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Dispersive liquid-liquid microextraction based on deep eutectic solvent for elemental impurities determination in oral and parenteral drugs by inductively coupled plasma optical emission spectrometry

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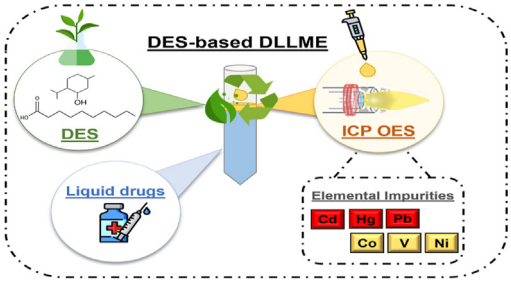
HIGHLIGHTS

- An eco-friendly DLLME procedure using a synthesized hydrophobic DES is proposed.
- Application of a new, safe, cheap and biodegradable DES as extractant solvent.
- DES-based DLLME for extraction/preconcentration of six elements from liquid drugs.
- Synergistic combination of DLLME and ICP OES for trace elemental determination.
- A sensitive DES-based DLLME-ICP OES method meeting the green analytical principles.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple, fast, sensitive and green pretreatment method for determination of Cd, Co, Hg, Ni, Pb and V in oral and parenteral drug samples using inductively coupled plasma optical emission spectrometry (ICP OES) has been developed. According to United States Pharmacopoeia (USP), those metals must be reported in all pharmaceutical products for quality control evaluation (i.e., elemental impurities from classes 1 and 2A of USP Chapter 232). To improve the analytical capabilities of ICP OES, a dispersive liquid-liquid microextraction (DLLME) has performed using a safe, cheap and biodegradable deep eutectic solvent (DES) as extractant solvent (a mixture of 2:1 M ratio of DL-menthol and decanoic acid). Seven parameters affecting the microextraction efficiency have carefully optimized by multivariate analysis. Under optimized conditions, the DES-based DLLME-ICP OES procedure improved limit of quantitation (LOQ) values on range from 12 to 85-fold and afforded an enrichment factor on average 60-times higher than those obtained to direct ICP OES analysis. Consequently, LOQ values for Cd, Co, Hg, Ni, Pb and V have been on average 10-times lower than target limits recommended for drugs from parenteral route of administration. Trueness has evaluated by addition and recovery experiments following USP recommendations for three oral drug samples in liquid dosage form and three parenteral drugs. Recovery and RSD values have been within the range of 90–109% and 1–6%, respectively. All

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analytes were below the respectives LOQ values, hence, lower than the limits proposed by USP Chapter 232.

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1. Introduction

The presence of elemental impurities as compounds in drug products can potentially have adverse health effects and therefore must be carefully monitored. According to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [1] and USP Chapter 232 [2], permissible daily exposures (PDE) values for elemental impurities are established for pharmaceuticals from three routes of administration (i.e., oral, parenteral, and inhalational). These target elements are also grouped into four main categories based on the toxicity and their likelihood of occurrence: class 1 (As, Cd, Hg, Pb), class 2A (Co, Ni, V), class 2B (Ag, Au, Ir, Os, Pd, Rh, Pt, Ru, Se, Tl), and class 3 (Ba, Cu, Cr, Li, Mo, Sb, Sn).

Class 1 elements are extremely toxic to humans whereas class 2A elements have relatively high probability of occurrence in pharmaceuticals. Thus, both classes must be evaluated in all potential sources of contamination. On the other hand, classes 2B and 3 show lower toxicity and a reduced probability of occurrence in pharmaceuticals, so they may be excluded from the risk assessment unless they are intentionally added during the manufacture of excipients or other components of drug products [1–3]. Considering the target-limits recommended for classes 1 and 2A, higher PDE values for Co, Ni and V are suggested for drugs administered via oral route. Nonetheless, for parenteral medications, the PDEs for the above-mentioned elements are 10-times lower and, along with elements from class 1, range from 2 to 20 μg day^{-1} [2].

Currently, Green Analytical Chemistry (GAC) [4] concept is of high importance and developing green analytical methods is a current challenge to modern analytical chemistry. Green alternatives, based on GAC and White Analytical Chemistry (WAC) [5] principles would include practices such as preferential use of multi-analyte or multi-parameter methods, low-toxic and biodegradable reagents, reducing reagent consumption and waste production, increased safety of the analyst, and an increasing degree of integration, automation, simplification, and miniaturization of analytical procedures [4–6]. Thus, the implementation of GAC and WAC principles needs to be properly balanced with functionality of the method based on analytical efficiency expressed by validation criteria (trueness, precision, and sensitivity), reason why most of recently introduced green analytical procedures [7–12] are characterized by not only environmental friendliness, but also relatively high sensitivity, simplicity, time-saving and low-cost analysis.

An effective sample preparation procedure is crucial to accurate elemental determination in complex matrices using spectroanalytical techniques [13,14]. For this reason, several sample preparation for pharmaceutical products (e.g., drugs, excipients, and active pharmaceutical ingredients) have been developed for elemental determination by ICP-based methods [3,15]. These procedures significantly depend on the dosage form of the drug (i.e., tablet, pill or liquid) and include different sample preparations [15–19]. Moreover, based on the target-limits from USP Chapter 232, when analytical instrument is not sensitive enough for direct analyte quantification at trace/ultra-trace levels, a specific procedure entailing an effective extraction/preconcentration methodology prior to quantification is also required [20].

Accordingly, dispersive liquid-liquid microextraction (DLLME) is a successful extraction technique in which few microliters of a water-immiscible and a dirpersive solvents form a cloudy solution when injected into an aqueous sample, and after centrifugation, the extractant solvent containing the analytes are separated from the aqueous phase enabling high enrichment factor [21]. This solvent-minimized sample preparation has gained increasing research interest due to their advantages, including simplicity of operation, high speed, high extraction efficiency with matrix effect free, low cost, and minimum requirements for sample and organic solvents [21–23]. Despite the several advantages of DLLME, two main drawbacks have been described: the use of disperser solvent, which usually decreases the partition coefficient of analytes, and the dispersion difficulties of some extractant solvents into the samples. Therefore, vortex and ultrasound-assisted DLLME have been developed [21] since they did not require a disperser solvent. Despite its several advantages, one of the main obstacles for DLLME is the suitable selection of a appropriate extraction solvent considering its effectiveness, availability, cheapness and which meets the green principles [4,6]. On this regard, deep eutectic solvents (DESs) have recently surged as one of the most promising alternatives to the use of harmful organic solvents [22,24,25].

