractant should have melting point close to room temperature, high extraction efficiency, less toxic and its solubility in the aqueous phase should be low. For the above reasons, 1-decanol, 1-undcanol, and 1-dodecanol solvents were selected. At first, each of the solvents was used as an extractant, and then their double mixture with a specific ratio was used. The results in Fig. 1(A) shows that the extraction efficiency of deferasirox with each solvent alone is not more than 55 %, but when two of the solvents are mixed in a particular ratio, the extraction efficiency increases. Although the extraction efficiencies are comparable in all double extraction systems, in the 1-undcanol/1-decanol double extraction system, the extraction efficiencies are slightly better, and the standard deviation is low. As a result, 1-undcanol/1-decanol double system was selected as the optimal double extraction system.

3.2. Proportion of double extractants

In this research, the most suitable proportion of double extractants was obtained to achieve high extraction efficiency. For this purpose, the double extractants were obtained by using 1-undecanol and 1-decanol with different ratios of 1:1, 1:2, 1:3, 2:5, 3:7, 2:1, 5:2 and 7:3. Experiments in Fig. 1(B) show that 1-undecanol and 1-decanol at a 1:1, 2:1, 5:2 and 7:3 M ratios could not form DSS. The mixture of 1-undecanol and 1-decanol in other ratios has a positive effect on the extraction of the deferasirox. However, double extractant obtained from a mixture of 1-undecanol and 1-decanol in a 2:5 ratio, has higher extraction efficiency and lower standard deviation. Therefore, the 2:5 ratio was selected as the optimum ratio of 1-undecanol and 1-decanol.

3.3. Selection of double extractant volume

The previous section shows that 1-undcanol and 1-decanol with a 2:5 vol ratio are the best double-solvent system. The volume of this dual extraction system plays a significant role in the enrichment factor and extraction efficiency of deferasirox. To study the effect of double extractant volume on the extraction efficiency of deferasirox, different volumes of double extractant (20, 30, 40, 50, 60 and 70 μL) were tested to select the optimum volume of double extractant to be applied in subsequent experiments. According to the results in Fig. 2(A), at volumes less than 40 μL, the contact surface is not high, and the extraction efficiency is low. Not enough volume is obtained for injection into the HPLC, and the repeatability is significantly reduced. At volumes more significant than 40 μL, the dual extractant cannot quickly accumulate on the surface of the solution, and the enrichment factor decreases due to the dilution effect. Thus, in order to have a high extraction recovery and good repeatability, 40 μL of double extractant was selected asthe optimum volume.

