Dispersive liquid–liquid microextraction of Cd, Hg and Pb from medicines prior to ICP OES determination according to the United States Pharmacopeia

Fernanda C. Pinheiro,[a,b*] Miguel Ángel Aguirre,[b] Joaquim A. Nóbrega[a] and Antonio Canals[b]

[a]Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil
[b]Department of Analytical Chemistry and Food Sciences, University Institute of Materials, Faculty of Science, University of Alicante, P.O. Box 99, 03080, Alicante, Spain
[*]Corresponding author: fernandac.pinheiro@hotmail.com

Abstract

A simple, sensitive and matrix effect free analytical method for simultaneous determination of Cd, Hg and Pb in drug samples (i.e., commercial dosage tablets) by inductively coupled plasma optical emission spectrometry (ICP OES) has been developed. According to the United States Pharmacopoeia (USP) Chapter 232, those metals are considered elemental impurities from class 1 and they must be assessed in pharmaceutical production as well as in quality control evaluation. In order to increase the sensitivity of the analysis, a dispersive liquid-liquid microextraction (DLLME) was performed and seven factors affecting analyte extraction were optimized by multivariate analysis. The microvolume of analyte enriched phase was directly introduced into the plasma using a multinebulizer, providing a high enrichment factor. When compared to conventional ICP OES analysis, DLLME improves limits of quantitation (LOQ) values on average 40-fold for all analytes. Consequently, LOQ values were significantly lower than their permissible daily exposures for oral drugs. Accuracy was evaluated by addition and recovery experiments following USP recommendations in eight commercial drug samples. Recovery and RSD values were within the range of 90-108% and 1-9%, respectively.

Keywords Inductively coupled plasma optical emission spectrometry; Microwave-assisted digestion; Dispersive liquid–liquid microextraction; Tablet and pill drugs; Contaminants

1. Introduction

In the pharmaceutical field, safety and efficacy of medicines are fundamental issues. On this matter, the monitoring of elemental impurities provide assurance of the quality of pharmaceuticals products since some elements can possess unwanted pharmacological–toxicological effects [1–3]. For this purpose, two guidelines have been recently recommended by the United States Pharmacopeia (USP): (i) Chapter 232, Elemental Impurities Limits [4], and (ii) Chapter 233, Elemental Impurities Procedures [5]. Chapter 232 specifies 24 elemental impurities and their toxicity limits considering the oral permissible daily exposure (PDE) values of three drug categories (i.e., oral, parenteral and inhalation drugs) [4]. Chapter 233 describes analytical procedures for elemental determination using two spectroanalytical methods: inductively coupled plasma optical emission spectrometry (ICP OES) or inductively coupled plasma mass spectrometry (ICP-MS) [5].

Although the drafting process of these two chapters started in 2010, a new version of the general Chapter 232 in a strict compliance with the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Q3DR1) guideline [6,7] was published in 2016 [8], became official one year later [9] and was only implemented in 2018. According to ICH and USP requirements [4–6] the PDE values for elemental impurities (target elements) are grouped into four main categories: class 1 (Cd, Pb, As, Hg), class 2A (Co, V, Ni), class 2B (Tl, Au, Pd, Ir, Os, Rh, Ru, Se, Ag, Pt), and class 3 (Li, Sb, Ba, Mo, Cu, Sn, Cr). These categories are based on the toxicity of target elements, their likelihood of occurrence and route of administration. Chapter 232 [4] and ICH [6] also provide guidance on which of those 24 elemental impurities must be tested for.

The elements from class 1 are considered toxic to humans and have limited or no use in the manufacture of pharmaceuticals. Their presence in pharmaceuticals typically comes from commonly raw materials and must be evaluated in all finished pharmaceutical products and in potential sources of contamination, for instance active pharmaceutical ingredients and excipients [1–3,6]. Even at low concentration levels, the heavy metals Cd, Hg and Pb pose a serious health risk when used for pharmaceutical purposes [3,6,10,11]. Cadmium in their inorganic forms are considered carcinogenic to humans [12] and, although Hg and Pb are not classified as carcinogenic, these elements may cause severe toxicological and hematopoietic effects [10–12]. Due to their high toxicity, low PDE values are recommended for these target elements [4,5].

ICP based methods enable fast multi-elemental analysis with high sensitivity, accuracy and robustness [13–15]. On one hand, considering the low PDE values recommended for the above-mentioned elements, the majority of the proposed ICP based methods for elemental impurities determination are focused on ICP-MS analysis [1–3,15]. On the other hand, ICP OES should be considered a suitable analytical method for this purpose since its higher availability, in contrast to the higher instrumentation cost of ICP-MS. In order to reach enough sensitivity for determination of these elements in drug samples using ICP OES, a preconcentration step prior to measurement could be used [16,17].