The first DES application in metal liquid-phase microextraction (LPME) was used to the extraction of Cd and Pb in edible oils [26]. Thereafter, the combination of DESs with DLLME was rapidly developed, but is still seldom applied to elemental detection techniques [22]. DESs are defined as mixtures of two or more safe, cheap, renewable and biodegradable components with a melting point close to room temperature in most cases. Their synthesis are carried out between hydrogen bond acceptors (HBAs), such us quaternary ammonium salts, and hydrogen bond donors (HBDs) such as phenols, amines, carboxylic acids, or alcohols [21,22,25,27]. They are also known as cheap analogues of ionic liquids due to their advantages, including low toxicity, high thermal stability, ease of synthesis and low cost [25,27,28].

Application of microextraction techniques coupled with a multi-element technique perfectly meets most of the specified principles [4,5]. Other approaches to perform DLLME process in more eco-friendly way include application of a non-hazardous extractant solvent and multivariate analysis for reducing experiments. In view of the above, this study aimed to develop a simple, cheap, fast and green sample preparation procedure based on DLLME using a synthesized DES (i.e., DL-menthol and decanoic acid 2:1 M ratio) for the simultaneous extraction and preconcentration of Cd, Co, Hg, Ni, Pb, and V at trace levels from oral and parenteral drug samples for subsequent measurement by ICP OES. In order to increase the sensitivity of the ICP OES for determination of these elements following USP requirements, parameters affecting the extraction efficiency were carefully optimized by multivariate analysis.

2. Experimental

2.1. Instrumentation

Experiments were performed using an Agilent 720-ES inductively coupled plasma optical emission spectrometer (Agilent Technologies, Melbourne, Australia) operating in axial viewing mode. Argon (99.9992%, Carburos Metálicos, Barcelona, Spain) was

used in all measurements. Plasma operating conditions used in ICP OES are shown in Table 1. The RF applied power and nebulizer gas flow rate were optimized to obtain a compromise between maximum sensitivity and best precision for the majority of the emission lines tested. In case of organic extract uptake rate, it was the minimum liquid flow required to measure all emission lines analyzed. A centrifuge (model 2690/5, Nahita Centrifuges, Beriain, Spain) was used to accelerate the phase separation and a pH-meter (Crison Instrument, Barcelona, Spain) with a combined glass electrode was used for pH measurements. NemrodW statistical software (NemrodW® v.2007/2010, LPRAI, Marseille, France) was used to construct the experimental designs and evaluate the optimization results.

For the characterization of hydrophobic DES, infrared spectra were measured on a Jasco FT/IR-4100 Fourier Transform Infrared (FT-IR) spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker AC-300 (300 MHz) or AC-400 (400 MHz) NMR spectrometers in proton coupled mode. Differential Scanning Calorimetry (DSC) analyses were performed on a Mettler Toledo equipment, model TGA/SDTA851e/LF/1600. In DSC, the samples were continuously purged with 50 mL min^{-1} of nitrogen. About 6 mg of each compound was crimped in an aluminum standard melting pot and analyzed under dynamic nitrogen atmosphere by heating (5 °C min^{-1}) and cooling (5 °C min^{-1}) cycles between −10 and 100 °C.

2.2. Reagents and standard solutions

Experiments were performed using concentrated high purity grade HNO_3 (Merck, Darmstadt, Germany) and ultrapure water, resistivity higher than 18.2 MΩ cm, (Millipak-40 Filter Unit 0.22 mm NPT, Bedford, MA, USA). Complexing agent (8-Hydroxyquinoline (8-HQ), purity ≥98%, Sigma-Aldrich, Steinheim, Germany) solution of 16% m v^{-1} was prepared by dissolving the appropriate amount of reagent in ethanol (99.9%, AppliChem, Darmstadt, Germany) and acetic acid glacial (99.8%, Scharlau Chemie, Barcelona, Spain) at a ratio of 4:1 v v^{-1}. Analytical reference solutions used for ICP OES calibrations and for addition and recovery experiments were prepared by appropriate dilutions of 1000 mg L^{-1} of Cd, Co, Hg, Ni, Pb, and V (High Purity Standards, Charleston, SC, USA) in 0.07 mol L^{-1} HNO_3 medium. External calibration was used for all ICP OES analysis. The concentrations of the analytical solutions used for calibration for direct ICP OES analysis were 0.5, 1, 1.5, 2, 3, and 4 mg L^{-1} for all analytes and 5, 15, 30, 60, 125, and 250 μg L^{-1} of Cd, Co, Hg, Ni, Pb and V for DLLME-ICP OES. To minimize contamination all laboratory glassware were kept in 10% v v^{-1} nitric acid solution for 24 h before use.

For the synthesis of hydrophobic DES, DL-menthol (purity ≥98%) provided by Alfa-Aesar™ (Tewksbury, MA, United State) was used as hydrogen bond acceptor (HBA) for the DES. Decanoic acid (purity ≥98%) provided by Sigma-Aldrich was used as hydrogen bond donor (HBD). Reagents were used without any further purification.

Table 1
Operating parameters used in Agilent 720-ES ICP OES.

Instrument parameter	Value
RF applied power (kW)	1.2
Plasma gas flow rate (L min^{-1})	15
Auxiliary gas flow rate (L min^{-1})	1.5
Nebulizer gas flow rate (L min^{-1})	0.75
Organic extract uptake rate (μL min^{-1})	50
Nebulizer	OneNeb® Series 2
Spray chamber	Cyclonic spray chamber
Number of replicates	3
Analyte	**Emission line (nm)[a]**
Cd	226.502 II
Co	238.892 II
Hg	253.652 I
Ni	216.555 II
Pb	220.353 II
V	311.837 II

[a] I: Atomic line, II: Ionic line.

2.3. Synthesis of hydrophobic DES

The hydrophobic DES formed by decanoic acid and DL-menthol was synthesized by simply mixing DL-menthol (2 mol) with decanoic acid (1 mol) at 60 °C under argon atmosphere, stirring the mixture until a clear and homogenous liquid was formed (usually 30 min).

2.4. Samples and sample preparation

Three oral drug samples (OA-OC) and three parenteral drug samples (PA-PC) in liquid dosage form were analyzed. More details about these samples are presented in Table 2. The maximum permissible daily dose (MDD) for each drug was consulted on the package leaflet. These drugs are intended to be used orally or parentally for different disorders and are accessible to population without prescription. All analyzed samples were purchased in local pharmacies in San Vicente del Raspeig, Alicante, Spain. Before DLLME, oral and parenteral drug samples were 10-fold diluted with distilled-deionized water after adjusting pH.

