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Development of preconcentration strategies for the simultaneous ultratrace determination of As, Cd and Pb in foods by ICP-OES: knotted-reactor vs dispersive liquid-liquid microextraction.

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Abstract

Determination of As, Cd and Pb in food samples by means of inductively coupled plasma optical emission spectrometry (ICP-OES) is challenging due to detection capabilities being close to the maximum levels established by current international food security policies. This work evaluates the benefits and drawbacks of knotted reactor extraction (KR) and dispersive liquid-liquid microextraction (DLLME) for the simultaneous ultratrace determination of the above-mentioned elements by ICP-OES. To this end, ICP-OES experimental conditions were optimized to minimize the negative effects of organics on plasma characteristics. Next, both KR and DLLME were optimized using experimental design for the simultaneous As, Cd and Pb preconcentration. KR-

and DLLME-ICP-OES methods were compared and applied to the analysis of different food samples, representative of the commodities regulated by the EU policy. Results in this work show that both KR and DLLME allow successful toxic element analysis in foods according to current EU policies. Nevertheless, DLLME is a more attractive approach than KR. First, DLLME allows the simultaneous determination of As, Cd and Pb, while KR is just limited to the last two elements, since As-complexes are not efficiently retained within the system. When compared to conventional ICP-OES analysis (i.e., no preconcentration), DLLME improves limits of detection (LOD) on average 40-fold for As, Cd and Pb whereas KR improves it just 10-fold. For both methodologies, LOD improvement is derived by the preconcentration procedure as well as the beneficial effect of organics on aerosol generation and transport to the plasma regarding aqueous samples. Finally, DLLME affords higher sample throughput and consumption index than KR.

Keywords: Metals, food analysis, knotted-reactor, dispersive liquid-liquid microextraction, inductively coupled plasma, optical emission spectrometry

Introduction

Inductively coupled plasma optical emission spectrometry (ICP-OES) is widely employed in food sciences for major, minor and trace elemental analysis due to its outstanding figures of merit: (i) good accuracy and precision; (ii) low limits of detection (in the order of μg L^{-1}); (iii) high dynamic range; and (iv) multi-element capabilities.[1] Since the conventional sample introduction system in

ICP-OES operates with liquid samples, a preliminary preparation step is usually required to analyze foods. Thus, form solid samples, a previous acid digestion step is generally required. Beverages could be directly introduced into the plasma but, most of the times, also require a preliminary sample treatment (e.g. filtration, dilution and even acid digestion) to mitigate both spectral and non-spectral interferences.[1] Determination of toxic elements (e.g., As, Cd, Pb, etc.) in foods by means of ICP-OES use to be troublesome since detection capabilities achieved by commercial instrumental techniques are usually close (or even above) to the maximum allowed levels established by current international food security policies, particularly those from the European Union (EU).[2] Consequently, after sample decomposition, an additional extraction-preconcentration treatment is necessary for the accurate determination of toxic elements in foods.[3,4]

Preconcentration based on the retention of metallic complexes on the inner walls of a polytetrafluoroethylene (PTFE) knotted reactor (KR) has been successfully employed to improve the analytical figures of merit of ICP-OES.[5] The sample and a chelating agent solution are mixed under appropriate experimental conditions to favor analyte complex retention within the KR due to changes in the flow direction caused by the knots, which push the analyte complex particles towards the tubing walls. Next, the analyte complex is eluted by the appropriate solvent prior to ICP-OES analysis. Though organic solvents are particularly beneficial for complex elution, inorganic eluents (e.g. HNO_3, HCl, etc.) are required to avoid interferences due to organics and even plasma extinction.[6,7] This preconcentration methodology has been mostly applied to elemental analysis in water and biological samples but scarcely to food

samples.[5] One of the few examples is the case of Lara et al., who successfully applied KR to Cd determination in wine using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol as the chelating agent.[6] Therefore, the analytical potential of KR for elemental analysis in foods is still unclear. On this regard, coupling KR to ICP-OES has been focused on the determination of single elements, not taking advantage of the multielemental capability of ICP-OES.

In recent years, dispersive liquid-liquid microextraction (DLLME) has become a very popular extraction/preconcentration technique prior to atomic spectrometric determinations due to its high enrichment factors, simplicity, high sample throughput, low cost and sustainability (i.e. minimum reagents requirements and waste generation). In DLLME for the preconcentration of metallic ions, the sample is first conditioned with a chelating agent and buffer solution. Next, the extraction solvent is injected into the sample with the aid of a third solvent which acts as a dispersing agent, resulting in a cloudy emulsion. Because of the large contact surface area between the aqueous and organic phases, the analyte-chelate complex is transferred into the organic droplets. Finally, the cloudy emulsion is centrifuged and the organic phase is removed for analysis by means of an elemental detection technique.