On this regard, preconcentration approaches based on microextraction techniques, particularly dispersive liquid-liquid microextraction (DLLME), have been extensively used since their advantages, including simplicity, speed, ease of use, low cost and high enrichment factors using an extremely low extractant solvent volume [17–19]. Traditional DLLME involves the use of a mixture of solvents (i.e., extractant and disperser solvents) which are injected into the aqueous sample forming a cloudy solution. The dispersion of extraction solvent accelerates the analyte

extraction and after a centrifugation step is possible to collect an aliquot of the enriched extractant [18–20]. In order to eliminate the disperser solvent and to enhance the extractant phase dispersion, vortex-assisted DLLME has been employed [18].

After the DLLME procedure, the low extractant solvent volume is generally dissolved in another miscible organic solvent before the introduction of extract using pneumatic nebulization, nevertheless, this step can deteriorate the enrichment factor achieved during the preconcentration. Moreover, the introduction of organic matrices into the argon plasma can cause severe matrix effects and also the formation of carbon deposits on the plasma torch. In order to address these challenges, a multinebulizer has been successfully used for the simultaneous introduction of organic and aqueous solutions for preventing the formation of carbon deposits [21–24]. This novel multinebulizer incorporates two independent liquid inlets into a single nebulization body with a common nebulization gas inlet and a unique outlet orifice allowing that two liquids, miscible or immiscible, be mixed at the tip of the nebulizer [24]. Hence, a microvolume of analyte enriched extract (without further dilution) and aqueous solution can be simultaneously introduced into the plasma by independent channels, reducing carbon deposits on the torch without decreasing the enrichment factor.

To our knowledge, this is the first report which an extraction methodology is applied for drug samples to elemental impurities determination in accordance with ICH guidelines and USP chapters. It is well-known that ICP-MS afford suitable sensitivity for the ultratrace determination of the elemental impurities. However, given the larger number of laboratories that already employ ICP OES, this study aimed to develop a simple DLLME procedure for the simultaneous preconcentration of Cd, Hg and Pb in drug samples for subsequent measurement by ICP OES.

2. Experimental

2.1. Reagents and standard solutions

To minimize contamination all laboratory glassware were kept in 10% v v^{-1} nitric acid solution for 24 h and then washed with ultrapure water before use. 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). Sodium diethyldithiocarbamate (DDTC, 99%, Sigma-Aldrich, Steinheim, Germany) was used as complexing agent. Buffer solutions were prepared by dissolving the appropriate amount of sodium acetate (Panreac Químicas S.A., Castellar del Vallés, Spain) at pH 4 and 6 and sodium phosphate (Scharlau, Barcelona, Spain) at pH 9. Toluene (99.9%, Sigma Aldrich) and 1-octanol (99.9%, Sigma Aldrich) were used as extracting solvent. 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, Hg and Pb (High Purity Standards, Charleston, SC, USA) in 0.14 mol L^{-1} HNO_3 medium.

2.2. Instrumentation

A pHmeter (Crison Instrument, Barcelona, Spain) with a combined glass electrode was used for pH measurements. A centrifuge (model 2690/5, Nahita Centrifuges, Beriain, Spain) was used to accelerate the phase separation. 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 S.A, Barcelona, Spain) was used in all measurements. Plasma operating conditions used in ICP OES are shown in Table 1.

Introduction of extract (i.e., analyte enriched phase) were performed using a multinebulizer (MultiNeb®, Ingeniatrics, Seville, Spain) [24]. This multinebulization device is an advanced version of another previous prototypes already described [25]. It presents two independent liquid inlets and two different types of peristaltic tubes were used depending on the solution introduced. In the liquid inlet where the analyte enriched phase was introduced, a peristaltic tube compatible with most petroleum-based products (F-4040-A, id. 0.25 mm, Ismatec, Switzerland) was used. In the other one where an ultrapure water was continuously pumped, a Tygon® peristaltic tubes (R-3607, id. 0.76 mm, Ismatec) was employed. During the optimization, standard solutions containing 500 µg L^{-1} (concentration within the linear range) of Cd, Hg and Pb were used. NemrodW statistical software (NemrodW® v.2007/2010, LPRAI, Marseille, France) was used to construct the experimental designs and evaluate the results.

2.3. Samples and sample preparation

Eight drug samples in solid dosage form (A-H) were analyzed: A) metformin hydrochloride, used for diabetes treatment; B) losartan potassium, used for hypertension treatment; C) orfenadrine citrate, monoidratated dipirone and caffeine anidra, used as muscle relaxant and analgesic; D) sodium dipyrone, used as analgesic; E) nimesulide, used as anti-inflammatory; F) omeprazole, used for benign (gastric or duodenal) peptic ulcers treatment; G) levothyroxine sodium, used for thyroid treatment; and H) diclofenac sodium, paracetamol, carisoprodol and caffeine, used for rheumatism treatment. All analyzed samples were classificated as oral administration route and were purchased in local pharmacies in São Carlos, São Paulo, Brazil and in San Vicente del Raspeig, Alicante, Spain.