2.5. Dispersive liquid–liquid microextraction procedure

A volume of 8.0 mL of the 10-fold diluted sample, at pH 3.4 and 8-HQ concentration of 1.0% m v^{-1}, was transferred to 10-mL glass tubes. Then, 70 μL of the DES extractant solvent was added, and the mixture was shaken using a vortex shaker for 3 min. After shaking, the solution was centrifuged at 3000 rpm for 4 min to separate the organic and aqueous phases. Fifty microliters of the organic extract (at the top of the solution) was collected from the glass tube using a micropipette and directly inserted into the ICP OES without furthermore dilution. Fig. 1 shows a schematic representation of the general optimized DES-based DLLME procedure. During the optimization of the extraction conditions, standard solutions containing 100 μg L^{-1} of all analytes in 0.07 mol L^{-1} HNO_3 medium were used.

2.6. Addition and recovery tests according to USP requirements

Addition and recovery experiments were performed according to *J* values (expressed in μg L^{-1}), which were calculated based on the specific PDE value for each element (in μg day^{-1}) considering oral or parenteral route of administration, divided by the MDD (in mL day^{-1}) and the dilution factor (DF) adopted during sample preparation [2,29] as follows $J = PDE/(MDD \times DF)$. Table 3 shows PDE and *J* values for all analytes for oral and parenteral drug samples considering the specific MDD of each medication, as indicated in the package insert (Table 2), and DF of 10-fold. According to *J* values for each analyte, all drug samples were spiked in triplicate with concentrations of 0.5*J* and 1.5*J* in order to verify the trueness of the DES-based DLLME procedure. Considering oral drug samples, due to *J* values are higher than the proposed working range (i.e., 5.0–250 μg L^{-1}), additional dilution was required. For Hg, Co, V and Ni, *J* values were 5, 10, 10 and 20-times lower, respectively. For parenteral drugs, only the *J* values for Ni were 2-times lower.

Table 2
Active principle, function, indication and maximum daily dose (MDD) for oral and parenteral drug samples analyzed.

Drug sample	Active principle	Function	Indication	MDD (mL day^{-1})
OA	ibuprofen	anti-inflammatory	10–12 years, 30–40 kg	40
OB	paracetamol	analgesic and antipyretic	9–10 years, 25–32 kg	19.2
OC	metamizol magnesium	analgesic and antipyretic	≥15 years, >53 kg	10
PA	metamizol magnesia	analgesic and antipyretic	≥15 years, >53 kg	12.4
PB	diclofenac sodic	anti-inflammatory	≥18 years	9
PC	dexketoprofen	anti-inflammatory	≥18 years	6

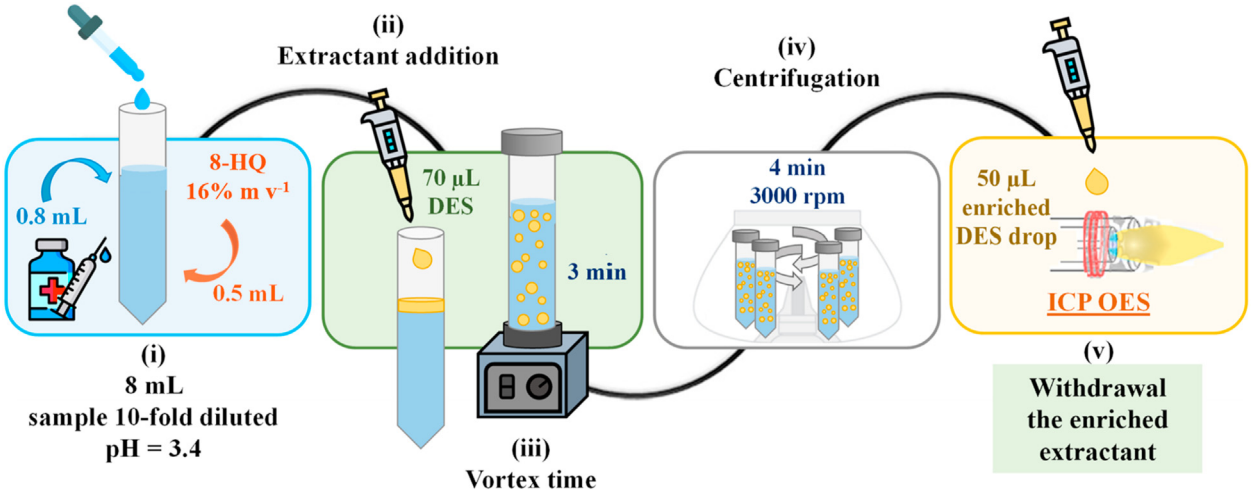

Fig. 1. Schematic representation of the DES-based DLLME procedure for preconcentration of Cd, Co, Hg, Ni, Pb and V in parenteral and oral drug samples.

Table 3
Class [1,2], PDE [2] and *J* values (µg L^{-1}) for Cd, Co, Hg, Ni, Pb and V for oral and parenteral drug samples.

Analyte	Class	Oral drug samples				Parenteral drug samples			
		PDE (µg day^{-1})	*J* values			PDE (µg day^{-1})	*J* values		
			OA	OB	OC		PA	PB	PC
Cd	1	5	13	26	50	2	16	22	33
Pb	1	5	13	26	50	5	40	56	83
Hg	1	30	75	156	300	3	24	33	50
Co	2A	50	125	260	500	5	40	56	83
V	2A	100	250	521	1000	10	81	111	167
Ni	2A	200	500	1042	2000	20	161	222	333

3. Results and discussion

3.1. Characterization of hydrophobic DES

To confirm the structure of DES, FT-IR spectra of pure DL-menthol, pure decanoic acid, and DES were examined and results are presented in Fig. 2. In the spectrum of pure DL-menthol, absorptions corresponding to the tension and flexion –OH (3309, 1454 cm^{-1}, respectively) and the absorption corresponding to the tension C–O (1029 cm^{-1}) were observed. In the spectrum of pure decanoic acid, the COO–H and C=O vibrations were positioned at 3351 and 1727 cm^{-1}, respectively. All these characteristic peaks were also found in DES FT-IR spectrum at the same position, demonstrating that the DES is comprised of DL-menthol and decanoic acid.

Regarding to ^{1}H NMR experiments on DES, it was possible to see a clear interaction between the alcohol substituent (R^1-OH) of the DL-menthol and the proton of the decanoic acid (R^2-CO_2H), since a significant shift in the signals of both was observed in comparison with pure starting materials (Fig. 3, compare a, b and c). These results indicated the successful synthesis of the hydrophobic DES. In order to see whether the 8-hydroxyquinoline (8-HQ) influenced in the DES structure, several ^{1}H NMR experiments were carried out. As it was expected for a compound with hydrogen donor capacity, an interaction between the DES and the 8-HQ was detected since a shift and a change in the shape of the signal of the DES were observed (Fig. 3, compare c, d and e). In case of the alcohol substituent (R^3-OH) of the 8-HQ, the signal is overlapped with the signals corresponding to the aromatic protons (Fig. 3d).

