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Synergistic combination of natural deep eutectic solvent and chemical vapor generation for trace determination of As, Cd, Hg and Pb in drug samples by inductively coupled plasma optical emission spectrometry

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ABSTRACT

A new and environmentally friendly analytical method for simultaneous determination of As, Cd, Hg and Pb in drug samples by inductively coupled plasma optical emission spectrometry (ICP OES) has been developed. In order to increase the sensitivity of the analysis, a multinebulizer has been used for chemical vapor generation (CVG) after a dispersive liquid-liquid microextraction (DLLME) procedure using a natural deep eutectic solvent (NADES) as extractant solvent. The factors affecting analyte extraction and on-line chemical vapor generation have been optimized by multivariate analysis. Under optimized conditions, DLLME-CVG-ICP OES improved limit of quantitation (LOQ) values on average 50-fold higher compared with direct ICP OES analysis and afforded an increase of the sensitivity (i.e., enrichment factor) on average 25-times higher than those obtained with CVG-ICP OES analysis. According to the United States Pharmacopoeia (USP) Chapter 232, it means LOQ values are on average 4.3-times lower than their respective 0.3*J* values for the target-elements from class 1. Trueness has been evaluated by recovery experiments following USP recommendations for two oral drug samples in solid dosage form (i.e., commercial dosage tablets). In addition, the greenness of the developed method has been evaluated using the AGREEprep metrics, showing an excellent green character since it includes the miniaturization of the sample preparation procedure using a reduced volume (i.e., few microliters) of a non-hazardous extractant solvent for multielemental analysis.

Introduction

According to the new regulations for elemental impurities published by the United States Pharmacopoeia (USP) (i.e., the Chapters 232 [1] and 233 [2]) in a strict compliance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [3], permissible daily exposures (PDE) values for 24 elemental impurities are established for pharmaceuticals as well as analytical procedures for these determinations in drug products. Even at low concentration levels, the target elements from class 1 (i.e., As, Cd, Hg and Pb), also called as "Big Four", are considered extremely toxic to humans and must be evaluated in all potential sources of contamination [3–5].

Chapter 233 establishes two analytical methods for the evaluation of elemental impurities levels: inductively coupled plasma optical emission spectrometry (ICP OES) or inductively coupled plasma mass spectrometry (ICP-MS) [2]. Nonetheless, considering the low target-limits recommended for class 1, the majority of the proposed argon-based plasma spectroanalytical methods are focused on ICP-MS analysis [5–7].

In view of the importance of the greener analytical methods development, the 12 main Green Analytical Chemistry (GAC) principles were formulated in 2013 by Gałuszka et al. [8]. in order to express a willingness to care for the human safety and the environment during the development of analytical procedures. Lately, Red-Green-Blue (RGB) [9] and White Analytical Chemistry (WAC) concepts [10] were proposed in order to properly balance the greenness of the method with its potential

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applicability based on analytical efficiency expressed by validation criteria. Therefore, green analytical methods have to be not only environmental friendliness, but also present high sensitivity, simplicity and low-cost analysis. One of the objectives proposed by both GAC and WAC concepts is the increasing degree of automation, simplification, and miniaturization of analytical procedures. In that regard, the application of microextraction techniques coupled with a multi-elemental technique perfectly meets some of these green principles [8–10].

Accordingly, dispersive liquid-liquid microextraction (DLLME) is a successful extraction technique in which few microliters of a water-immiscible organic solvent are dispersed in fine drops into the aqueous sample to extract the analytes of interest from aqueous phase, enabling high enrichment factor [11,12]. A cloudy solution is formed because of the dispersion of the extractant solvent into the aqueous phase, leading to a great contact surface area. Finally, the phases are separated by centrifugation, and the enriched organic phase is collected and analyzed. In order to enhance the extractant phase dispersion, vortex-assisted DLLME has been employed [11].