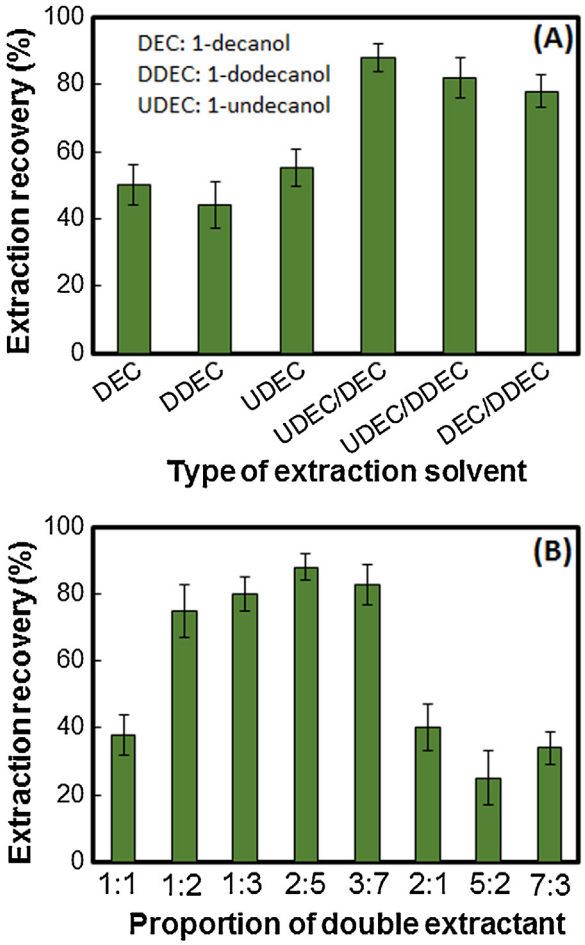

Fig. 1. Effect of the different types of extractant (A) and proportion of double extractant (B) on the extraction recovery of the deferasirox. Extraction conditions: volume of the sample solution, 5 mL; pH, 5.5; volume of the extraction solvent, 50 μL; stirring speed, 1000 rpm; extraction time, 20 min; room temperature.

3.4. Selection of sample solution pH

The sample solution pH will affect the existence form of the deferasirox in the sample solution, so the pH will play an important role in the extraction efficiency. To evaluate the effect of sample solution pH, various experiments were performed by different pH of aqueous solution (1 – 10). Other experimental conditions were kept constant. Fig. 2 (B) shows that the deferasirox can hydrolyzein acidic and alkaline media while it is stable at pH = 5 – 6. Thus the pH of sample solution was fixed at 5.5 by a phosphate buffer.

3.5. Effect of ionic strength

For evaluating the ionic strength on the performance of DLLME–DSS, some experiments were performed by different con-

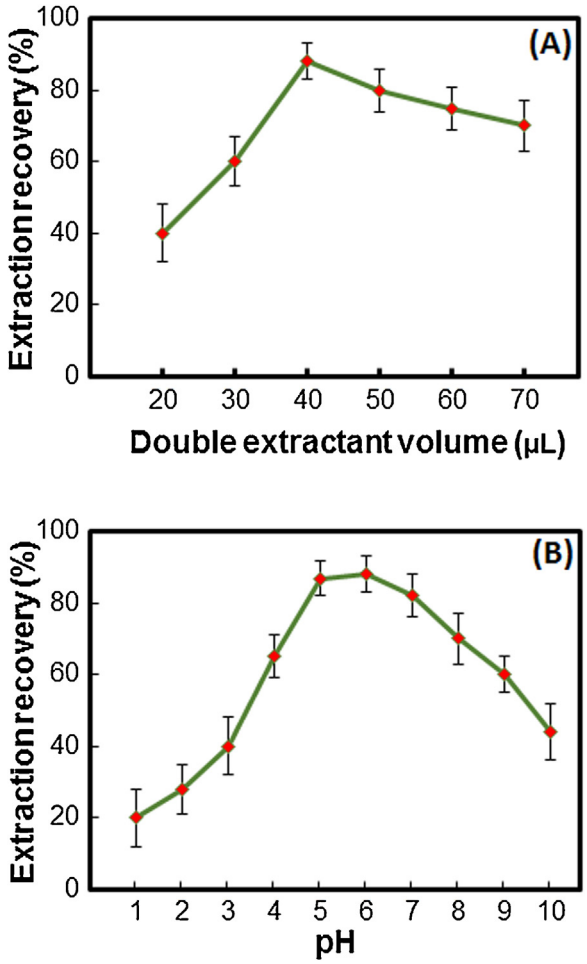

Fig. 2. Effect of the double extractant volume (A) and sample solution pH (B) on the extraction recovery of the deferasirox. Extraction conditions: as in Fig. 1; types of extractant, 1-undecanol/1-decanol; proportion of 1-undecanol/1-decanol, 2:5.

centrations of NaCl (0–10 %). The results indicated that with increasing NaCl from 0 to 4 %, the extraction of deferasirox remain almost constant, because two opposite effects occur in the extraction efficiency, which neutralize each other's effect. On the one hand, with increasing NaCl, the polarity of the aqueous phase increases and reduces the solubility of the deferasirox in the aqueous phase, thus increasing the extraction efficiency (salting-out). On the other hand, increasing the salt reduces the solubility of double extractant and increases the volume of the obtained organic phase and the dilution effect occurs. At concentrations greater than 4 %, the dilution effect prevails on salting-out and the extraction efficiency decreases. As a result, all the extraction experiments were carried out without salt addition.