Sample preparation for drugs in solid dosage form was performed based on previously proposed works for microwave-assisted sample digestion [26,27]. All samples were ground and homogenized using pestle and mortar and masses of approximately 500 mg were microwave-assisted digested in triplicate using a volume of 7 mL of 2 mol L^{-1} HNO_3. An Ethos 1 microwave oven (Milestone, Sorisole, Italy) was used. The heating program was applied in two steps: (1) 15 min to reach 220 °C, (2) 15 min at 220 °C, and (3) an additional 15-min cooling step. A maximum 1.5 kW of microwave power was applied. Subsequently, digests were diluted to 25 mL with distilled-deionized water (final dilution of 50-fold) after adjusting the pH. The samples not completely digested were centrifuged for 2 min at 3000 rpm for sedimentation of residual solids.

2.4. Dispersive liquid–liquid microextraction procedure

A 8.0 mL aliquot of the digested sample, at pH 6 and DDTC concentration of 1.0% m v^{-1}, was transferred to 10-mL glass tubes. Then, 100 µL of the extractant solvent (i.e., toluene) was added, and the mixture was shaken using a vortex shaker for 3 min. After shaking, the solution was centrifuged at 3000 rpm for 2 min to separate the two phases, with the analyte enriched phase at the top of the solution. After centrifugation, the analyte enriched phase was at the top of the solution (a toluene layer) and 80 µL of the organic phase was collected from the glass tube using a micropipette and directly inserted into the ICP OES without further dilution. During the organic phase sampling, the toluene layer could be evenly collected separately from the aqueous phase A schematic representation of the general DLLME procedure is presented in Fig. 1.

185 ## 2.5. Evaluation of accuracy according to USP requirements

186 According to the USP Chapter 233 accuracy must be evaluated by addition and recovery
187 experiments with acceptable recoveries ranging from 70 to 150% of the spiked value at
188 concentrations ranging from 0.5*J* to 1.5*J* values for each target element, considering up to 20%
189 of repeatability [4,5]. In this case, the *J* value (also named target limit) is the concentration of the
190 element(s) in µg g^{-1} of interest at the target limit, appropriately diluted to the working range of
191 the instrument. Thus, *J* values were calculated according to oral PDE values specific for each
192 target element (i.e., 5.0, 30 and 5.0 µg day^{-1} for Cd, Hg and Pb, respectively) divided by the
193 maximum daily dose (MDD) and the dilution factor (DF), i.e. $J = PDE/(MDD \times DF)$ [4,5]. The
194 MDD ranged from 0.23 to 10 g day^{-1} for all samples analyzed. For that, the MDD of 10 g day^{-1}
195 was adopted for all samples to obtain the minimal *J* value that can be determined. In this work,
196 therefore, considering the MDD of 10 g day^{-1} and the DF of 50 (i.e., 500.0 mg of sample in 25.00
197 mL), the added concentrations (i.e., 0.5*J* to 1.5*J* values) were 5.0 and 15 µg L^{-1} for Cd and Pb;
198 and 30 and 90 µg L^{-1} for Hg.
199

200 # 3. Results and discussion

201 ## 3.1. Optimization of dispersive liquid–liquid microextraction

202 The multivariate optimization of the DLLME procedure was proceeded into two
203 complementary steps: (i) a Plackett-Burman design was employed as screening approach to
204 identify between significant and non-significant factors, followed by (ii) a central composite
205 design (CCD) to obtain optimal values for the significant factors. In both steps, the experiments
206 were randomly performed in order to nullify the effect of extraneous or nuisance factors using
207 standard solutions containing 500 µg L^{-1} of all analytes. Seven factors at two levels were

evaluated on the Plackett-Burman design. The DLLME variables investigated and their low (-) and high (+) levels are described in Table 2. The results of the Plackett-Burman design are visualized using Pareto charts of the standardized effect in Fig. 2.

In the Pareto charts (Fig. 2a-c) the gray bars indicate variables presenting a significant effect on DLLME procedure, while non-significant factors are indicate by white bars. The bars to the right indicate a positive effect, i.e., favorable condition at higher values of that factor, while the opposite effect is indicate by bars to the left. In general, DLLME was favored when using toluene as extractant solvent at high values of DDTC concentration, extraction time and centrifuge speed and low centrifugation time. Unfortunately, the use of 1-octanol does not satisfy the threshold limit established by the USP (data not shown), and therefore, toluene was used as extractant solvent. Only the factors (1) extractant solvent; (2) extractant solvent volume; and (3) sample pH showed a significant effect on the Plackett-Burman experiment.