Other approaches to perform DLLME process in more eco-friendly way include application of a non-hazardous extractant solvent, (e.g. supramolecular solvents, ionic liquids, deep eutectic solvents (DESs) or switchable solvents). On this regard, DESs have recently surged as one of the most promising alternatives to the use of hazardous organic solvents and as a cheap analogues of ionic liquids. The DESs are easily prepared by hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) using two or more unexpensive, renewable and biodegradable components [13–15]. When the compounds that formed the DES are natural components such as sugars, alcohols, amino acids, organic acids and choline derivatives, they are called Natural Deep Eutectic Solvents (NADES). NADES fully represent green chemistry principles [16].

Despite the advantages of preconcentration approaches based on microextraction techniques, one of the best-known effects of organic matrices introduction into the argon plasma is the formation of carbon deposits on the plasma torch. In order to face these challenges, the combined use of multinebulizer-based systems and ICP OES analysis has been successfully applied for samples with high organic contents [17–19]. This multinebulizer allows the simultaneous introduction of organic and aqueous solutions into the plasma, thus reducing carbon deposits and also correcting for matrix effects. This implies an important advantage over conventional sample introduction systems since it does not require the continuous cleaning of ICP components or the use of expensive additional components such as cooled spray chambers or an auxiliary oxygen supply [19].

Additionally, multinebulizer-based systems also allow the on-line chemical vapor generation (CVG) directly into the nebulizer without using any additional device (e.g., gas phase separation). Generation of volatile species is a powerful and widely employed methodology for determination at trace or ultra-trace level of selected elements coupled with spectroanalytical techniques [20]. For instance, CVG procedure has been widely employed for addressing elements such as arsenic when analytical instrument is not sensitive enough for direct analyte quantification [18,21].

In view of the above, this study aimed to propose a simple, sensitive and eco-friendly methodology based on the simultaneous preconcentration of As, Cd, Hg and Pb at trace levels from drug samples for subsequent measurement by CVG-ICP OES. In order to increase the sensitivity of the ICP OES, a DLLME using a non-hazardous extractant solvent (i.e., NADES) was performed to extract the analytes of interest and a multinebulizer has been used for CVG in situ at the nebulizer tip.

An extraction/preconcentration step prior to measurement has been previously developed for oral drug samples in solid dosage form [22] and oral/parenteral drug samples in liquid dosage form [23] to analyze the target elements from class 1, however, in contrast to this study, neither of these works could determine arsenic. In addition, some studies about DLLME-CVG have been published for the determination of trace metals by atomic absorption spectrometry (AAS) [24–26] or atomic fluorescence spectrometry (AFS) [27,28]. Nonetheless, studies on DLLME-CVG together with ICP OES have not been published yet. For that reason, and to the best of our knowledge this is the first study for the determination of all class 1 elements (i.e., including arsenic) combining the preconcentration step and the chemical vapor generation in a multinebulizer prior ICP OES analysis.

Experimental

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, obtained by a PURELAB flex 3 purification system (Elga LabWater, High Wycombe, UK). 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 (99.8 %, Scharlau Chemie, Barcelona, Spain) at a ratio of 4:1 v v^{-1}. Buffer solutions were prepared by dissolving the appropriate amount of sodium acetate (Panreac Químicas S. A., Castellar del Vallés, Spain) in acetic acid and ultrapure water in order to obtain pH values of 2 and 4. Thiourea (Scharlau Chemie, Barcelona, Spain) and sodium borohydride ($NaBH_4$, Panreac Químicas S.A, Barcelona, Spain) solutions of 1.0 % m v^{-1} were prepared by dissolving the appropriate amount of the reagents in the prepared buffer solution and ultrapure water, respectively. The synthesis and characterization of hydrophobic NADES used as extracting solvent was previously described by Pinheiro et al. [23], whereupon DL-menthol (purity ≥ 98 %, Alfa-Aesar™, Tewksbury, MA, United State) and decanoic acid (purity ≥ 98 %, Sigma-Aldrich) were used as hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), respectively.