Regarding to DSC experiments, several samples with different proportion of DL-menthol and decanoic acid were prepared by simple mixing the two components and grinding them until a homogeneous mixture was obtained. Those samples were analyzed by DSC. With the melting point of each one, a phase diagram was plotted, showing a eutectic point for a molar ratio 2:1 DL-menthol:decanoic acid (Fig. 4).

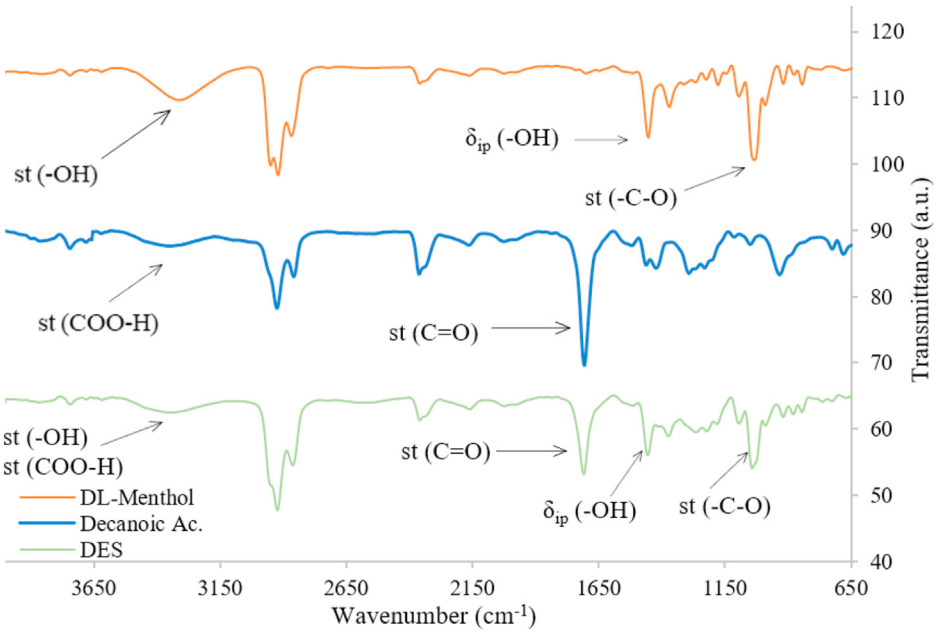

Fig. 2. FT- IR spectra of pure DL-menthol, pure decanoic acid and DES (i.e., DL-menthol and decanoic acid 2:1 M ratio) mixture.

3.2. Optimization of dispersive liquid–liquid microextraction

One of the main objectives of this work is to propose an eco-friendly extraction method optimization. To this end, the multivariate approach for optimization should follow the principles of Green Analytical Chemistry [4], since the number of experiments was significantly reduced with the consequent reduction on the consumption of samples, reagents, energy, among others. Due to the several factors affecting the DLLME procedure, the application of multivariate optimization design helps to determine the best model of the relationship between them, as well as the optimal experimental conditions, mainly considering the simultaneous determination of different analytes [30]. Thus, the multivariate optimization of the DES-DLLME procedure was performed using a Plackett-Burman design for screening approach to identify between significant and non-significant factors followed by a central composite design (CCD) to obtain optimal values for the significant factors.

The seven DLLME factors evaluated on the Plackett-Burman design and their low (−) and high (+) levels, respectively, were (i) DES volume (50 and 100 μL); (ii) sample pH (2 and 4); (iii) 8-HQ concentration (0.50 and 1.0% m v^{-1}); (iv) extraction time (1 and 3 min); (v) centrifugation time (2 and 4 min); (vi) centrifuge speed (2000 and 3000 rpm); and (vii) ionic strength, NaCl concentration (0 and 5% m v^{-1}). Pareto charts of the standardized effect show the results of the Plackett-Burman design for different elements (Fig. 5) and their responses in Table S1).

Considering all analytes, DLLME was favored without adding NaCl (i.e., negative effect) and at high levels (i.e., positive effects) of 8-HQ concentration, extraction time, centrifuge time and centrifuge speed, except to Pb for centrifuge speed factor (Fig. 5). All these factors showed a non-significant effect on DLLME of all analytes. Additionally, the factors (i) DES volume (for Co, Hg, Ni and V) and (ii) sample pH (for all analytes) showed a significant effect on signal intensities. Generally, the sample pH and extractant solvent volume are factors extremely significative for metal extraction procedures [21,30] because the pH has direct influence on the complexation step and the extractant solvent volume affects the enrichment factor of analytes [21,23,25].

Therefore, a central composite design (CCD) was performed to optimize DES volume and sample pH. The different level values chosen in the CCD were: (i) DES volume (50, 57, 75, 93, and 100 μL), and (ii) sample pH (2.0, 2.3, 3.0, 3.7, and 4.0). The response surfaces obtained are shown in Fig. 6 and their responses in Table S2. The optimized DES volume and sample pH for extraction of different analytes were: 66 μL and 3.3 for Cd, 69 μL and 3.4 for Co, 71 μL and 3.4 for Hg, 73 μL and 3.5 for Ni, 69 μL and 3.4 for Pb, 72 μL and 3.5 for V. As no significant differences in optimum sample pH and the DES volume for each element were obtained, the average of those values (i.e., DES volume of 70 μL and pH at 3.4) were selected as the most favorable conditions for all analytes. Therefore, the optimized conditions for simultaneous extraction of Cd, Co, Hg, Ni, Pb and V were: sample pH of 3.4, 8-HQ concentration of 1.0% m v^{-1}, 70 μL of DES as extractant solvent, vortex time of 3 min, centrifugation time of 4 min and centrifugation speed of 3000 rpm.

3.3. Analytical performance for DES-based DLLME-ICP OES method

Table 4 summarizes the analytical figures of merit obtained by developed DES-based DLLME-ICP OES method and direct ICP OES analysis for determination of Cd, Co, Hg, Ni, Pb, and V in oral and parenteral drug samples. The enrichment factors (EF) were defined as the ratio of the calibration curve slope with and without the preconcentration procedure. The correlation coefficients (r) obtained for all DLLME-ICP OES calibration curves ranged from 0.9985 to 0.9996 and EF values ranged from 22 to 86, showing good linearity and significant increase in sensitivity for all analytes.