Due to pH influence on the complexation step, its evaluation is indispensable in metal extraction procedures [28]. In case of DDTC, pH values below 3.95 favors the protonated form of DDTC (pKa = 3.95 [29]), therefore limiting chelate formation. Moreover, high pH values could also have a negative effect on extraction, since analytes can form hydroxides decreasing the amount extracted. In turn, the extractant solvent volume infers directly in the enrichment factor of the analytes [18,19]. By increasing the extractant solvent volume to a certain degree, the extraction efficiency is increased. However, further increases could cause a dilution effect, resulting in a decrease in the enrichment factor.

In order to optimize both significant factors, a central composite design (CCD) was performed. Both factors were investigated at five levels as described in Table 3 and the response surfaces obtained for the different elements are shown in Fig. 3. Optimum conditions for each

response surface were calculated and the lowest level of extractant solvent volume was obtained for all analytes (i.e., 100 μL of toluene) and pH values at 6.1, 6.3 and 5.2 for Cd, Hg and Pb were obtained, respectively. On that basis pH 6.0 (average of those values) and an extractant solvent volume of 100 μL were selected as the most favorable conditions for all analytes. In summary, the optimized conditions for simultaneous DLLME of Cd, Hg and Pb were: DDTC concentration 1.0% m v^{-1}, 100 μL of toluene as extractant solvent, pH 6.0, extraction time of 3 min, centrifugation time of 2 min and a centrifugation speed of 3000 rpm.

3.2. Analytical performance for DLLME-ICP OES method according to the USP requirements

The proposed microextraction procedure provided a significant increase in sensitivity for all elements. Table 4 summarizes the analytical figures of merit of the developed DLLME-ICP OES method and conventional ICP OES analysis for determination of Cd, Hg and Pb in drug samples. Coupling DLLME to ICP OES is particularly challenging due to spectral and non-spectral interferences caused by organic solvents [17–19]. On this regard, in the multinebulization device used, water is continuously introduced into the plasma. This advantage over conventional nebulization system facilitates the introduction of organic solvents that are not so compatible with plasma, avoiding the need of continuous cleaning of torch and injector tube [21,24].

3.2.1. Linearity, sensitivity and precision

Two calibration curves were performed: (i) conventional ICP OES analysis (i.e., without DLLME procedure) using five calibration points with working range from 1.0 to 5.0 mg L^{-1} for all analytes, and (ii) DLLME-ICP OES using seven calibration points with working range from

2.5 to 120 µg L^{-1} for Cd, Hg and Pb. According to the USP Chapter 233, measurement of at least three calibration standards in the working range between 0.3*J* and at least 1.5*J* for each target element are recommended [5]. In case of the developed analytical method, the working range was set from 0.25*J* to 2.0*J* for simultaneous determination of Cd, Hg and Pb. The correlation coefficients (r) obtained for all DLLME-ICP OES calibration curves ranged from 0.9980 to 0.9996, showing good linearity. The enrichment factor (EF) values for each analyte were calculated as the ratio between sensitivity values with and without DLLME procedure. High EF values were obtained, ranging from 55 to 72.

The repeatability was estimated from six independent measurements of samples spiked at 0.5*J* and 1.5*J* of each target element. The relative standard deviations obtained were ranged from 1.6 to 5%. Obtained repeatability values were significantly lower than 20% of RSD stated by the USP Chapter 233 for repeatability [5].

3.2.2. Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) were calculated according to Eurachem guidelines[30] 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 and 3 for LOQ and LOD, respectively. The LOQ values for conventional ICP OES analysis were all higher than the target-limits. In turn, the LOQ values for Cd, Hg and Pb using DLLME are 36, 33 and 6-times, respectively, lower than their respective *J* values. This means a LOQ improvement on average 24-fold. Following USP recommendations, LOQ values $\leq 0.3J$ are suggested as acceptance criteria [5]. Once LOQ values achieved for Cd, Hg and Pb were 11, 10 and 2-times lower than their

0.3*J*, respectively, it may be inferred that the proposed DLLME-ICP OES method is suitable to meet USP requirements even using drugs in tablets form with MDD higher than 10 g day^{-1}.