Analytical reference solutions used for ICP OES calibrations and for recovery experiments were prepared by appropriate dilutions of 1000 mg L^{-1} of As, Cd, Hg, and Pb (High Purity Standards, Charleston, SC, USA) in 0.07 mol L^{-1} HNO_3 medium. The concentrations of all analytes were 200, 250, 300, 350, 400, 450, and 500 μg L^{-1} for ICP OES and CVG-ICP OES, and 2.5, 5, 15, 30, 45, 90, and 120 μg L^{-1} for DLLME-CVG-ICP OES and ICP-MS. External calibration was used for all the analysis. For the optimization of the extraction conditions, standard solutions containing 500 μg L^{-1} of all analytes were used. Recovery experiments were performed according to *J* values which were calculated according to oral PDE values specific for each target element (i.e., 15, 5.0, 30 and 5.0 μg day^{-1} for As, Cd, Hg and Pb, respectively) divided by the maximum daily dose (MDD) and the dilution factor (DF), (i.e. $J = PDE/(MDD \times DF)$) [1,2]. A MDD of 10 g day^{-1} was adopted for all samples to obtain the minimal *J* value that can be determined [22]. All drug samples were spiked before microwave-assisted digestion in triplicate with concentrations of 0.5*J* and 1.5*J* in order to check the accuracy of the developed analytical procedure. Therefore, considering the MDD of 10 g day^{-1} and the DF of 50 mL g^{-1} 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; 15 and 45 μg L^{-1} for As; and 30 and 90 μg L^{-1} for Hg.

Samples and sample preparation

Two drug samples in solid dosage form (i.e., A and B) were analyzed. A) omeprazole, used for benign (gastric or duodenal) peptic ulcers treatment; and, B) levothyroxine sodium, used for thyroid treatment. All analyzed samples were classified as oral administration route and were purchased in local pharmacies in San Vicente del Raspeig, Alicante, Spain. Sample preparation for drugs in solid dosage form (i.e., 500 mg) was performed based on previously proposed works for microwave-assisted sample digestion [22,29]. Digests were diluted to 25 mL with pure water (final dilution of 50-fold) after adding 8-HQ and thiourea at final concentration of 1.0 % m v^{-1} for both reagents and adjusting the

pH at 4.4 using a buffer solution of sodium acetate and acetic acid.

Dispersive liquid–liquid microextraction procedure

An 8.0 mL aliquot of the digested sample, at pH 4.4 and 8-HQ and thiourea at final concentration of 1.0 % m v^{-1}, was transferred to 10-mL glass tubes. Then, 80 μL of the extractant solvent (i.e., NADES) was added, and the mixture was shaken using a vortex shaker for 1 min. After shaking, the solution was centrifuged at 1460 *g*-force for 3 min to separate the two phases, with the analytes enriched phase at the top of the solution. After centrifugation, 60 μL 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. A schematic representation of the general DLLME procedure is presented in Fig. 1.

Instrumentation and on-line chemical vapor generation

A vortex agitator (Reax Top, Heidolph Instruments, Schwabach, Germany) was used to favor the dispersion of the extractant, a centrifuge (Mixtasel-BL, J.P. Selecta, Barcelona 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. Experiments were performed using an Agilent 720-ES inductively coupled plasma optical emission spectrometer (Agilent Technologies ICP OES, Melbourne, Australia) operating in axial viewing mode. For comparison purpose, direct ICP-MS analysis was performed using an Agilent 7700x. Argon (99.9992 %, Carburos Metálicos, Barcelona, Spain) was used in all measurements. Plasma operating conditions used in ICP OES are shown in **Table S1**. The selected conditions provided the highest signal for the set of emission lines evaluated simultaneously.