Limits of detection (LOD) and quantification (LOQ) were calculated according to Eurachem guidelines [31] considering the analyte concentration corresponding to the obtained standard deviation (i.e., determined by 10 consecutive measurements of the blank) at low levels multiplied by a factor k. The IUPAC default value for k is 10 for LOQ and 3 for LOD [32]. Following USP Chapter 233, LOQ values $\leq 0.3J$ are suggested as acceptance criteria since accuracy must be demonstrated at lower spiked concentrations of $0.5J$ for each target element [29]. In this context, the LOQ values for

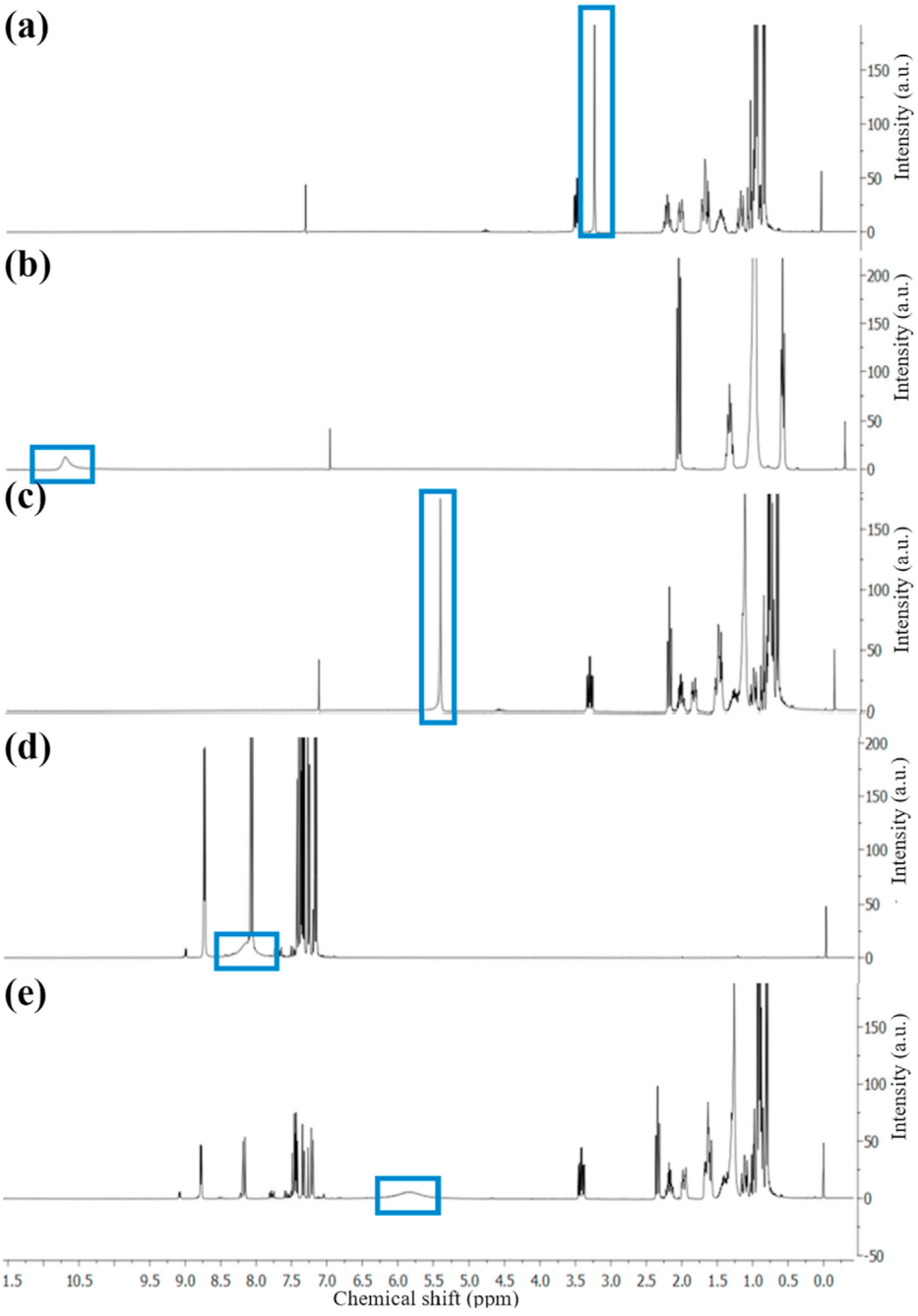

Fig. 3. ^{1}H NMR spectra of **(a)** pure DL-menthol, **(b)** pure decanoic acid, **(c)** DL-menthol:decanoic acid (2:1) mixture, **(d)** pure 8-HQ, **(e)** DL-menthol:decanoic acid (2:1) mixture and 8-HQ.

direct ICP OES analysis were all higher than 0.3*J* for all elements for parenteral drug samples. Due to the higher PDE values recommended for oral route of administration, the LOQ values obtained for oral samples using ICP OES without DLLME were higher than 0.3*J* for all elements for sample OA; for Cd, Co, Hg and Pb for sample OB; and for Cd and Pb for sample OC.

Limits of quantification values for Cd, Co, Hg, Ni and V using DES-based DLLME were 26, 6, 3, 16, 3 and 9-times lower than their respective 0.3*J* values for Cd, Co, Hg, Ni, Pb and V, respectively, for sample PA (i.e., lower *J* values among all analytes and all drug samples analyzed). Consequently, it may be inferred that the LOQ values obtained for DES-based DLLME of Cd, Co, Hg, Ni and V are suitable to meet USP requirements even using oral liquid drugs with MDD higher than 40 mL day^{-1} and for Pb with MDD until 40 mL day^{-1}. For parenteral route of administration, considering the simultaneous determination of six analytes, the LOQ values obtained for DLLME of Hg and Pb are suitable to meet USP requirements using parenteral drugs with MDD until 30 mL day^{-1}.

The repeatability was estimated from six independent measurements of sample spiked at 8.0 and 24 μg L^{-1} of all analytes. These values were selected considering the sample with lower 0.5*J* and 1.5*J* values among all elements (i.e., parenteral drug sample PA). Repeatability ranged from 3 to 6%, values significantly lower than 20% of RSD stated by USP Chapter 233 [29].