Coupling DLLME to ICP-OES is particularly challenging due to spectral and non-spectral interferences caused by organics.[8,9] Several strategies have been proposed to deal with these interferences: (i) solvent evaporation followed by acid reconstitution;[10] (ii) water back-extraction;[11] (iii) dilution with an appropriate solvent;[12] and (iv) the use of non-conventional sample introduction systems (e.g. electrothermal vaporization).[13] Nevertheless, these strategies increase the complexity of the analysis, raise costs and reduce sample throughput. Martinez

et al.[14] have recently demonstrated that some organic extracts used in DLLME (e.g. chloroform, 1-undecanol and 1-butyl-3-methylimidazolium hexafluorophosphate) can be directly analyzed by means of ICP-OES. These authors observed that, with a thorough selection of the experimental conditions, LODs in ICP-OES were indeed enhanced by the preconcentration itself, but also by the higher analyte transport efficiency caused by the organic solvents employed.[15,16] As with KR extraction-preconcentration, DLLME has been scarcely applied to multielemental analysis in food samples coupled to ICP-OES.[17,18]

The goal of this work was to improve ICP-OES analytical figures of merit for the simultaneous ultratrace determination of As, Cd and Pb levels in foods after a preconcentration step based on KR and DLLME. First, ICP-OES experimental conditions were thoroughly optimized to operate with organic extractants usually employed with both preconcentration methodologies, thus taking advantage of the benefits of their physicochemical properties on aerosol generation and transport to the plasma. Next, KR and DLLME extraction conditions were optimized for the simultaneous determination of As, Cd and Pb in a single run. Finally, KR- and DLLME-ICP-OES methods were applied to several food samples (i.e. chocolate, mussels, rice and wine) representative of commodities regulated by EU food policies.

Experimental

Chemicals

Tetrahydrofuran (THF, 99%), acetone (≥99.5%) and methanol (≥99.9%) were obtained from Honeywell (New Jersey, USA). 1-decanol (99%), 1-

undecanol (99%), decanoic acid (98%), acetonitrile (99%), sodium citrate tribasic dihydrate (99%), ammonium pyrrolidine dithiocarbamate (APDC) (99.9%), potassium iodide (>99%), sodium thiosulphate (>99.99%) and As (III), Cd (II) and Pb (II) monoelemental standard solutions (1000 mg L^{-1}) were purchased from Sigma-Aldrich (Steinheim, Germany). Absolute ethanol (99.9%), nitric acid (69% w w^{-1}), sodium dihydrogen phosphate (≥99.5%), sodium chloride (≥99.5%), disodium hydrogen phosphate (≥99.5%), glacial acetic acid (99.7%), sodium acetate (99%) and 1-propanol (≥99.5%) were obtained from Panreac (Barcelona, Spain). Finally, citric acid (≥99.5%) was provided by VWR (Radnor, USA).

Samples

Four different food samples covering different type of matrices were analyzed in this work: (i) mussels (*Mytilus edulis chilensis,* Chile); (ii) rice (La Fallera, Spain); (iii) red wine (Caño Viejo, Spain, alcoholic content: 10% w w^{-1}); and, (iv) chocolate (Nestle, Spain, cocoa content: 44% w w^{-1}). These foods are representative of commodities regulated for toxic metals by the EU.[2]

Sample preparation

Mussel samples were dried at 60 ºC for 48 h in a heating stove (model SE70SDB, San Jor, Buenos Aires, Argentina). All the solid samples were grinded using an electric grinder (model MO-8100A, Ultracomb, China). Mussels, rice and chocolate samples were digested in a microwave oven (model Start D, Milestone, Italy) using the experimental conditions recommended by the manufacturer (Table 1). After sample decomposition,

digests were quantitatively transferred into 20 mL volumetric flasks and neutralized with NaOH solution. Finally, all samples (solid digests and untreated wine) were subjected to the corresponding extraction/preconcentration methodology (i.e., KR or DLLME). Experimental conditions for both KR and DLLME were optimized by means of experimental design.[19] Data analysis was carried out using Statgraphics® centurion 16.1.11 32-bit software (Statpoint Technologies, USA).

Knotted reactor extraction

In the present work, opposite to that previously reported in ICP-OES,[5] the KR was operated off-line. An overview of KR operation is shown in Fig. 1.A. First, 0.5 M KI (1 mL) and 0.2 M $Na_2S_2O_3$ (0.5 mL) were added to a 4 mL sample aliquot previously placed into a 10 mL vial. This mixture was left to stand for 10 min and pH was adjusted with the corresponding buffer solution (i.e. acetic acid). Thus, all the As present in the sample was in the appropriate oxidation state (III) to react with the chelating agent. Next, the sample (flow rate 2.0 mL min^{-1}) and an APDC solution (flow rate 0.5 mL min^{-1}) were mixed with the aid of a T-joint and the mixture was loaded to a PTFE knotted reactor (i.d. 500 µm, 334 cm length) in which the analyte-APDC complex was retained. Next, the complex was eluted with 150 µL of an appropriate organic solvent (methanol, ethanol, 1-propanol, acetic acid and acetonitrile) and transferred to an Eppendorf tube. Finally, a cleaning step was carried out using a 3% w w^{-1} nitric acid solution, which was circulated for 2 minutes at a rate of 3 mL min^{-1}.