Introduction of enriched organic phase was performed using a multinebulizer (MultiNeb®, Ingeniatrics, Seville, Spain) [19,30]. It presents two independent liquid inlets and two different types of peristaltic tubes were used depending on the solution introduced. The channel 1 (i.e., flow rate: 50 μL min^{-1}) was used to aspirate $NaBH_4$ solution and the channel 2 (i.e., flow rate: 50 μL min^{-1}) was employed to introduce the extract (i.e., analyte enriched phase). A scheme of the system is shown in **Figure S1**. The liquid streams are mixed at the tip into the nebulizer and the aerosol of the mixture of the liquids exits by the unique hole generating the conditions for CVG. Then, the volatile species of interest generated in the process are transported through the spray chamber to the plasma without any separation of gas and liquid phases. In the liquid inlet where the analyte enriched organic phase was introduced (i.e., channel 2), 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 $NaBH_4$ solution was continuously pumped (i.e., channel 1), a Tygon® peristaltic tubes (R-3607, id. 0.76 mm, Ismatec) was employed.

Results and discussion

Optimization of DLLME-CVG procedure

In order to increase the analytical performance of ICP OES, a CVG after a DLLME was performed. The liquid streams, (i.e., the analyte enriched organic extract and the reducing agent ($NaBH_4$)) were mixed at the tip into the nebulizer and the aerosol of the mixture of the liquids enable the formation of the volatile species from the microextraction previously performed. The thiourea was used as a reducing agent in order to ensure that all arsenic present in the sample or in the extract (after the microwave-assisted digestion) was reduced to the appropriate oxidation state (i.e., As III) to react with the chelating agent (i.e., 8-HQ) [20]. Additionally, the addition of thiourea enhances the vapor generation efficiency [31].

Due to the several factors affecting the DLLME-CVG procedure, mainly considering the simultaneous determination of different analytes through the formation of their respective volatile species, the application of multivariate optimization design helps to determine the optimal experimental conditions for both procedures (i.e., DLLME and CVG), employing a reduced number of experiments [32]. Thus, the multivariate optimization of the DLLME-CVG procedure was performed using a Plackett-Burman design for screening approach to identify significant and non-significant factors followed by a central composite design (CCD) to obtain optimal values for the significant factors. The eight factors evaluated on the Plackett-Burman design and their low and high levels, respectively, were (i) NADES volume (100 and 200 μL); (ii) sample pH (2 and 4); (iii) $NaBH_4$ concentration (0.50 and 1.0 % m v^{-1}); (iv) 8-HQ concentration (0.50 and 1.0 % m v^{-1}); (v) thiourea concentration (0.50 and 1.0 % m v^{-1}); (vi) extraction time (1 and 3 min); (vii) centrifugation time (1 and 3 min); and (viii) centrifugation speed (650

