Article

Development of a Dispersive Liquid−Liquid Aerosol Phase Extraction Method for the Quantification of Ag, Cd, Cu, Ni, and Pb in Seawater by Inductively Coupled Plasma Optical Emission Spectroscopy

Nuria Guijarro-Ramírez, Raquel Sánchez,* and José-Luis Todolí

Cite This: *ACS Omega* 2024, 9, 29422−29430

Read Online

ACCESS | Metrics & More | Article Recommendations | SI Supporting Information

ABSTRACT: The dispersive liquid−liquid aerosol phase extraction (DLLAPE) method was applied for the determination of Ag, Cd, Cu, Ni, and Pb in seawater samples by inductively coupled plasma optical emission spectroscopy (ICP-OES). Key parameters such as sample pH and extractant concentration were systematically evaluated, with ammonium O,O'-diethyldithiophosphate (DDTP) identified as the optimal chelating agent. Optimal extraction conditions were achieved at pH 2.5 for Ag, Cu, Ni, and Pb, while Cd extraction efficiency was found to be pH independent. The extractant concentration did not greatly improve the extraction efficiency. Furthermore, the influence of nebulizer gas flow rate and extraction time was evaluated, achieving the maximum extraction yield at 0.6 L min^{-1} and 120 s, respectively.

1. Sample collection
2. Sample preparation: DLLAPE extraction
3. Analysis by ICP-OES
4. Data treatment
5. Method application

- Ag, Cd, Cu, Ni and Pb
- Analysis of ten seawater samples collected from coastal areas of Alicante (Spain)

The method was evaluated for accuracy and bias through recovery studies, and the results showed that most elements had recovery rates close to 100% with relative standard deviation values in between 3 and 9%. However, in the case of Ag and Ni, 1.184 and 1.089 correction factors were, respectively, applied to compensate for the bias. Moreover, the procedural limits of quantification (pLOQs) found for Ag, Cd, Cu, Ni, and Pb were 0.4, 0.14, 0.2, 0.2, and 0.2 μg L^{-1}, respectively. The in-house validation of the method provided expanded uncertainty values lower than 6% for all elements except for Ag (16.6%). Finally, the application of the method to real seawater samples from coastal areas in Alicante and San Juan (Spain) confirmed its suitability for trace metal analysis in complex marine matrices, underscoring its potential for environmental monitoring and research.
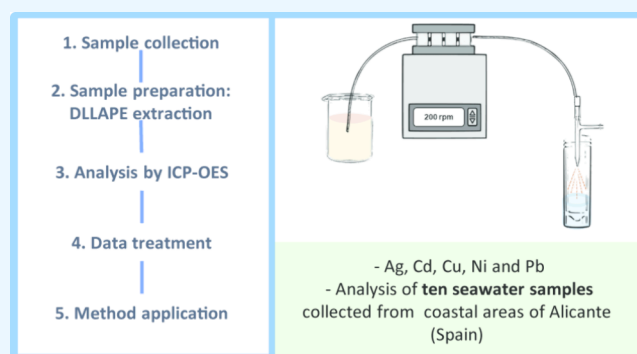
1. INTRODUCTION

Metals are inherently present in the biosphere, including marine environments, where they play vital roles in the growth and development of marine organisms. Elements such as zinc, copper, and cobalt are essential nutrients; however, their beneficial presence becomes detrimental when concentrations escalate to toxic levels. Human activities have long contributed to the elevated presence of metals in coastal and estuarine ecosystems, posing a threat to environmental integrity and the well-being of marine life.[1−5] The accumulation of metals in marine ecosystems, particularly in seawater, highlights urgent concerns for biodiversity and the health of marine organisms. Therefore, there is a need for monitoring and controlling their levels to safeguard marine habitats and the quality of seawater.