Dispersive liquid–liquid microextraction

Supramolecular solvents have been selected as the extraction media since they are more environmentally friendly than the traditional volatile organic solvents (e.g. chloroform, etc.) employed in DLLME. These solvents are nanostructured liquids spontaneously generated from aqueous or hydro-organic solutions of amphiphiles through a self-assembly process known as coacervation.[20,21] In this work, three supramolecular solvents were generated by combining THF with different surfactants (i.e. 1-decanol, 1-undecanol and decanoic acid). Fig. 1.B shows a scheme of the DLLME experimental procedure. First, following the procedure described in the previous section, 4 mL sample aliquots were treated with KI and $Na_2S_2O_3$ and the mixture was spiked with a buffer solution to adjust the pH and 100 µL of APDC (2%). Next, a mixture of THF and the corresponding surfactant was added to the sample with a 2.5 mL glass syringe (Hamilton s/1000, USA). A cloudy solution was formed and, after centrifugation for 90 s at 3,130 g (Auxilab Nahita 2690, Beriáin, Spain), the micelle upper layer was transferred to an Eppendorf tube, where it was diluted prior to ICP-OES analysis due to its high viscosity. To this end, different dilution solvents (methanol, ethanol, 1-propanol, acetonitrile and acetic acid) and extractant:solvent ratios (1:0.25 to 1:3) were investigated.

ICP-OES instrumentation

ICP-OES measurements were performed using an Agilent 720 ICP-OES instrument (Santa Clara, USA) with axial viewing, under the operating conditions reported in Table 2. Sample introduction was achieved using a concentric pneumatic nebulizer (Seaspray, Glass Expansion, Australia) and a cyclonic spray chamber (Cinnabar, Glass Expansion, Australia). Samples were

introduced into the system with the aid of a flow injection manifold (Model V-451, Upchurch Scientific, Silsden, United Kingdom) equipped with a 25 µL loop valve and an in-house prepared 300 µL plastic syringe with a PEEK coated quartz capillary needle (200 µm i.d., PEEKSIL, Upchurch, Oak Harbor, Washington, USA). Samples were introduced into a 1% w w^{-1} HNO_3 carrier stream controlled by a peristaltic pump (Model Minipuls 3, Gilson, France). Arsenic I 193.696 nm, Cd II 214.439 nm and Pb II 220.353 nm were the monitored wavelengths for each analyte. Signal acquisition was performed by means of the transient signal (TRS) software of the ICP-OES instrument. Microsoft Excel® software was employed for manual signal integration.

For comparison, digested samples and untreated wine were also analyzed by means of ICP-MS as described elsewhere.[22]

Results

Knotted-reactor extraction

Coupling KR to ICP-OES

Due to the detrimental effects of organic solvents on the plasma discharge, inorganic solutions are usually employed to elute the analyte complexes retained within the KR.[6,7] Nevertheless, by the appropriate selection of the experimental conditions, spectral and non-spectral interferences caused by organics could be mitigated. In fact, operating organic solvents could be potentially beneficial to improve the analytical figures of merit in comparison to inorganic acid solutions since organics affords higher aerosol generation and analyte transport.[14] In the present work, the possibility of applying organic solvents with KR in ICP-OES was investigated. To this end, several modifications were introduced in the experimental arrangement usually

employed in ICP-OES.[5] First, the KR was operated off-line, resulting in a higher flexibility regarding experimental conditions (e.g. carrier flows, elution solvents, etc.). On the other hand, to improve plasma stability operating with organic solvents, eluates from the KR were introduced into the ICP-OES by means of a flow injection analysis (FIA)-manifold using a 1% w w^{-1} nitric acid solution as the carrier medium.[14]

First, the influence of the elution solvent on analyte signal in ICP-OES was evaluated. To this end, a 1 mg L^{-1} As(III), Cd(II) and Pb(II) standard solution was preconcentrated within the KR according to the experimental procedure described in the Experimental section. Next, the analyte complexes were eluted with 150 µL of different organic solvents (methanol, ethanol, 1-propanol, acetonitrile and acetic acid) and the extracts were directly analyzed by ICP-OES. For the sake of comparison, a standard sample in 10% w w^{-1} nitric acid solution was also measured, since this solution has been usually employed for analyte elution with KR in ICP-OES.[6,7] Fig. 2 shows Cd II 214.439 nm signal profiles obtained with the different organic eluents tested. Data in Fig. 2 reveals that operating methanol, ethanol and acetonitrile affords stronger memory effects that the remaining tested eluents (i.e., 1-propanol, acetic acid and 10% w w^{-1} nitric acid solutions). In addition, a very unstable plasma is observed when introducing methanol, ethanol or acetonitrile into the plasma due to the higher volatility of these three solvents against 1-propanol, acetic acid and 10% w w^{-1} nitric acid.[23,24] Analyte signals for 1-propanol and acetic acid solutions were significantly higher than those obtained with 10% w w^{-1} nitric acid (1.7 and 1.8-fold, on average, for 1-propanol and acetic acid, respectively). These results are the expected, taking into account the physicochemical properties (i.e. surface

tension) of these solvents.[15,16,24] Similar findings were observed for Pb and, therefore, acetic acid was selected as the analyte elution solvent for the determination of these elements operating the KR.

Finally, no conclusive results were obtained for As. The emission signal for this element (As I 193.696 nm) was very poor, regardless of the KR operating conditions. Alternatively, As concentration was increased up to 15 mg L^{-1} to perform the optimization, but no significant improvement was achieved. This behavior is explained considering that the As-APDC complex retention within the KR is poor (i.e. 18%)[25] and the low sensitivity of As in ICP-OES. Therefore, As determination by means of KR-ICP-OES was discarded.