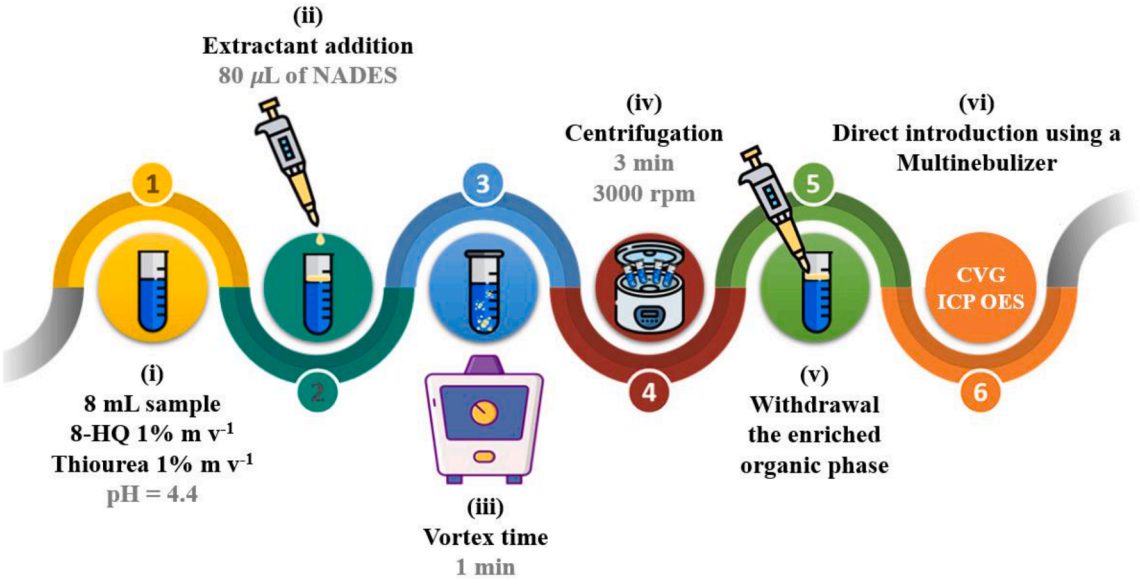

Fig. 1. Scheme of the DLLME-CVG-ICP OES procedure for preconcentration of As, Cd, Hg and Pb in drug samples.

and 1460 *g*-force). Pareto charts of the standardized effect show the results of the Plackett-Burman design for different elements in **Figure S2**.

In general, DLLME-CVG was favored at low level of extraction time (i.e., negative effect, indicate by bars to the left) and high levels of $NaBH_4$, 8-HQ and thiourea concentrations, centrifugation time and centrifugation speed (i.e., positive effects, indicate by bars to the right). All these factors showed a non-significant effect on DLLME-CVG for all the analytes (i.e., indicate by blue bars). In its turn, the DLLME-CVG variables (i) NADES volume (for Cd, Hg and Pb) and (ii) sample pH (for Cd and Pb) showed a significant effect on signal intensities. As shown in previous works [22,23], both factors are extremely significative for metal extraction procedures [11], since the extractant solvent volume affects the enrichment factor of analytes (further increases of the extractant solvent volume could cause a dilution effect, resulting in a decrease in the enrichment factor) and the pH has direct influence on the complexation step. In case of 8-HQ, it is poorly soluble in aqueous medium at pH range from 5.0 to 9.9, moreover, high pH values could also have a negative effect on extraction, since analytes can form hydroxides decreasing the amount extracted. In turn, considering low pH values evaluated (i.e., pH 2 and 4), positive effects on extraction could be achieved at a pH close to the first pKa of 8-HQ (i.e., 5.13) [33].

A central composite design (CCD) was performed to both significant factors. The different level values chosen in the CCD were: (i) NADES volume (79.3, 100, 150, 200, and 220.7 μL), and (ii) sample pH (1.6, 2.0, 3.0, 4.0 and 4.4). The response surfaces obtained are shown in **Figure S3**. Based on the response surfaces, the optimal values of both factors were low NADES volumes and high pH values for all analytes. Therefore, the optimized conditions for simultaneous extraction of all analytes were: sample pH of 4.4, 80 μL of NADES, $NaBH_4$ concentration of 1.0 % m v^{-1}, 8-HQ concentration of 1.0 % m v^{-1}, thiourea concentration of 1.0 % m v^{-1}, extraction time of 1 min, centrifugation time of 3 min and centrifugation speed of 1460 *g*-force.

Table 1
Analytical figures of merit for As, Cd, Hg and Pb determination in drug samples using DLLME-CVG-ICP OES.

	Emission line (nm)			
	As (188.980)	Cd (214.439)	Hg (194.164)	Pb (220.353)
Working range (μg L^{-1})	2.50-120	2.50-120	2.50-120	2.50-120
Calibration equation	y = (37.7 ± 0.9)*x* + (29 ± 53)	y = (383 ± 10)*x* – (842 ± 633)	y = (49.3 ± 1.6)*x* + (95 ± 96)	y = (35.4 ± 1.1)*x* + (228 ± 67)
r[a]	0.997	0.996	0.995	0.995
Sensitivity (cps L μg^{-1})[b]	37.7 ± 0.9	383 ± 10	49.3 ± 1.6	35.4 ± 1.1
EF[c]	11	53	12	23
LOD (μg L^{-1})	0.7	0.4	0.6	0.5
LOQ (μg L^{-1})	2	1.5	2	1.8
USP LOQ (μg L^{-1})[d]	≤9	≤3	≤18	≤3
Repeatability 0.5*J* (RSD%)[e]	9	10	10	8
Repeatability 1.5*J* (RSD%)[f]	8	9	9	8

[a] Correlation coefficient (seven calibration points);
[b] Slope ± standard deviation;
[c] Enrichment factor;
[d] LOQ values ≤0.3*J*.
[e] Mean value for six replicate analyses of spiked solution with 15, 5, 30 and 5 μg L^{-1} of As, Cd, Hg and Pb, respectively.
[f] Mean value for six replicate analyses of spiked solution with 45, 15, 90 and 15 μg L^{-1} of As, Cd, Hg and Pb, respectively.