Taking into account the low target limits for elements from class 1 [4,6], Table 5 summarizes analytical methodology previously reported for Cd, Hg and Pb determination in pharmaceutical samples using ICP OES. Considering MDD of 10 g day^{-1}, the LOQ values obtained for Cd [26,31,32], Hg [26] and Pb [26,31–33] were higher than 0.3*J* (*i.e.*, LOQ established by USP). As it can be noted, none of the aforementioned analytical methods meet the USP requirements for these three analytes at the same time. There are only two analytical methods with comparable LOQ values [34,35]. In the first work [34], the low LOQ values were achieved using a dilution factor lower than 30-fold. Generally for conventional sample introduction by pneumatic nebulization using ICP OES, maximum total dissolved solids recommended is lower than 1% m v^{-1} [13]. In addition, the low dilution factor can induce severe matrix effects, and therefore, effects on aerosol transport and plasma properties should be carefully assessed [13]. In the second study [35], the authors used an ultrasonic nebulizer with a relative high sample consumption (i.e., 1.9 mL min^{-1}), being the main disadvantage the high cost of the ultrasonic nebulizer.

3.2.3. Accuracy

The trueness was evaluated by addition and recovery experiments performed taking into account *J* values as USP Chapter 233 recommendation [5]. All samples were spiked at levels equivalent to 0.5*J* and 1.5*J* for Cd, Hg and Pb in order to check the trueness of the method (Table 6). All analytes were below their respective LOQ values for all samples analyzed. This pattern was also observed in previous studies with commercial drug samples in solid dosage form [26,27,31,33–35]. Consequently, all samples are within the limits recommended by the USP Chapter

232 taking into account the maximum daily dose of each medicine as indicated in the package insert, i.e., lower than 10 g day^{-1} for tablets drugs. Recovery values ranged from 90 to 108% were observed by spike experiments at both levels based on acceptable recoveries established from 70 to 150% [5]. No matrix effects were observed for DLLME-ICP OES measurements and the repeatability was demonstrated by a precision ≤9% RSD (n = 3) considering all samples.

4. Conclusions

The developed DLLME procedure combined with ICP OES was successfully applied to the simultaneous extraction/preconcentration of Cd, Hg and Pb for trace determination of the above-mentioned elements using ICP OES after a microwave-assisted acid digestion of drug samples using dilute nitric acid. Analytical performance was well validated in the terms of linearity, LOQ, repeatability, and accuracy in accordance with the USP Chapter 233. Pharmaceutical sample preparation using dilute nitric acid solutions provide safer operation and reduced acid consumption. Posteriorly, DLLME affords high enrichment factors, simplicity and sustainability once reagents requirements and waste generation are extremely minimized. When compared with conventional ICP OES analysis, DLLME-ICP OES affords a significant increase of sensitivity showing an enrichment factor on average of 65-fold. Consequently, considering the benefits of direct analysis of organic phase using a multinebulization based system and the appropriate multivariate optimization of DLLME, suitable sensitivity to follow USP requirements for determination of Cd, Hg and Pb using ICP OES was achieved. Therefore, DLLME-ICP OES methods can be seen as a promising alternative for trace elemental analysis in drug samples according to ICH guidelines and USP chapters.

Author contributions

Fernanda C. Pinheiro: conceptualization, methodology, validation, investigation, writing - original draft; Miguel Ángel Aguirre: conceptualization, methodology, validation, investigation, writing - review & editing; Joaquim A. Nóbrega: conceptualization, writing - review & editing, supervision, funding acquisition; Antonio Canals: conceptualization, writing - review & editing, supervision, project administration, funding acquisition.

Conflict of interest

All authors declared that they have no conflict of interest.