Optimization of ICP-OES experimental conditions

ICP-OES experimental conditions were optimized to operate with acetic acid extracts. Plasma RF power was kept close to the maximum available instrumental nominal value (i.e. 1400 W) to favor matrix decomposition as well as analyte atomization, ionization and excitation within the plasma. To evaluate the influence of both the nebulizer gas flow rate (Q_g) and sample uptake rate (Q_l) on signal emission, a 1 mg L^{-1} analyte standard solution in acetic acid was prepared. Fig. 3 shows the influence of Q_g on the Cd II 214.439 nm integrated signal at different Q_l values. Results indicate that analyte emission is favored when decreasing both Q_g and Q_l. This behavior can be explained in terms of aerosol generation and plasma robustness.[16,26] Similar findings were observed for Pb II 220.353 nm. From data gathered in Fig. 3, an optimum Q_g of 0.6 L min^{-1} and a Q_l of 0.4 mL min^{-1} were selected to analyze acetic acid extracts. These

conditions allowed the long-term ICP-OES operation with acetic acid eluates without formation of carbon deposits on the torch.

Optimization of Cd and Pb extraction conditions with KR

The experimental variables controlling metal extraction in the KR were optimized by means of a central composite design (CCD).[19] After checking previous studies in the literature[5-7] and some preliminary experiments, pH, APDC concentration, KR length and sample elution flow were identified as the most significant variables controlling Cd and Pb extraction. Each of the four variables selected were investigated in five levels: (i) pH (1; 3; 5; 7 and 9); (ii) APDC concentration (0.005% w w^{-1}; 0.27% w w^{-1}; 0.53% w w^{-1}; 0.80% w w^{-1} and 1.65 % w w^{-1}) (iii) reactor length (22 cm; 100 cm; 178 cm; 256 cm and 334 cm); and (iv) elution flow rate (0.11 mL min^{-1}; 0.23 mL min^{-1}; 0.34 mL min^{-1}; 0.45 mL min^{-1}; 0.56 mL min^{-1}). A total of 26 experiments were performed by triplicate using a standard 1 mg L^{-1} analyte solution (Table S1).

To evaluate the significance of each variable on Cd and Pb extraction, data was analyzed by ANOVA and the effects were summarized by means of the corresponding Pareto charts (Fig. 4). The ANOVA data analysis revealed that Cd and Pb extraction was dependent on pH, APDC concentration and reactor length, but not on sample elution flow. Extraction for both elements improved by decreasing solution pH as well as by increasing APDC concentration and reactor length. These results are the expected considering that these metal-APDC complex formation is favored by increasing the concentration of chelating agent at acidic pH values.[27] Similarly, a higher reactor length favors the metal chelate retention in the KR. Experimental data also revealed that there are

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some interactions among the investigated variables. Thus, Cd extraction significantly depended on two-factor interactions effects, pH/elution flow rate and APDC concentration/reactor length. On the other hand, Pb extraction significantly depended on pH/APDC concentration and APDC concentration/reactor length. Table 3 shows the optimum experimental conditions derived from the CCD model for Cd and Pb extraction. In general, optimal experimental conditions for both elements were rather similar, although some differences were found in the pH and the elution flow rate. Because of the simultaneous multi-elemental capabilities of ICP-OES, compromise pH and elution flow rate values had to be selected for the simultaneous determination of both elements in a single run. From the CCD model, it was predicted that Cd and Pb extraction would be reduced in 5% when operating at pH 1.2 and with an elution flow rate of 0.3 mL min^{-1}. These data were experimentally verified and, consequently, the above-mentioned pH and elution flow rate were selected accordingly for further studies.

Dispersive liquid-liquid microextraction

Coupling DLLME to ICP-OES

Supramolecular solvents based on the use of THF with either alcohols[28-30] or organic acids[31] have been employed in the literature for metal extraction by means of DLLME and atomic absorption spectrometry detection. To date, however, no previous attempt to apply these solvents in ICP-based techniques has been reported.

In this work, supramolecular solvents were prepared by combining THF with different surfactants, namely: (i) 1-decanol;[28,29] (ii) 1-undecanol;[30] and (iii)

decanoic acid.[31] Initially, supramolecular solvents were directly introduced into the ICP-OES instrument using a FIA manifold, but emission signals from As, Cd and Pb were highly irreproducible. These results are the expected, taking into account that supramolecular solvents are highly viscous substances[31] and, consequently, analytical figures of merit in ICP-OES can be compromised due to the negative influence of this physical property on aerosol generation.[15,16] To solve this problem, supramolecular solvents were diluted with methanol (1:1 proportion). Nevertheless, it was observed that both the FIA system and the nebulizer were quickly blocked after some injections with the decanoic acid-based micelles. The low solubility of decanoic acid in the 1% w w^{-1} nitric acid carrier solution (approximately 0.15 g L^{-1}) generates the precipitation of the compound along the sample introduction device. In fact, a decanoic acid precipitate was also visible inside the spray chamber. Consequently, decanoic acid-based supramolecular solvents were discarded for further studies. Supramolecular solvents based on 1-decanol and 1-undecanol were more attractive since these alcohols are liquids at room temperature and do not precipitate in the presence of the carrier solution.