3.6. Effect of extraction time

The extraction time is an important factor that may affect the analytes' extraction efficiency from aqueous solution into the DSS. In the DLLME–DSS, extraction time is defined as the time between injecting the double extractant and turn off the magnetic stirrer. The extraction time must be high enough to perform an effective extraction of the analytes. On the other hand, it must be low enough not to waste time. Thus, the variation in extraction recovery of deferasirox as a function of extraction time was studied in therange of 5–50 min. When the extraction time increased from 5 to 20 min, the extraction recovery of deferasirox was increased due to the mass transfer of analyte from cellular material to double extractant by diffusion and osmosis. However, the extraction recovery had no noticeable enhancement when the extraction time increasing from 20 min to 50 min (Fig. 3(A)). Therefore, 20 min was selected as the optimum extraction time.

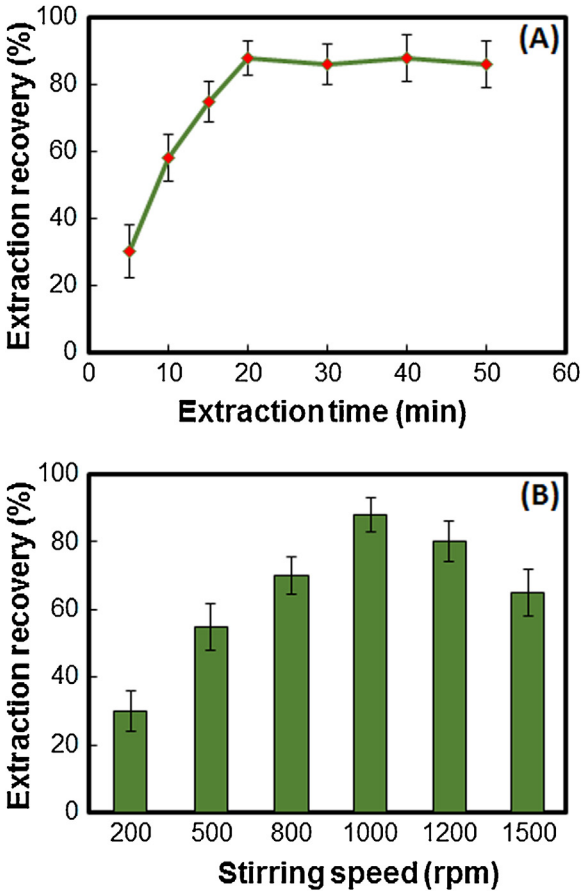

Fig. 3. Effect of the extraction time (A) and stirring speed (B) on the extraction recovery of the deferasirox. Extraction conditions: as in Figs. 1& 2.

3.7. Effect of stirring speed

Magnetic stirring can accelerate mass transfer and promote the dispersion of the double extractant into the sample solution; thus, stirring improves the extraction efficiency of the analyte in the double extractant. A proper increase in stirring speed can improve extraction efficiency. However, if the extraction speed is too high, the solution will spatter and organic droplets will be destroyed. Different stirring speeds (200, 500, 800, 1000, 1200 and 1500 rpm) were investigated. The stirring speed of 1000 rpm obtained the highest extraction efficiency (Fig. 3(B)). Thus, 1000 rpm was selected as the stirring speed.

3.8. Analytical performance

Validation of the DLLME–DSS method was carried out by investigating some analytical figures of merit such as linear dynamic range (LR), limit of detection (LOD), limit of quantification (LOQ), repeatability (intra–day), reproducibility (inter–day), extraction recovery (ER) and enrichment factor (EF). The characteristics of the calibration curve, summarized in Table 1, were obtained under the optimized conditions. Linearity was observed in the range of 0.2–200 μg L^{-1} for deferasirox with coefficient of determination (r^2) of 0.9988. The repeatability and reproducibility of the present method was calculated by using RSD (n = 7) having 100 μg L^{-1} of

Table 1
Analytical characteristics of DLLME–DSS–HPLC–UV for determination of deferasirox.