Analytical performance of DLLME-CVG-ICP OES method

Table 1 and **Table S2** summarizes the analytical figures of merit obtained by the developed DLLME-CVG-ICP OES method, direct ICP OES, CVG-ICP OES and ICP-MS for the determination of As, Cd, Hg, and Pb in drug samples. Different calibration curves were performed: (i) for direct ICP OES and CVG-ICP OES analyses, seven calibration points with working range from 200 to 500 μg L^{-1} were prepared; and (ii) seven calibration points from 2.5 to 120 μg L^{-1} were prepared for DLLME-CVG-ICP OES and ICP-MS analyses. Considering USP Chapter 233 recommendation [2], the working range was set from 0.25*J* to 2.0*J* for all target elements. The linear correlation coefficients (r) obtained for all DLLME-CVG-ICP OES calibration curves ranged from 0.995 to 0.997 and the enrichment factors values ranged from 11 (for As) to 53 (for Cd), showing significant increase in sensitivity for all analytes. The EF was defined as the ratio of both calibration curves slopes, (i.e., with and without DLLME).

Limits of detection (LOD) and quantification (LOQ) were calculated according to Eurachem guidelines [34] 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 10 for LOQ and 3 for LOD [35]. Following USP Chapter 233, LOQ values ≤0.3*J* are suggested as acceptance criteria [2]. In this context, the LOQ values for direct ICP OES and CVG-ICP OES analysis were higher than the target-limits established by the USP (i.e., data shown in **Table S2**).

In turn, the LOQ values using DLLME-CVG-ICP OES are lower than their respective 0.3*J* values for all elements with a LOQ improvement on average 50-fold compared with direct ICP OES analysis. The repeatability was estimated from six independent measurements of samples spiked at 0.5*J* and 1.5*J* of each target element and the relative standard deviations obtained were ≤10 %, values significantly lower than 20 % of RSD stated by USP Chapter 233 [2]. Consequently, it may be inferred that the analytical parameters obtained for DLLME-CVG-ICP OES method are suitable to meet USP requirements for effective determination of class 1 target-elements.

Furthermore, the LOQs obtained using ICP-MS are only between 7 and 18 times lower compared with DLLME-CVG-ICP OES, and the sensitivity was increased in an average of 175 times using ICP-MS. Therefore, taking into account that both analysis methodologies meet the requirements of the United States Pharmacopoeia, and the acquisition and maintenance costs of the ICP-MS are very high, the developed method is a great alternative for the analysis of As, Cd, Hg and Pb in pharmaceutical samples.

Drug samples analysis according to USP requirements

Preliminary analysis shown that the four analytes were below their respective LOQ values for both samples analyzed (Table 2). Consequently, all samples are within the limits recommended by the USP Chapter 232 taking into account the maximum daily dose of each medicine (i.e., lower than 10 g day^{-1} for tablets drugs). In order to assess the trueness of the developed analytical procedure, all samples were spiked before microwave-assisted digestion with concentrations of 0.5*J* and 1.5*J* for As, Cd, Hg and Pb [2]. Recovery values ranged from 91 to 102 % at both levels and the repeatability showed RSD values lower than 8 % ($n = 6$) considering both samples. Therefore, no matrix effects were observed for DLLME-CVG-ICP OES, and external calibration was used. The application of multinebulization-based system for CVG, and consequently the additional water from $NaBH_4$ solution eliminated possible carbon deposit at the torch of the plasma and spectral interferences created by carbon compounds and therefore, prevents the resulting loss of sensitivity and precision.