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Figures

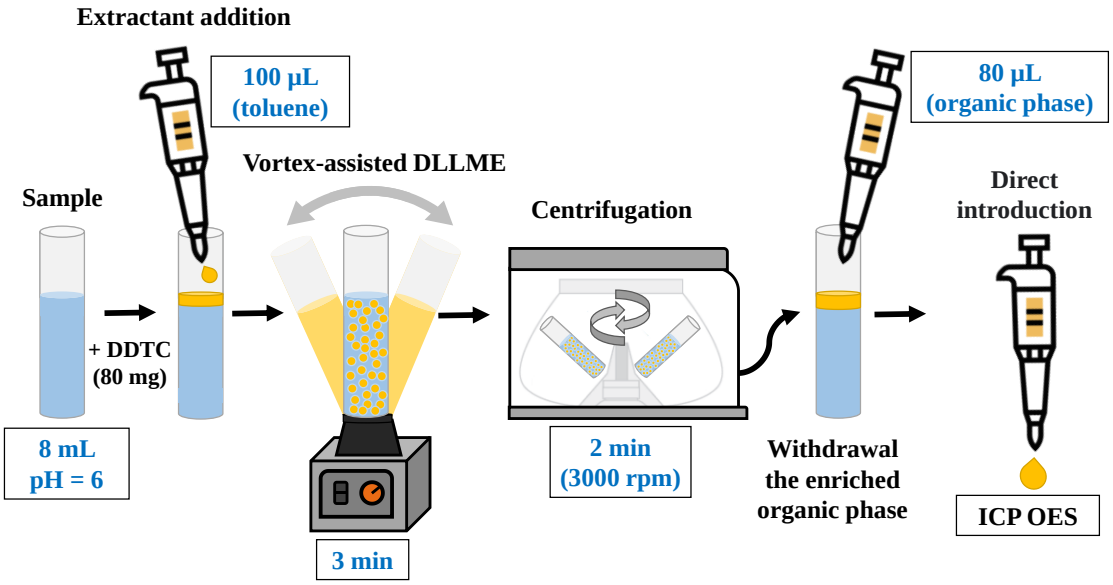

Fig. 1 Scheme of the DLLME procedure for preconcentration of Cd, Hg and Pb in drug samples.

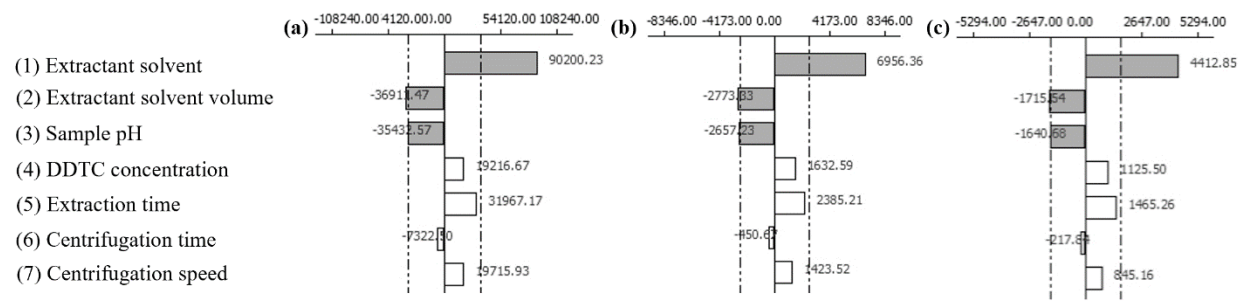

Fig. 2 Pareto charts obtained in the screening study of the experimental variables affecting the DLLME of **(a)** Cd, **(b)** Hg and **(c)** Pb. (■) significant effect; (□) insignificant effect. Analyte concentration of 500 μg L^{-1}.