Parameter	Analytical feature
Linear range ($\mu g\ L^{-1}$)	0.2-200
RSD% (Intra-day, n = 7, by using internal standard at concentration of 100 $\mu g\ L^{-1}$ for deferasirox)	3.8
RSD% (Inter-day, n = 7, by using internal standard at concentration of 100 $\mu g\ L^{-1}$ for deferasirox)	5.7
RSD% (Intra-day, n = 7, without using internal standard at concentration of 100 $\mu g\ L^{-1}$ for deferasirox)	4.5
RSD% (Inter-day, n = 7, without using internal standard at concentration of 100 $\mu g\ L^{-1}$ for deferasirox)	6.2
r^2 (by using internal standard)	0.9988
r^2 (without using internal standard)	0.9965
Limit of detection ($\mu g\ L^{-1}$) (S/N = 3, n = 7)	0.06
Limit of quantification ($\mu g\ L^{-1}$) (S/N = 10, n = 7)	0.2
Extraction recovery (%)	88.2
Enrichment factor	147

deferasirox and were 3.8 and 5.7 %, respectively. The LOD (S/N = 3) and LOQ (S/N = 10) were 0.06 $\mu g\ L^{-1}$ and 0.2 $\mu g\ L^{-1}$, respectively. The EF and ER% of the method were 147 and 88.2 %, respectively, at the concentration level of 100 μgL^{-1} of deferasirox and the sample volume of 5.0 mL.

3.9. Analysis of blood samples

The efficiency of the DLLME–DSS method was validated with the monitoring of the deferasirox in blood of patients with thalassemia. Blood samples were taken from a 22-year-old woman as a healthy volunteer who had not previously received deferasirox (control) and three patients with thalassemia who were being treated at Dr. Mohamad Kermanshahi Hospital. The first case was a 12-year-old boy with beta-thalassemia with a blood ferritin level of 1300 who had been taking deferasirox oral tablets for two months and two 360 mg tablets a day. The second case involved a 24-year-old man with beta-thalassemia with a blood ferritin level of 1500 who had been taking deferasirox oral tablets for six months and three 360 mg tablets a day. The third case was a 26-year-old man with beta-thalassemia with a blood ferritin level of 2500 who had been taking deferasirox oral tablets for one year and 360 mg four tablets a day. Samples were taken twice from each of the three selected patients, once 3 h before taking the first deferasirox tablet (-3) and the second time 3 h after taking the first deferasirox tablet (+3). Consumed dosage based on body weight and blood ferritin level was between 20 and 40 mg/kg body weight per day. Samples were separated by gender and age, and analyzed 48 h after sampling. Results in Table 2 show that all blood samples contain deferasirox with different concentrations in the range of 12.3 ± 0.5–51.2 ± 3.6 mg L^{-1}. Fig. 4 shows the obtained chromatograms of direct injection of deferasirox and mifepristone standard at concentration level of 1.00 mg L^{-1}, blood sample taken from 26-year-old man and the corresponding spiked ones at concentration level of 30.0 $\mu g\ L^{-1}$ for deferasirox.

To validate the method and evaluate the effects of the matrix, all samples were spiked with standard solution of deferasirox. Relative recovery of deferasiroxin spiked samples at different concentrations is shown in Table 2, ranging from 92 to 106 %. These results demonstrate that the blood matrices, in our present context, have no significant effect on DLLME–DSS–HPLC–UV for determination of deferasirox.

3.10. Comparison of DLLME–DSS with other methods

The proposed DLLME–DSS method is compared with other analytical methods for extraction and preconcentration of different drugs in biological samples and the results are summarized in Table 3. As shown in Table 3, the detection limit of this method is lower than other methods and consumption of organic solvent is greatly reduced. The RSD and linear range of the proposed method are superior to those reported before. Compared to other methods, the extraction time is relatively short except for the DLLME method.