Table 2
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-B) according to the *J* value using DLLME-CVG-ICP OES.

Sample	Added concentration	Found concentration As	Cd	Hg	Pb
A	-	<LOQ	<LOQ	<LOQ	<LOQ
	0.5*J*[a]	14.9 ± 0.9 (99 ± 6)	4.6 ± 0.2 (92 ± 4)	28.8 ± 1.6 (96 ± 6)	4.7 ± 0.2 (95 ± 4)
	1.5*J*[b]	44 ± 3 (98 ± 8)	14.0 ± 0.5 (94 ± 4)	86.3 ± 1.6 (95.9 ± 1.9)	13.8 ± 0.8 (92 ± 6)
B	-	<LOQ	<LOQ	<LOQ	<LOQ
	0.5*J*[a]	14.4 ± 1.1 (96 ± 7)	5.1 ± 0.4 (102 ± 7)	27.4 ± 1.1 (91 ± 4)	4.9 ± 0.2 (100 ± 5)
	1.5*J*[b]	43.7 ± 0.9 (96 ± 2)	13.8 ± 0.6 (92 ± 5)	83 ± 2 (92 ± 3)	14.5 ± 0.3 (97.1 ± 1.8)

[a] 0.5*J*: Spiked digest with 15, 5.0, 30 and 5.0 μg L^{-1} of As, Cd, Hg and Pb, respectively; 1.5*J*: Spiked digest with 45, 15, 90 and 15 μg L^{-1} of As, Cd, Hg and Pb, respectively.

Comparison with other analytical methods

Table 3 summarizes analytical methodology previously reported for As, Cd, Hg and Pb determination in pharmaceutical samples using ICP OES. Taking into account that the oral drug samples in solid dosage form (i.e., pills and tablets) need more sophisticated procedures for sample decomposition, and consequently, a high dilution factor of sample solution (i.e., ratio between final volume and the sample mass), the LOQ values obtained for As [29,36], Cd, Hg [22,29] and Pb [22,29,36] by direct ICP OES analysis were higher than 0.3*J* (i.e., LOQ suggested by USP). Menoutis et al. [37]. obtained comparable LOQ values to 0.3*J* by direct ICP OES analysis, however, this can be explain since the authors used a high cost ultrasonic nebulizer with a relative high sample consumption (i.e., 1.9 mL min^{-1}). Similar to this proposed work, an extraction/preconcentration step prior to ICP OES measurement has been developed for oral drug samples in solid dosage form after microwave-assisted acid digestion [22]. Despite the satisfactory limits achieved, the main disadvantage is the use of a less ecological solvent when compared to NADES. Furthermore, the developed method in this work [22] was not useful for the determination of arsenic.

On the other hand, considering oral drug samples in liquid dosage form, with a simpler sample preparation procedure with lower dilution factor, the LOQ values obtained for As, Cd and Hg [38] and for Cd, Hg and Pb [23] by direct ICP OES analysis were respectively lower and higher than 0.3*J*. It can be explained because *J* values were calculated depending on the specific MDD (i.e., PDE remain constant for each analyte) of each medication, and considering some medicines with low MDD [38], the target-limits established by the USP are consequently higher. In addition, the same NADES was used as extractant solvent at the work carried out by Pinheiro et al. [23]. Although a lower LOQ was obtained for Cd, higher limits were obtained for Hg and Pb, and arsenic could not be determined. The arsenic trivalent form does not form complexes with 8-HQ, therefore, in this work, the use of thiourea as reducing agent has facilitated its extraction, and the CVG generation increased the sensitivity enabling to measure it in accordance to the threshold limit established by the USP. Finally, the analytical greenness of each method was calculated through the AGREEprep [39] metrics (**Figure S4**). It is possible to see that the method developed in this work is the greenest (i.e., 0.40) with the exception of the work carried out by Pinheiro et al. [23], where an AGREEprep value of 0.46 was reached. Nevertheless, this higher score was obtained because the evaluated sample was liquid and digestion step was not needed avoiding the consumption of nitric acid and energy.