Since the analytical figures of merit of ICP-based techniques strongly depend on the physicochemical properties of the sample matrix,[15,16] the influence of the solvent employed to dilute the supramolecular solvent on analyte signal in ICP-OES was examined. In addition to methanol, four solvents were tested: (i) ethanol; (ii) 1-propanol; (iii) acetonitrile; and (iv) acetic acid. For each of them, 1:1 extractant:solvent mixtures were prepared containing 1 mg L^{-1} of As, Cd and Pb. Fig. 5 shows the influence of the dilution solvent on the integrated net analyte signals for the 1:1 diluted 1-decanol-based micelles. The

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highest signal for all the analytes was obtained operating with acetic acid, followed by the alcohols and acetonitrile. Differences among the tested solvents were mainly related to blank corrections, since all the mixtures yielded similar raw signals. Comparable findings were also observed using 1-undecanol as surfactant. A priori, higher signals would have been expected for alcohols and acetonitrile due to their higher volatility, which theoretically favors aerosol transport efficiency.[15,16] Experimental data suggest, however, that volatility is not critical for the selection of the dilution solvent due to the low dilution factors employed in this work. Based on the results presented in Fig. 5, acetic acid was selected to dilute the supramolecular solvent. Additionally, the influence of the supramolecular solvent:acetic acid ratio on emission signals was also examined. To this end, extractant:acetic acid ratios ranging from 1:0.25 to 1:3 were investigated. All the assayed mixtures had a fixed amount of analyte (1 mg L^{-1}), thus allowing the evaluation of the influence of the physicochemical properties of the matrix on aerosol generation and transport. For all the analytes, emission signals improved with dilution up to a 1:1 ratio and remained constant with further acetic acid additions (Fig. S1). These data suggest that dilution is beneficial to improve aerosol generation, probably due to a reduction of sample viscosity. Nevertheless, from a practical point of view, high dilution of DLLME extracts is a drawback, due to its negative effect on sensitivity and limits of detection (LOD). When increasing DLLME extract dilution from 1:0.5 to 1:1, analyte concentration is decreased by 50% but signal improvement is just 43%. In this work, a 1:0.5 dilution ratio was selected as a compromise between analyte figures of merit and sample handling.

Optimization of ICP-OES experimental conditions

ICP-OES experimental conditions were optimized to operate with the 1:0.5 supramolecular solvent:acetic acid mixtures. The influence of both Q_g and Q_l on the analyte signal for DLLME was similar to that previously observed for pure acetic acid extracts with the KR (Fig. S2). These results are the expected, considering that organic extracts are introduced into the plasma with the same sample introduction system. Consequently, Q_g and Q_l were respectively fixed at 0.6 L min^{-1} and 0.4 mL min^{-1}.

Optimization of As, Cd and Pb extraction conditions with DLLME

A CCD design was employed for a detailed optimization of extraction conditions with DLLME. Some preliminary experiments were performed to evaluate the influence of the surfactant nature (1-decanol and 1-undecanol) on analyte extraction. It was observed that metal extraction was almost independent of the selected surfactant and, hence, 1-decanol was selected for further studies. According to these preliminary experiments and previous works[32], pH, APDC concentration, THF volume and surfactant mass were identified as the main relevant variables controlling metal extraction. Each of the four variables selected were investigated in five levels: (i) pH (0; 3; 6; 9 and 12); (ii) APDC concentration (0.0% w w^{-1}; 0.10% w w^{-1}; 0.25% w w^{-1}; 0.40% w w^{-1} and 0.55 % w w^{-1}) (iii) THF volume (0.03 mL; 0.15 mL; 0.28 mL; 0.40 mL and 0.53 mL); and (iv) surfactant mass (0 mg; 80 mg; 160 mg; 240 mg and 320 mg). A total of 26 experiments were performed by triplicate using a 1 mg L^{-1} standard analyte solution (Table S2). Pareto charts show that the most significant variables on metal extraction depended on the studied analyte (Fig. 6).

Extraction recoveries for Cd and Pb were favored by increasing pH, APDC concentration and THF volume as well as by decreasing surfactant mass. On the other hand, only THF exerts a (positive) significant effect on As extraction. In either case, it was noticed that the investigated variables were not orthogonal, since analyte extraction was also dependent on two-factor interaction effects. Table 3 shows the optimum experimental conditions derived from the CCD model for As, Cd and Pb extraction by means of DLLME. As shown in Table 3, significant differences on the optimum pH and surfactant mass values for each element are obtained. Thus, for instance, Cd and Pb extraction is maximum at pH values around 6, whereas As requires highly acidic conditions (pH = 1.8). As discussed for KR extraction, the CCD model was examined to select a compromise set of experimental conditions for the simultaneous analysis of all the analytes in a single run. Table 3 gathers the compromise DLLME experimental conditions selected for As, Cd and Pb extraction. It was observed that, under those conditions, extraction efficiency for all the analytes was reduced 10% on average in comparison with the optimum conditions for each element.

Comparison between KR and DLLME

To date, no previous attempt is found in the literature to compare the analytical figures of merit afforded by KR- and DLLME in ICP-OES under a similar set of experimental conditions. Table 4 summarizes analytical figures of merit afforded by both methodologies for As, Cd and Pb determination. LODs were calculated from the analyte calibration graph after the corresponding preconcentration treatment, according to IUPAC's recommendation as 3 times

the standard deviation of the blank signal divided by the calibration curve slope. The enhancement factor (EF) is defined as the ratio of the calibration curve slope with and without the extraction-preconcentration step. Finally, the consumptive index (CI) is defined as the ratio between the sample volume and EF.[33] From data gathered in Table 4, it can be concluded that DLLME is a more attractive sample preparation strategy than KR for metal analysis by means of ICP-OES. First, it allows the simultaneous determination of As, Cd and Pb whereas KR is limited to the last two elements. On the other hand, DLLME is more efficient preconcentrating metals since, despite DLLME organic extracts require a dilution step for ICP-OES analysis, it still yields a higher EF. Consequently, DLLME presents an improved LOD (on average 3-fold) and CI in comparison with the KR. An additional benefit of DLLME regarding KR is the higher sample throughput, due to its simpler experimental arrangement. Finally, no significant differences were observed on the dynamic ranges between both strategies.