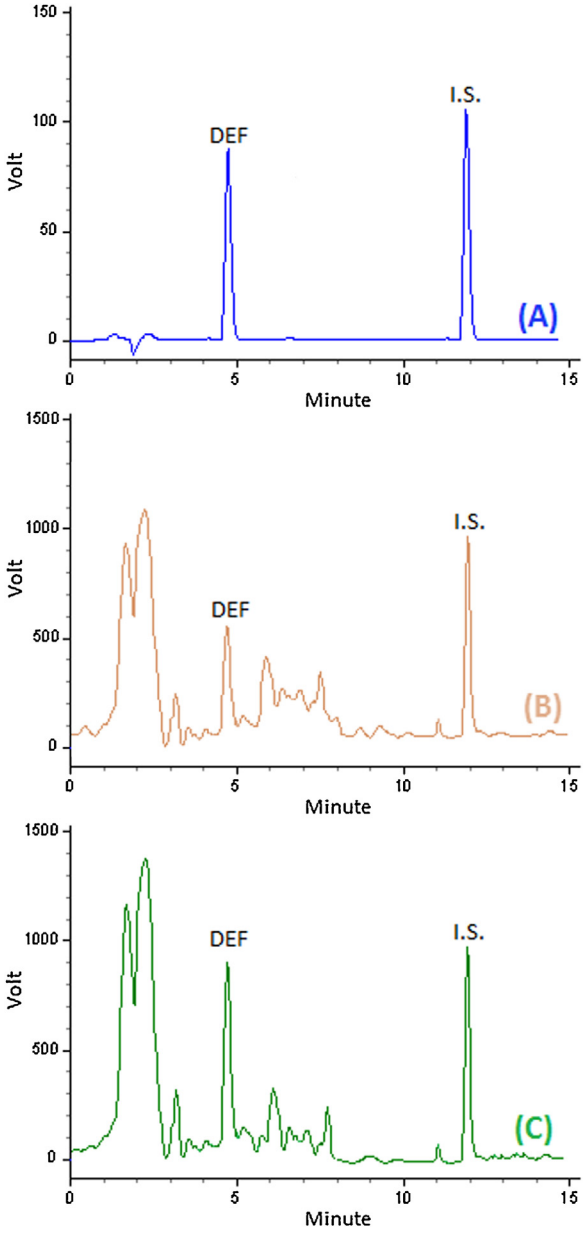

Fig. 4. Chromatograms of direct injection of deferasirox and mifepristone standards at concentration level of 1.00 mg L^{-1} (A), blood sample taken from 26-year-old man (B) and the corresponding spiked ones at concentration level of 30.0 $\mu g\ L^{-1}$ for deferasirox (C) obtained by using DLLME–DSS combined HPLC–UV. Peak identification: (DEF) deferasirox, (I.S.) internal standard.

Table 2
Determination of deferasirox in blood samples and relative recovery of spiked deferasirox in these samples.[a].

Blood samples	Time of sampling (h)	Concentration, mean ± SD[b] (n = 3) (mg L^{-1})	Added (mg L^{-1})	Found, mean ± SD (n = 3) (mg L^{-1})	Relative recovery (%)
Taken from a 22-year-old female (healthy volunteer)	–	n.d.[c]	50	52.5 ± 2.6	105
Taken from a 12-year-old male	−3	12.3 ± 0.5	10	21.6 ± 1.4	93
	+3	32.4 ± 2.2	10	42.5 ± 2.3	101
Taken from a 24-year-old male	−3	19.5 ± 1.3	20	40.1 ± 2.8	103
	+3	42.0 ± 2.8	20	61.6 ± 3.5	98
Taken from a 26-year-old male	−3	39.6 ± 1.7	30	71.5 ± 4.1	106
	+3	51.2 ± 3.6	30	78.8 ± 3.6	92

[a] These data are based on the diluted volumes of blood samples and dilution effect was considered for calculation of them.
[b] Standard deviation.
[c] Not detected.