Conclusions

The developed NADES-based DLLME procedure combined with CVG-ICP OES was successfully applied to trace determination of As, Cd, Hg and Pb in medicine samples. Additionally, the combination of (i)

Table 3
Comparison of ICP OES-based methods developed for target-elements from class 1 determination.

Pharmaceutical sample	Sample mass (mg)	Sample preparation procedure	Sample preparation details	DF[a] method	Quantification limit (0.3*J*)[b] As	Cd	Hg	Pb	AGREEprep score	Reference
Pills and tablets	200	MW-AD[c]	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	0.8 (1.8)	0.4 (0.6)	1.2 (1.8)	0.7 (0.6)	0.24	37
Pills and tablets	100	MW-AD[c]	5 mL of 3HNO_3:1HCl v v^{-1}; final digest volume of 50 mL	500	33 (18)	2.6 (3.6)	10 (24)	114 (3.6)	0.24	36
Pills and tablets	500	MW-AD[c]	7 mL of 2 mol L^{-1} HNO_3; final digest volume of 50 mL	100	17 (4.5)	5.4 (1.5)	21 (9)	39 (1.5)	0.30	29
Liquid drugs	NA	DA[d]	10-fold dilution in 0.14 mol L^{-1} HNO_3	10	10 (15)	1.0 (4.8)	7.0 (30)	18 (4.8)	NA	38
Liquid drugs (oral and parenteral)	NA	DA[d]	10-fold dilution in 0.14 mol L^{-1} HNO_3	10	NA	17 (1.6)	72 (23)	49 (1.6)	0.46	23
		DA[d] + DLLME[e]				0.2 (1.6)	3.0 (23)	4.0 (1.6)		
Pills and tablets	500	MW-AD[c]	7 mL of 2 mol L^{-1} HNO_3; final digest volume of 25 mL	50	NA	12 (3)	70 (18)	70 (3)	0.27	22
		MW-AD[c] + DLLME				0.3 (3)	1.8 (18)	1.6 (3)		
Pills and tablets	500	MW-AD[c]	7 mL of 2 mol L^{-1} HNO_3; final digest volume of 25 mL	50	159 (9)	36 (3)	122 (18)	94 (3)	0.40	This work
		MW-AD[c] + DLLME[e]			2.0 (9)	1.5 (3)	2.0 (18)	1.8 (3)		

[a] Dilution factor, considering sample mass, final digest volume and further sample dilutions before analysis;
[b] Calculated based on the *J* values specified by the referenced works and the dilution factor of the method;
[c] Microwave-assisted acid digestion in closed vessel;
[d] Dilution in aqueous solution;
[e] NADES-based DLLME. NA: Not applicable.

microwave-assisted digestion procedure using dilute nitric acid solution, (ii) multivariate approach for optimization of the DLLME-CVG procedure, (iii) the reduction of extractant volume and (iv) the biodegradable nature of the NADES used as extractant solvent follows most of the principles of GAC and WAC concepts. DLLME-CVG-ICP OES affords a significant increase of sensitivity showing an enrichment factor on average of 25-fold compared to CVG-ICP OES analysis. Posteriorly, the volatile species formed at the tip of the multinebulizer increases the sensitivity, avoiding problems in the transport of the analytes to the plasma. Analytical performance was well validated in accordance with the USP Chapter 233, and consequently, the results proved to be sensitive and reliable enough for the determination of target elements from class 1 by ICP OES following USP requirements. For all the above reasons, the developed method is a great alternative to the existing methods because it has a greener character compared with previous works. Furthermore, it meets the requirements of the USP due to the increased sensitivity for all the analytes from class 1 (i.e., As, Cd, Hg and Pb) through CVG, and in comparison with previous works, it is also capable of performing arsenic analysis through the use of thiourea.

Declaration of Competing Interest

All authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this manuscript.

Data availability

No data was used for the research described in the article.

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