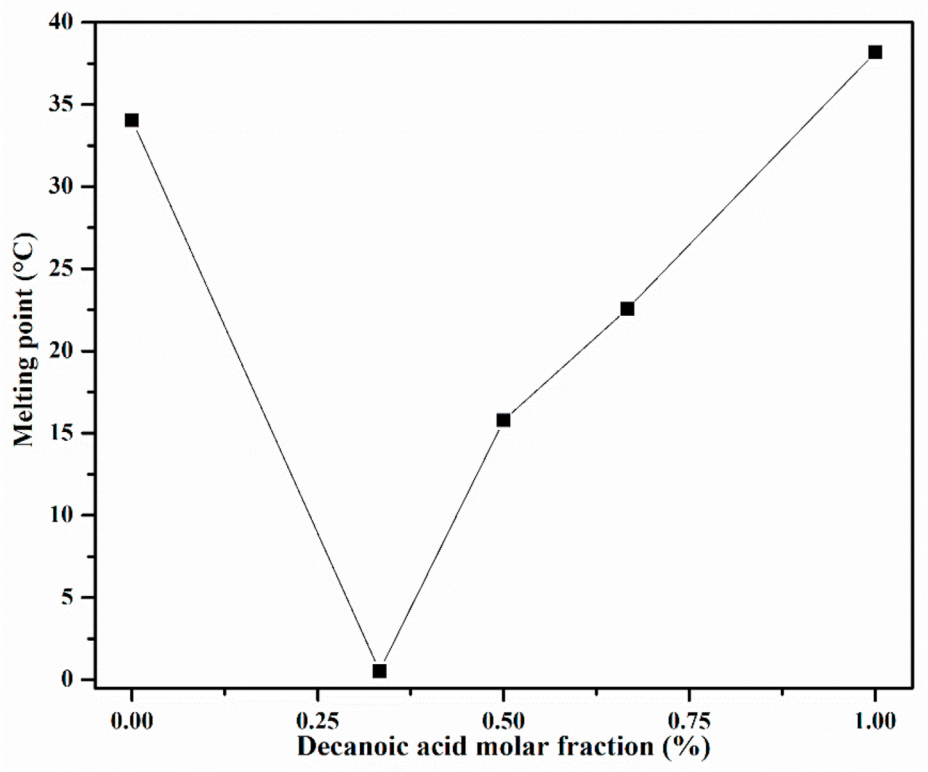

Fig. 4. Phase diagram for DL-menthol:decanoic acid eutectic mixture.

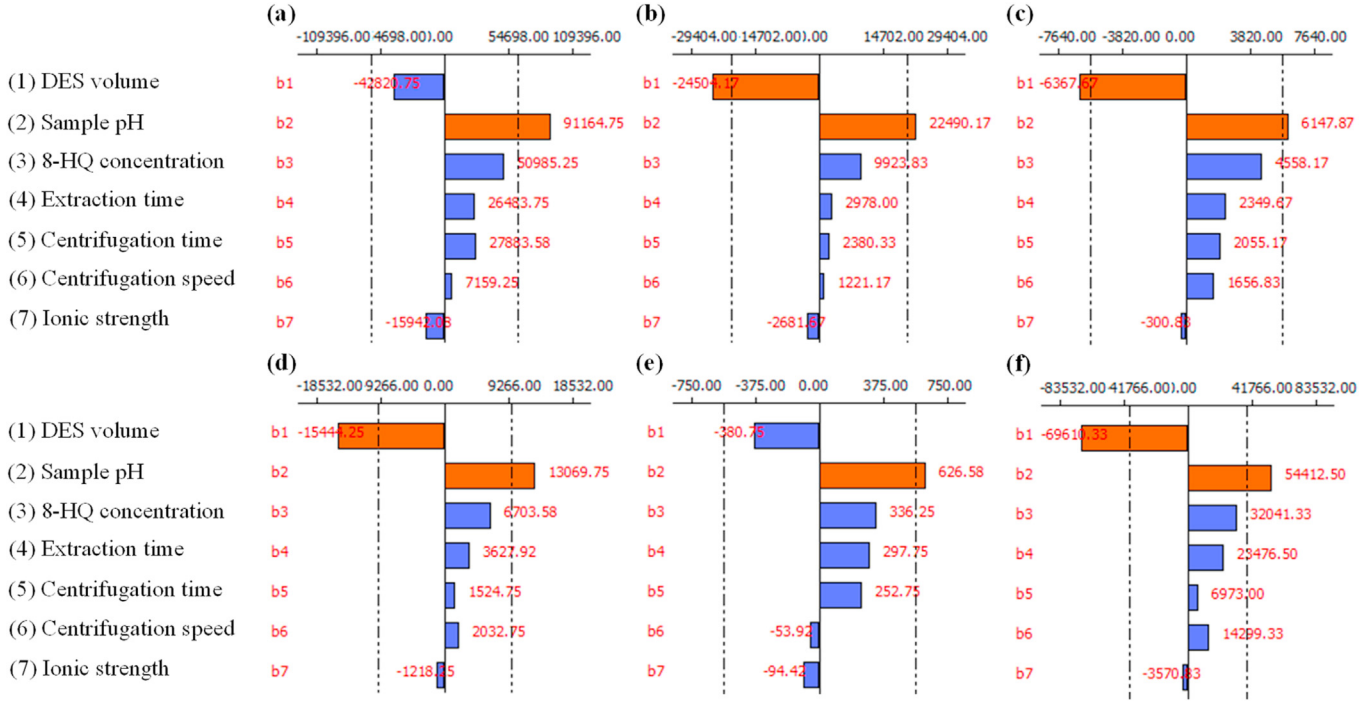

Fig. 5. Pareto charts obtained in the screening study of the main factors affecting the DLLME of **(a)** Cd, **(b)** Co, **(c)** Hg, **(d)** Ni, **(e)** Pb and **(f)** V. (■) Significant effect; (■) Non-significant effect. Bars to the right indicate a positive effect and bars to the left indicate a negative effect. Analyte concentration of 100 μg L^{-1}.

3.4. Addition and recovery tests according to USP requirements

All analytes were below their respective LOQ values for all oral and parenteral drug samples analyzed, hence, the analyzed samples are within the limits suggested by USP Chapter 232 [2] taking into account the MDD of each parenteral and oral drug. According to the USP Chapter 233 [29] analytical procedures must demonstrate accurate spike recoveries between 70 and 150% of the