As expected by EF values, both KR and DLLME approaches significantly improve LODs for As, Cd and Pb regarding direct analysis in ICP-OES (i.e., without any extraction procedure). Thus, the LOD improvement achieved by the KR for Cd and Pb was 12- and 10-fold. Regarding DLLME, the LOD improvements for As, Cd and Pb were 40-, 45- and 35-fold, respectively. These LOD improvements can be attributed to: (i) the preconcentration itself; (ii) the beneficial effect of the organic extractant on aerosol generation and transport in comparison to conventional aqueous solutions;[14] and (iii), particularly for As, carbon influence on analyte excitation-ionization mechanism.[34]

Analytical figures of merit afforded by both KR and DLLME have also been compared with those previously reported in the literature operating these strategies with ICP-OES detection (Table 5). First, no comparison was feasible for As, since this element has not been previously studied with ICP-OES. Regarding KR preconcentration, analytical figures of merit for Cd and Pb are worse than those reported by Lara et al.[6] and Olsina et al.[7], but it should be considered that an ultrasonic nebulizer was employed to improve aerosol generation and transport in those works. On the other hand, Cd and Pb data for DLLME was similar to those found in previous works but using less sample volume (4 mL). However, one of the main advantages presented by the herein proposed method is its robustness for the analysis of food samples, which represent far more complex matrices than the aqueous and liquid matrices studied in previous works.

Methods validation

European conformity guidelines for analytical methods of food contaminants were employed to validate both KR and DLLME methodologies.[35] To this end, four food samples were analyzed, namely: (i) chocolate; (ii) mussels; (iii) rice; and (iv) wine. These samples were selected to cover different matrices, thus allowing the evaluation of the selectivity and robustness of each sample preparation strategy under different experimental conditions. All the samples, with the exception of wine, were subjected to an acid digestion procedure in a MW oven before the corresponding extraction-preconcentration treatment. Calibration was performed by means of matrix-matched standards. Thus, standards for digested sample analysis were prepared simulating the acid

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content present after the digestion treatment (nitric acid 10% w w^{-1}), whereas standards for wine analysis were prepared containing the most relevant organic and inorganic components (ethanol 12% v v^{-1} and 1000 mg K L^{-1}) in wine.

The accuracy of the methods was evaluated by means of recovery tests (Table S3). Food samples were spiked with known amounts of As, Cd and Pb for a final concentration of 600 µg kg^{-1}. European Union guidelines establish that trueness of the measurements for analyte concentration levels above 10 µg Kg^{-1} is successfully assessed when the recovery values are within -20% to 10%.[35] According to this criterion, and with independence of the considered sample, quantitative recoveries for all the elements were obtained operating both with a KR and DLLME (80-102%). Alternatively, the analyzed food samples were simultaneously analyzed by ICP-MS without preconcentration (Table S4). Calibration was also carried out with matrix-matched standards. For mussels, results afforded by KR- and DLLME-ICP-OES agreed with those obtained in ICP-MS. No comparison was feasible for the remaining samples since the LODs achieved by KR- and DLLME-ICP-OES were not low enough to quantify the As, Cd and Pb levels present. The repeatability was determined by analyzing six replicates of each food sample on the same day for each methodology. Relative standard deviation (RSD) values for Cd and Pb in KR treatment were in the range of 3-5%. Similar values were found for As, Cd and Pb with DLLME. The reproducibility (inter-assay precision) of each methodology was evaluated as the RSD of the measurements obtained for six replicates on three different days. In this case, RSD values for both strategies ranged from 5 to 10%

Limits of detection afforded by both methodologies are below the maximum levels stablished by the EU[2] (Table 6) for chocolate, mussels, rice and wine samples and, hence, they are suitable for food monitoring purposes. The potential use of KR and DLLME for As, Cd and Pb determination in other foods regulated by the EU 1881/2006 directive has also been evaluated. To this end, a theoretical LOD was calculated for each method assuming an acid digestion treatment of 0.5 g sample and dilution up to 25 mL (i.e. similar experimental conditions to those employed in this work). In general, except for children destined commodities, LODs obtained with DLLME-ICP-OES would be low enough to quantify As, Cd and Pb. Regarding KR-ICP-OES, this strategy is more limited for metal/metalloid control in foods, since it does not allow As quantification and Cd and Pb detection capabilities are lower than those afforded by DLLME (Table S5).