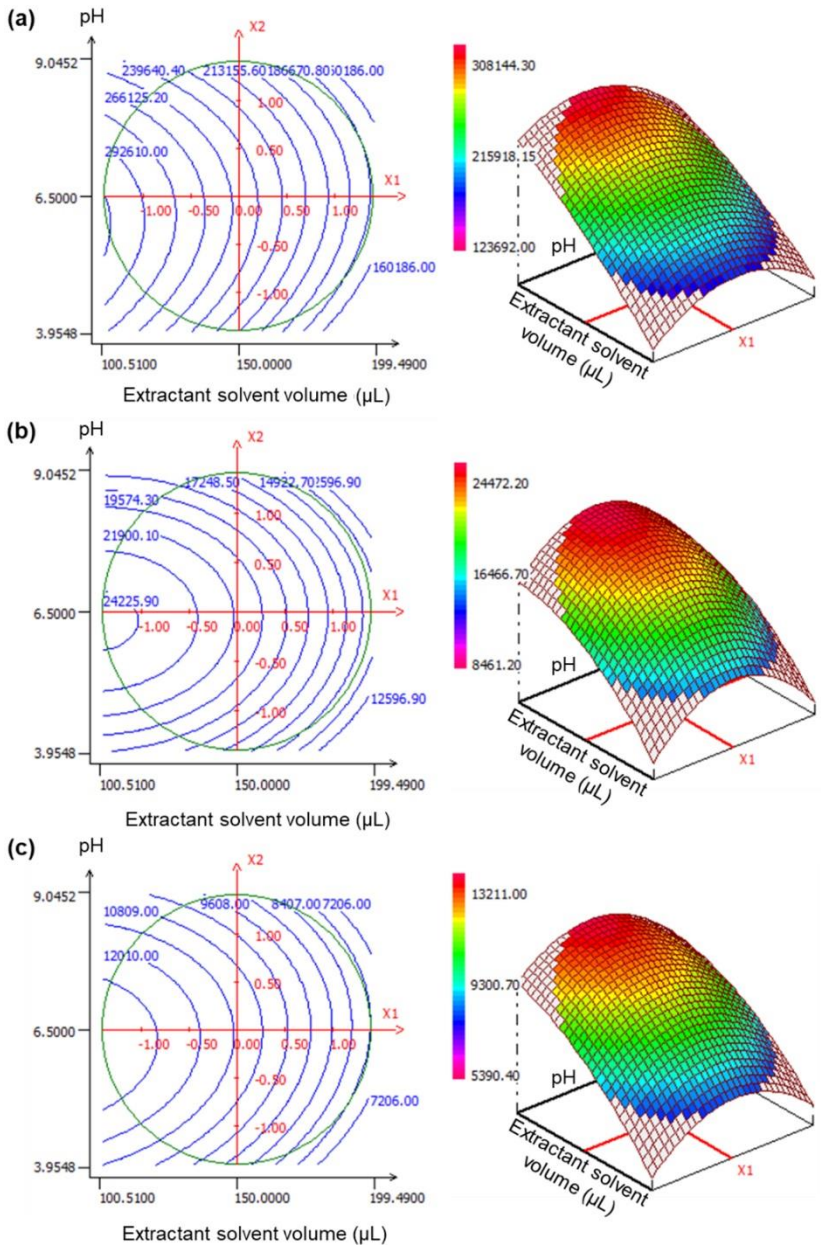

Fig. 3 Response surface from central composite design for **(a)** Cd, **(b)** Hg and **(c)** Pb. Analyte concentration of 500 µg L^{-1}.

461 **Tables**

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463 **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
Aqueous solution uptake rate (µL min^{-1})	200
Nebulizer	MultiNeb®
Spray chamber	Cyclonic spray chamber
Number of replicates	3
Analytes	Emission line (nm)[a]
Cd	226.502 II
Hg	253.652 I
Pb	220.353 II

464 [a] I: Atomic line, II: Ionic line.
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475 **Table 2** Factors and levels of the Plackett-Burman design

Factor	Level	
	Low (-)	High (+)
Extractant solvent	1-octanol	toluene
Extractant solvent volume (μL)	100	200
Sample pH	4	9
DDTC concentration (% m v^{-1})	0.5	1
Extraction time (min)	1	3
Centrifugation time (min)	2	4
Centrifuge speed (rpm)	2000	3000

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Table 3 Factors and levels of the central composite design

Factor	Level			Star points (α = 1.41)	
	Low (-)	Central (0)	High (+)	-α	+α
Extractant solvent volume (μL)	115	150	185	100	200
Sample pH	4.7	6.5	8.3	4.0	9.0

Table 4 Analytical figures of merit for Cd, Hg and Pb determination using DLLME-ICP OES and conventional ICP OES analysis

	Emission line (nm)		
	Cd (226.502)	Hg (253.652)	Pb (220.353)
ICP OES			
Linear range (μg L^{-1})	1000 - 5000	1000 - 5000	1000 - 5000
r[a]	0.9994	0.9990	0.9994
Sensitivity (cps L μg^{-1})[b]	10.85 ± 0.14	0.99 ± 0.02	0.455 ± 0.006
LOD (μg L^{-1})	4	20	20
LOQ (μg L^{-1})	12	70	70
DLLME-ICP OES			
Linear range (μg L^{-1})	2.50 - 120	2.50 - 120	2.50 - 120
r[c]	0.9996	0.9994	0.9980
Sensitivity (cps L μg^{-1})[b]	734 ± 11	54.4 ± 1.0	32.6 ± 0.7
EF[d]	68 ± 2	55 ± 2	72 ± 2
LOQ (μ L^{-1})	0.3	1.8	1.6
USP LOQ ≤0.3*J* (μg L^{-1})	≤3	≤18	≤3
Repeatability 0.5*J* (RSD%)[e]	1.6	5	4
Repeatability 1.5*J* (RSD%)[f]	4	3	4

[a] Correlation coefficient (five calibration points).
[b] Slope ± standard deviation.
[c] Correlation coefficient (seven calibration points).
[d] Enrichment factor ± expanded uncertainty. Calculated as slope ratio between calibration curves with and without DLLME.
[e] Mean value for six replicate analyses of spiked solution with 5.0, 30 and 5.0 μg L^{-1} of Cd, Hg and Pb, respectively.
[f] Mean value for six replicate analyses of spiked solution with 15, 90 and 15 μg L^{-1} of Cd, Hg and Pb, respectively.