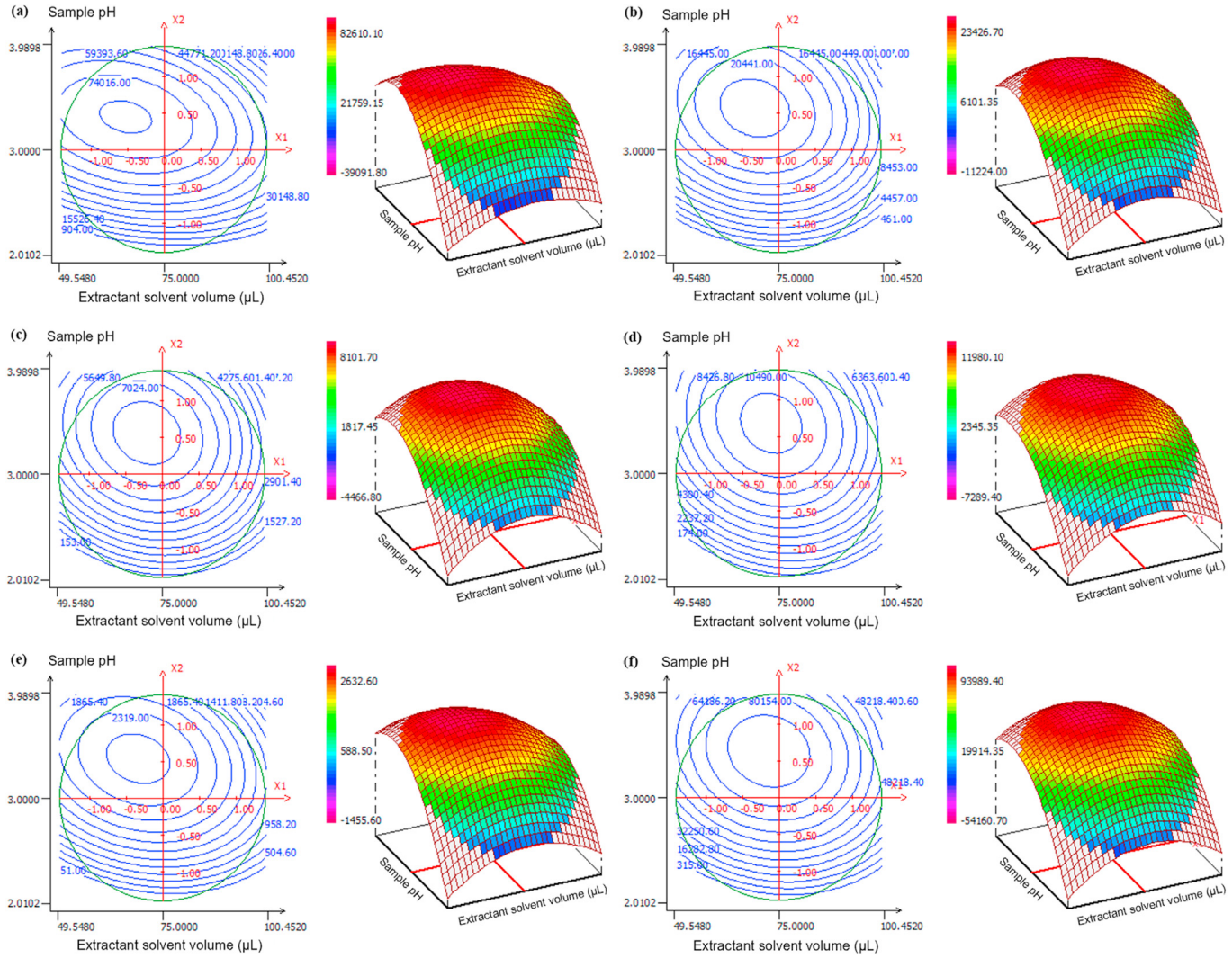

Fig. 6. Response surface from central composite design for **(a)** Cd, **(b)** Co, **(c)** Hg, **(d)** Ni, **(e)** Pb and **(f)** V. Analyte concentration of 100 µg L^{-1}.

spiked value at concentrations ranging from 0.5*J* to 1.5*J* of the value for each target element. Consequently, the samples were spiked at levels equivalent to 0.5*J* and 1.5*J* for all analytes in order to check the trueness of DES-based DLLME-ICP OES method (Table 5).

In order to group all analytes in a unique analytical working range, some addition levels (i.e., 0.5*J* and 1.5*J*) for some analytes were properly divided as previously mentioned in Section 2.6. The specific addition values for each analyte and sample were also presented in Table 5. Recoveries ranging from 90 to 109% were observed by spike experiments at both levels and the repeatability was demonstrated by a precision $\leq$6% RSD considering all oral and parenteral drug samples. No significant matrix effects were observed for ICP OES measurements; therefore, external calibration was used for all ICP OES analysis.

3.5. Comparison with other hydrophobic DES-based LPME procedures

According to our knowledge, this is the first report which a LPME procedure using a DES as extractant solvent is applied for elemental determination in drug samples. Hence, a comparison of the developed DES-based DLLME-ICP OES method with previously reported methods is shown in Table 6. The developed DES-based DLLME procedure is faster and simpler than DES-based liquid-phase microextraction procedures previously reported for extraction of metal ions in aqueous samples. A small volume of DES, i.e. lower than 100 µL, low extraction time and no disperser solvent are advantageous analytical characteristics of the developed method. In contrast to some reported methods that using ice bath for DES solidification [33,34], in the proposed DES-based DLLME procedure the organic extract is directly collected from the glass tube and immediately analyzed without furthermore sample preparation steps.

Considering all elements, the limit of detection of DES-based DLLME-ICP OES method by using only 8 mL of aqueous sample is better or similar to other methods. It is noted that for ETAAS methods [35,36], a higher sensitivity and enrichment factor were achieved, but these are monoelemental methods and the micro-extraction procedure was proposed just for one element at a time, i.e. Cd [35] and Pb [36]. In this sense, other important feature of the develop DES-based DLLME-ICP OES method are the relatively high number of analytes. In fact, a multi-analyte method is another principle of the Green Analytical Chemistry [4].

Table 4
Analytical figures of merit for Cd, Co, Hg, Ni, Pb and V determination in oral and parenteral drug samples using DES-based DLLME-ICP OES and direct ICP OES analysis.

	Emission line (nm)					
	Cd (226.502)	Co (238.892)	Hg (253.652)	Ni (216.555)	Pb (220.353)	V (311.837)
Direct ICP OES						
Linear range (μg L^{-1})	500–4000	500–4000	500–4000	500–4000	500–4000	500–4000
Calibration equation	y = 6.4x + 986	y = 4.1x + 401	y = 0.93x + 196	y = 1.84x + 181	y = 0.60x + 67	y = 15.1x + 1114
r[a]	0.9989	0.9992	0.9982	0.9982	0.9990	0.9989
Sensitivity (cps L μg^{-1})[b]	6.4 ± 0.2	4.1 ± 0.1	0.93 ± 0.03	1.84 ± 0.05	0.60 ± 0.01	15.1 ± 0.4
LOD (μg L^{-1})	5	30	20	70	15	30
LOQ (μg L^{-1})	17	90	70	200	50	100
DLLME-ICP OES						
Linear range (μg L^{-1})	5.0–250	5.0–250	5.0–250	5.0–250	5.0–250	5.0–250
Calibration equation	y = 546x + 2966	y = 214x + 178	y = 74x +16	y = 142x + 74	y = 13.4x +95	y = 677x - 3219
r[a]	0.9985	0.9992	0.9990	0.9993	0.9996	0.9990
Sensitivity (cps L μg^{-1})[b]	546 ± 9	214 ± 3	74 ± 1	142 ± 2	13.4 ± 0.1	677 ± 16
EF[c]	86 ± 2	52 ± 2	79 ± 3	77 ± 3	22.2 ± 0.7	45 ± 2
LOD (μg L^{-1})	0.05	0.6	0.8	0.9	1.2	0.8
LOQ (μg L^{-1})	0.2	2	3	3	4	3
USP LOQ (μg L^{-1})[d]	≤5	≤12	≤7	≤48	≤12	≤24
Repeatability 0.5*J* (RSD%)[e]	6	5	5	6	6	3
Repeatability 1.5*J* (RSD%)[f]	3	6	4	4	3	3