Conclusions

Results in this work demonstrate that both KR and DLLME could be combined with ICP-OES to control toxic elements in food samples according to current EU policies. The use of DLLME is clearly more advantageous than KR for the simultaneous determination of As, Cd and Pb in a single run. DLLME affords lower limits of detection (3-fold) and is a more efficient extraction-preconcentration methodology due to its higher enhancement factor, consumption index and sample throughput. KR is limited by the low retention efficiency of the analyte-chelate complex within the system, particularly for As. Irrespective of the extraction-preconcentration methodology, by the appropriate selection of experimental conditions, organic extracts could be directly analyzed

by ICP-OES. It simplifies the analytical procedure and increases the sample throughput. Interestingly, organics favors nebulization and analyte transport to the plasma in comparison to aqueous samples and, hence, analytical figures of merit are not only improved by the preconcentrating procedure but also due to the improvements on analyte transport efficiency to the plasma. Considering the benefits of direct analysis of organic media, as well as the multielemental capability of ICP-OES, there is no doubt that the combined used of extraction-preconcentration techniques and ICP-OES could be advantageous for ultratrace elemental analysis.

Conflicts of interest

There are no conflicts to declare

Acknowledgments

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Figure captions

Fig. 1. Schematic diagram of the experimental procedure employed with (A) KR and (B) DLLME.

Fig. 2. Influence of the elution solvent on the Cd II 214.439 nm emission signal profile with KR. ICP-OES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}. Analyte concentration: 1 mg L^{-1}.

Fig. 3. Influence of the nebulizer gas flow rate on the Cd I 214.439 nm integrated emission signal operating acetic acid at different Q_l with KR: (x) 0.4 mL min^{-1}; (▲) 0.7 mL min^{-1}; (■) 1 mL min^{-1}; and (●) 1.3 mL min^{-1}. Analyte concentration: 1 mg L^{-1}.

Fig.4. Pareto charts obtained in the optimization study of the main variables affecting (A) Cd and (B) Pb extraction with KR. Dotted vertical line corresponds to 95% confidence level. ICP-OES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}. Analyte concentration: 1 mg L^{-1}.

Fig. 5. Influence of the solvent employed for supramolecular solvent dilution on the integrated analyte emission signal. ICP-OES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}; supramolecular/dilution solvent ratio: 1:1; analyte concentration: 1 mg L^{-1}.

Fig. 6. Pareto charts obtained in the optimization study of the main variables affecting (A) As, (B) Cd and (C) Pb extraction with DLLME. The dotted vertical

lines correspond to the 95% confidence level. ICP-OES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}; supramolecular/acetic acid dilution ratio: 1:0.5; analyte concentration: 1 mg L^{-1}.

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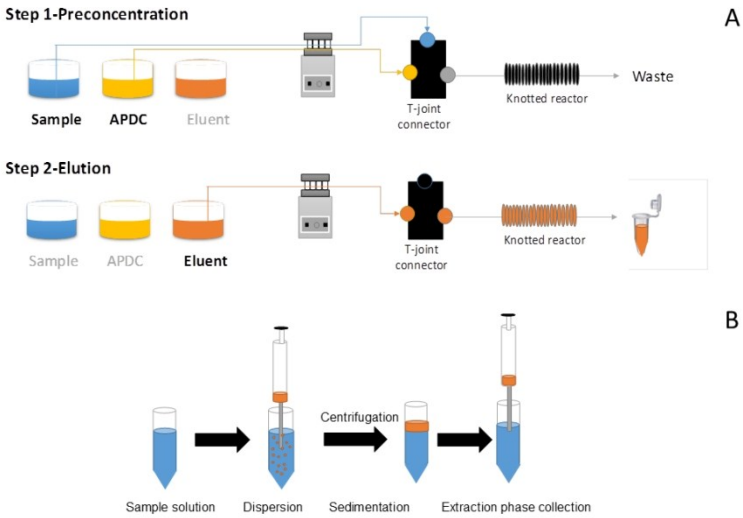

Figure 1

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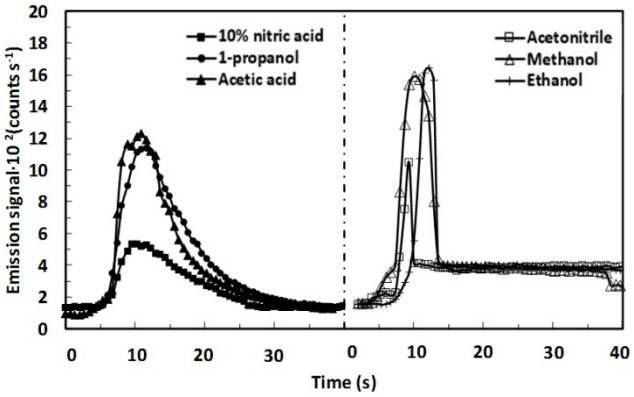

Figure 2

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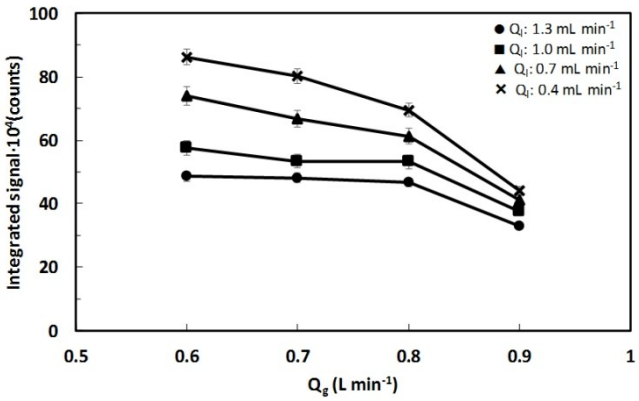