Table 3
Comparison of DLLME–SFO–HPLC–UV with other extraction methods for determination of different drugs in biological samples.

Methods	Aalyte	LOD[a] (μg L^{-1})	LR[b] (μg L^{-1})	RSD[c] %	EF[d]	Extraction time (min)	Extractant volume (μL)	Reference
VA–LC–MS/MS[e]	Deferasirox	10	40–40000	2.9–7.4	–	<10	800	[2]
SI–sweeping–FASS–MEKC[f]	Deferasirox	300	1000–20000	4.13–6.22	2	110	1000	[4]
PPE–HPLC–UV[g]	Deferasirox	78.1	78.1–40000	4.64	–	16	750	[6]
HILIC–ESI–MS[h]	Deferasirox	61	200–120000	1.8	–	<15	2000	[9]
DLLME–SFO–HPLC–UV[i]	Amphetamines	2–8	10–3000	6.2–7.8	117–125	10	330	[26]
DLLME–SFO–HPLC–UV	Opium alkaloids	0.5–5	1.5–1000	4.3–7.4	110.4–165	10	530	[27]
CCSHLLE–DLLME–SFO–HPLC–UV[j]	Amphetamines	0.5–2	1–3000	4–5	157–168	<20	1034	[35]
DLLME–SFO–HPLC–UV	Deferasirox	0.06	2–200	3.8	147	<20	40	This work

[a] LOD, limit of detection.
[b] LR, linear range.
[c] RSD, relative standard deviation.
[d] Enrichment factor.
[e] Vortex assisted–liquid chromatography–mass spectrometry.
[f] Short-end injection and sweeping with a field-amplified sample stacking–micellar electrokinetic chromatography.
[g] Protein precipitation extraction–high performance liquid chromatography–ultraviolet detection.
[h] Hydrophilic interaction liquid chromatography/positive ion electrospray mass spectrometry.
[i] Dispersive liquid–liquid microextraction based on the solidification of floating organic drop–high performance liquid chromatography–ultraviolet detection.
[j] Counter current salting-out homogenous liquid–liquid extraction and dispersive liquid–liquid microextraction–high performance liquid chromatography–ultraviolet detection.

However unlike the DLLME method, in this method the disperser solvent are not required and no centrifugeis required for the collection of extraction solvent. All these results indicate that DLLME–DSS is a simple, inexpensive and reproducible technique that can be used for the extraction and preconcentration of deferasirox in blood samples.

4. Conclusions

In this research for the first time, a DLLME based on double-solvent system combined with HPLC–UV has been proposed for the determination of deferasirox in blood of patients with thalassemia. The advantages of this method include a simple operational procedures, inexpensive, environmental friendly, dispersive-solvent-free and low solvent consumption. We expected this method will be a breakthrough in separation science for the extraction of different drugs in blood samples. In this method, the first two long alcohols of the typical chain are mixed in a particular ratio, and then it is injected into the sample solution, which is on the magnetic stirrer. In this case, the mixture of the two alcohol changes to new double-solvent aggregate. This new double-solvent system is used as an extractant, which has a higher extraction power than any of its components alone. In conclusion, DLLME–DSS combined with HPLC–UV can be used for the extraction and analysis of deferasirox in blood of patients with thalassemia.

Author statement

Deferasirox is an oral iron chelator that has been on the market since 2005 and has been a suitable replacement for injectable chelators. It is important to check the amount of this drug in the blood of patients due to side effects. In this study, a new dispersive liquid-liquid microextraction based on double-solvent system (DLLME–DSS) was applied to the extraction of deferasirox in the blood of patients with thalassemia prior to its analysis by high-performance liquid chromatography–ultraviolet detection (HPLC–UV). In this method, the first two long alcohols of the typical chain are mixed in a particular ratio, and then it is injected into the sample solution, which is on the magnetic stirrer. In this case, the mixture of the two alcohol changes to new extractant aggregate. This new double-solvent is used as an extractant, which has a higher extraction power than any of its components alone.

Declaration of Competing Interest

The authors report no declarations of interest.

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