[a] Correlation coefficient (six calibration points).
[b] Slope ± standard deviation.
[c] Enrichment factor ± expanded uncertainty. Calculated as slope ratio between calibration curves with and without DLLME.
[d] LOQ values ≤ 0.3*J* considering the sample with lower *J* values (i.e., parenteral drug sample PA).
[e] Mean value for six replicate analyses of spiked solution with 8 μg L^{-1} of all analytes.
[f] Mean value for six replicate analyses of spiked solution with 24 μg L^{-1} of all analytes.

Table 5
Recoveries and relative standard deviation (%, n = 3) obtained for the spiked in oral (OA-OC) and parenteral (PA-PC) drug samples at two different levels: 0.5*J* and 1.5*J* (i.e., spike in μg L^{-1}) using DES-based DLLME-ICP OES.

Analyte	Sample	Spike	Recovery	Analyte	Sample	Spiked	Recovery	Analyte	Sample	Spike	Recovery
Cd	OA	6	106 (3)	Co	OA	6	98 (5)	Hg	OA	8	91 (3)
		19	100 (6)			19	97 (4)			23	95 (4)
	OB	13	103 (5)		OB	13	101 (5)		OB	16	101 (6)
		39	105 (4)			39	100 (5)			47	95 (3)
	OC	25	93 (4)		OC	25	93 (4)		OC	30	95 (1)
		75	97 (4)			75	100 (3)			90	96 (5)
	PA	8	92 (2)		PA	20	92 (6)		PA	12	92 (1)
		24	92 (5)			60	99 (6)			36	97 (2)
	PB	11	90 (4)		PB	28	98 (3)		PB	17	102 (2)
		33	93 (5)			83	104 (1)			50	109 (3)
	PC	17	95 (2)		PC	42	99 (5)		PC	25	101 (5)
		50	100 (1)			125	103 (6)			75	110 (4)
Ni	OA	13	96 (5)	Pb	OA	6	93 (3)	V	OA	13	104 (1)
		38	108 (2)			19	107 (3)			38	95 (5)
	OB	26	100 (4)		OB	13	98 (2)		OB	26	95 (6)
		78	98 (5)			39	97 (6)			78	101 (6)
	OC	50	96 (2)		OC	25	96 (2)		OC	50	100 (1)
		150	99 (5)			75	96 (5)			150	96 (2)
	PA	40	94 (4)		PA	20	98 (3)		PA	40	94 (3)
		121	107 (6)			60	105 (2)			121	98 (6)
	PB	56	91 (2)		PB	28	100 (4)		PB	56	98 (5)
		167	106 (2)			83	92 (2)			167	106 (1)
	PC	83	107 (2)		PC	42	105 (1)		PC	83	106 (2)
		250	93 (4)			125	97 (2)			250	99 (2)

4. Conclusions

The developed sample pretreatment procedure based on DLLME of Cd, Co, Hg, Ni, Pb, and V from oral and parenteral drug samples prior to their determination by ICP OES is simple, fast and meets most of the green principles since it is a multianalyte method and it includes the application of a reduced volume of a non-hazardous extractant solvent (i.e., DES), among other. In addition, multivariate analysis is recommended as an environmentally friendly optimization approach of extraction conditions of both sample preparation and detection on spectrochemical analysis. DES-based DLLME-ICP OES method affords enrichment factors on average 60-fold in comparison with direct ICP OES analysis, consequently, the results was proved to be sensitive and reliable enough to follow USP requirements for determination of above-mentioned elements in drugs in liquid dosage form considering target-limits for oral and parenteral route of administration. While ICP-MS achieved suitable sensitivity for elemental ultratrace determination, the synergetic

Table 6
Comparison of analytical characteristics of the proposed method with some DES-based published methods for metal liquid-phase microextraction in aqueous samples.

Sample (amount, mL)	Analytes	Extraction technique	DES (molar ratio; amount, μL)	Extraction time (min)	Analytical technique	LOD (μg L^{-1})	EF	Reference
Human blood (10)	Hg (II)	DLLME	1-octyl-3-methylimidazolium chloride and 1-undecanol (1:2; 55)	8	ETAAS	0.1	112	[33]
Food and water (30)	Pb (II)	AA-LPME	ChCl:phenol (1:4; 600) with 800 μL of THF	NI	ETAAS	$0.6x10^{-3}$	60	[36]
Food and water (50)	Cd	UA-LPME	ChCl:phenol (1:4; 500) with 600 μL of THF	3	ETAAS	$0.2x10^{-4}$	100	[35]
Black tea, water and urine (20)	Cd, Cu, Ni, Pb	AA-LL-ELLME	ChCl:TNO[1] (1:2 with TEA 1:1; 100)	4	FAAS	0.3–1	67–69	[37]
Milk (5)	Cd, Cu, Pb	DLLME	Menthol:sorbitol:mandelic acid (1:2:1; 100)	NI	FAAS	0.4	NI	[34]
Liquid drugs (8)	Cd, Co, Hg, Ni, Pb, V	DLLME	DL-menthol and decanoic acid (2:1; 70)	3	ICP OES	0.05–1	22–86	This work

EF, enrichment factor; ETAAS, electrothermal atomic absorption spectrometry; AA-LPME, air-assisted liquid-phase microextraction; ChCl, choline chloride; THF, tetrahydrofuran; NI, not indicated; UA-LPME, ultrasonic assisted-liquid phase microextraction; AA-LL-ELLME, air-assisted ligandless emulsification liquid-liquid microextraction; TNO, 5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-ol; TEA, triethylamine; FAAS, flame atomic absorption spectrometry.

combination of DLLME and ICP OES can be considered an affordable option for trace elemental determination in medicines.

CRediT authorship contribution statement

Fernanda C. Pinheiro: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Visualization. **Miguel Ángel Aguirre:** Conceptualization, Methodology, Validation, Investigation, Writing – review & editing. **Joaquim A. Nóbrega:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Nerea González-Gallardo:** Methodology, Writing – original draft. **Diego J. Ramón:** Writing – review & editing, Supervision. **Antonio Canals:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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