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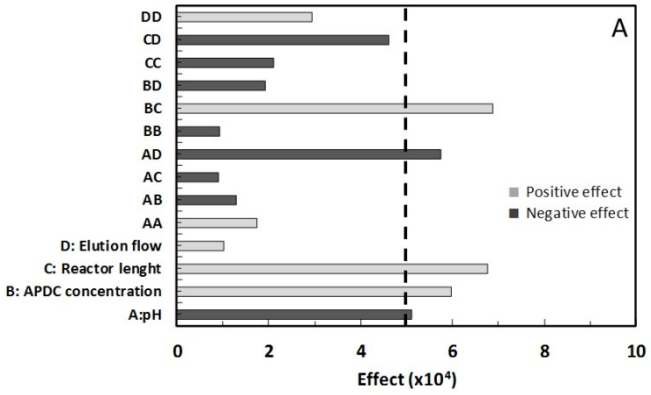

Figure 4.A

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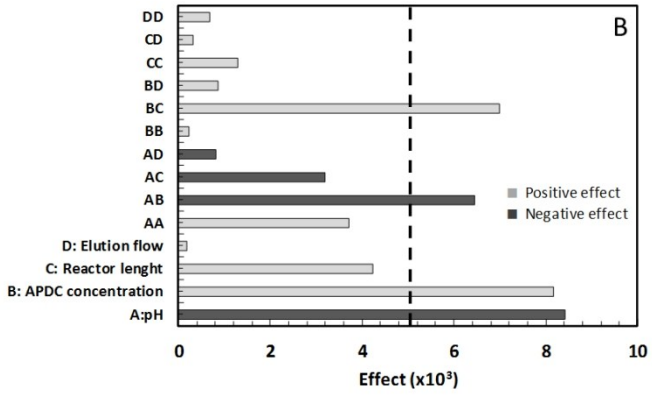

Figure 4.B

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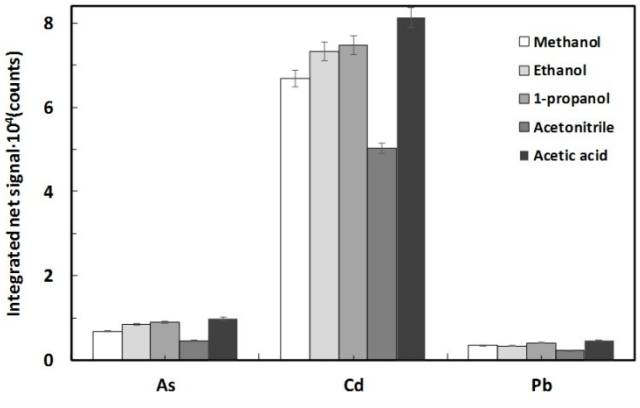

Figure 5

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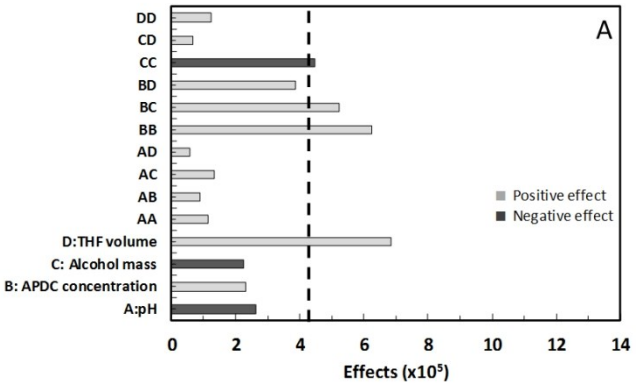

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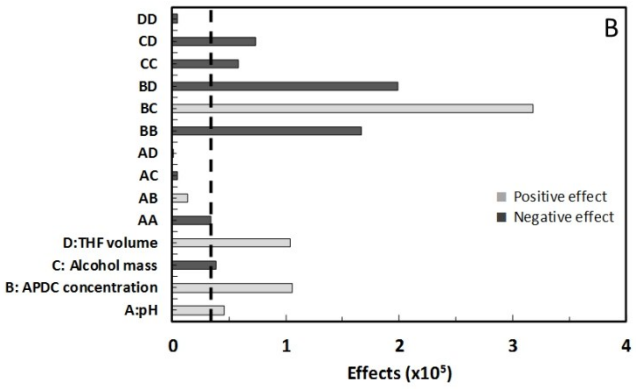

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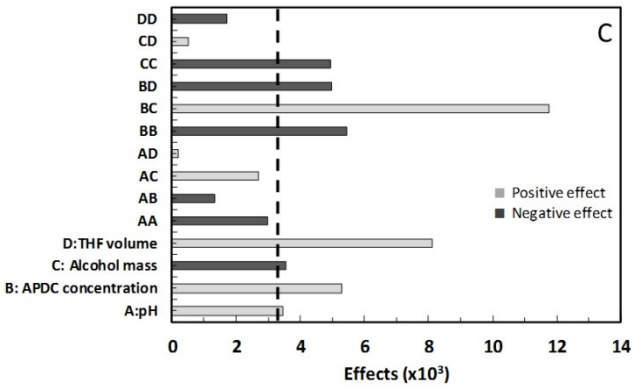

Figure 6.C

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