Table 5 Quantification limits for Cd, Hg and Pb of the ICP OES methods used for elemental impurities determination in pharmaceutical samples

Pharmaceutical sample	Sample mass (mg)	Sample preparation procedure	Sample preparation details	DF[a] method	Quantification limit ($\mu g\ L^{-1}$)			Reference
					Cd	Hg	Pb	
Pills and tablets	500	MW-AD[b]	7 mL of 2 mol L^{-1} HNO_3; final digest volume of 50 mL	100	5.4	21	39	[26]
Antibiotic tablets	200	CH-AD[c]	5 mL of 14 mol L^{-1} HNO_3 + 1 mL of H_2O_2 30% v v^{-1}; final digest volume of 25 mL	125	4.2	NA	64	[31]
Pills and tablets	100	MW-AD[b]	5 mL of $3HNO_3$:1HCl v v^{-1}; final digest volume of 50 mL	500	2.6	10	114	[33]
Levetiracetam	1000	MW-AD[b]	15 mL of 14 mol L^{-1} HNO_3 + 2 mL of H_2O_2 30% v v^{-1}; final digest volume of 25 mL	25	16	16	4	[32]
Lu tablets	450	MW-AD[b]	12 mL of $3HNO_3$:1HCl v v^{-1}; final digest volume of 13 mL	29	0.32	1.55	0.70	[34]
Aspirin and Lisinopril	200	MW-AD[b]	7 mL of 14 mol L^{-1} HNO_3 + 2 mL of HCl + 1 mL of H_2O_2 30% v v^{-1}; final digest volume of 50 mL	250	0.4	1.2	0.7	[35]
Pills and tablets	500	MW-AD[b] + DLLME	7 mL of 2 mol L^{-1} HNO_3; final digest volume of 25 mL	50	0.3	1.8	1.6	This work

NA: Not applicable.
[a] Dilution factor, considering sample mass, final digest volume and further sample dilutions before analysis.
[b] Microwave-assisted digestion in closed vessel.
[c] Conventional heating-assisted digestion in closed vessel.

Table 6 Found concentrations (mean ± standard deviation, μg L^{-1}, n = 3) and recovery values in parenthesis (mean ± RSD, %) obtained for the spiked in digested drug samples (A-H) according to the *J* value using DLLME-ICP OES. All analytes were below their respective LOQ values for all samples analyzed

Sample	Added concentration	Found concentration		
		Cd	Hg	Pb
A	0.5*J*	5.0 ± 0.2 (98 ± 2)	31 ± 2 (102 ± 5)	5.0 ± 0.3 (99 ± 6)
	1.5*J*	14.9 ± 0.7 (98 ± 4)	95 ± 3 (105 ± 4)	15.3 ± 0.9 (102 ± 6)
B	0.5*J*	5.2 ± 0.1 (99 ± 4)	30.3 ± 0.6 (101 ± 2)	4.6 ± 0.2 (91 ± 4)
	1.5*J*	14.9 ± 0.7 (97 ± 6)	87 ± 3 (97 ± 3)	14 ± 1 (91 ± 8)
C	0.5*J*	5.1 ± 0.3 (98 ± 2)	30 ± 2 (99 ± 8)	4.5 ± 0.3 (90 ± 6)
	1.5*J*	14.3 ± 0.9 (94 ± 7)	88 ± 7 (98 ± 7)	13.7 ± 0.8 (91 ± 5)
D	0.5*J*	5.0 ± 0.3 (100 ± 6)	29 ± 2 (95 ± 6)	5.2 ± 0.3 (103 ± 7)
	1.5*J*	15 ± 1 (100 ± 9)	85 ± 4 (95 ± 4)	15 ± 1 (101 ± 8)
E	0.5*J*	4.8 ± 0.3 (97 ± 6)	32 ± 1 (105 ± 4)	5.40 ± 0.09 (108 ± 2)
	1.5*J*	16 ± 1 (103 ± 7)	90 ± 7 (100 ± 8)	15 ± 1 (102 ± 9)
F	0.5*J*	5.0 ± 0.3 (99 ± 6)	30 ± 2 (100 ± 7)	5.0 ± 0.2 (101 ± 4)
	1.5*J*	15 ± 1 (98 ± 6)	93 ± 6 (104 ± 6)	14.9 ± 0.6 (99 ± 4)
G	0.5*J*	4.8 ± 0.1 (96 ± 3)	31 ± 2 (103 ± 6)	4.8 ± 0.2 (95 ± 3)
	1.5*J*	16.0 ± 0.1 (106 ± 1)	96 ± 2 (107 ± 3)	14.7 ± 0.8 (98 ± 5)
H	0.5*J*	5.13 ± 0.06 (103 ± 1)	29 ± 2 (98 ± 8)	5.0 ± 0.3 (99 ± 5)
	1.5*J*	15.4 ± 0.9 (103 ± 6)	88 ± 5 (97 ± 5)	14.7 ± 0.2 (98 ± 2)

0.5*J*: Spiked digest with 5.0, 30 and 5.0 μg L^{-1} of Cd, Hg and Pb, respectively.
1.5*J*: Spiked digest with 15, 90 and 15 μg L^{-1} of Cd, Hg and Pb, respectively.