The Water Framework Directive (WFD)[6] assumes a critical role in safeguarding the quality of the aquatic environment by effectively addressing the risks posed by priority chemical substances. These substances, including metals such as cadmium, nickel, and lead, have been demonstrated to adversely affect the chemical status of water bodies. In order

to ensure compliance with the environmental quality standards (EQSs) outlined in Daughter Directive 2013/39/EU,[7] the WFD provides a comprehensive list of priority substances, which include cadmium, nickel, and lead. The EQSs for Ni and Pb, expressed as a maximum allowable concentration (MAC), are 34 and 14 μg L^{-1}, respectively. For cadmium, the EQS values vary based on water hardness, ranging from 0.45 to 1.5 μg L^{-1}.[7]
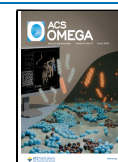
Nickel, cadmium, and lead pose significant threats to marine ecosystems due to their inherent toxicity. These metals could be absorbed by plankton or marine organisms, accumulating in their tissues and causing adverse effects on their health and aquatic ecosystems.[8−15] However, while copper is essential for

biological functions, excessive levels can become toxic,[16] requiring regulatory thresholds such as those established by the United States Environmental Protection Agency (EPA) for copper (4.8 μg L^{-1}) and silver (1.9 μg L^{-1}) in seawater.[17]

The determination of metals in seawater using inductively coupled plasma optical emission spectrometry (ICP-OES) presents significant challenges due to the complex matrix composition and trace concentrations of these elements. The high salinity of seawater, approximately 3.5%, induces matrix effects and spectral interferences that may adversely affect the accuracy and precision of the ICP-OES measurements. The complexity of matrices mainly composed by easily ionizable elements (EIEs), such as seawater, presents a challenging situation.[18−23] Generally, the presence of an inorganic matrix leads to either a decrease or an increase in the ICP-OES emission intensity depending on the operating conditions and the plasma observation height.[24] Additionally, the increase in matrix concentration tends to exacerbate interference, the degree of which varies with the specific emission wavelength chosen. Therefore, a previous sample preparation step is required to perform this kind of analysis. The simplest approach involves diluting the sample to mitigate matrix effects.[25,26] However, large dilution factors are often required, which can degrade the limits of detection. Consequently, various sample preparation methods have been developed to efficiently isolate the target analyte from the sample matrix, including liquid−liquid extraction,[27−29] adsorption columns,[30,31] ion exchange resins,[32−34] and coprecipitation.[35,36]

Conventional liquid−liquid extractions, which use vigorous agitation to create small droplets and increase the contact surface area to improve extraction efficiency, split the analyte between two immiscible phases according to the distribution law. To maximize the analyte recovery, extractive agents forming complexes with the metal ions may be added. Achieving efficient liquid−liquid extractions typically involves performing multiple extractions on the same sample, which requires a significant quantity of reagents, generates substantial waste, and extends the agitation and extraction times. Dispersive liquid−liquid microextraction (DLLME) has been introduced as an alternative, significantly reducing the volume of organic solvents needed.[29,37] In DLLME, a solvent-dispersing agent mixture is added to the sample, allowing dispersion of the extracting solution within the sample. Both phases are separated through centrifugation and filtration for subsequent analysis. Unfortunately, these additional steps increase the sample preparation time.[29,37] Recently, a dispersive liquid−liquid aerosol phase extraction method (DLLAPE) has been successfully applied for the analysis of water,[38] oil,[39,40] and biodiesel[41] samples. This innovative approach involves nebulizing one phase over another, eliminating the need for a dispersing agent and minimizing reagent consumption. The DLLAPE significantly shortens the extraction time compared to traditional methods, attributable to the extensive surface area created at the liquid−liquid interface, estimated at approximately 1 m^2 per cm^3 of extracting solution.[41]

This study aimed to develop, evaluate, and validate the analytical performance of the DLLAPE method for determining silver, cadmium, copper, nickel, and lead concentration in seawater samples using ICP-OES. The method validation assessed the repeatability, intermediate precision, trueness, and bias contributions to the expanded uncertainty. Finally, 10 seawater real samples were analyzed to demonstrate the method applicability for metal determination in complex marine matrices.

2. MATERIALS AND METHODS

2.1. Reagents and Samples.
A multielemental organic solution (100 mg kg^{-1}, Conostan S-21 + Be + K + Li + Sb,

Table 1. ICP-OES Operating Conditions

instrument parameter	cyclonic spray chamber	hTISIS spray chamber
temperature spray chamber (°C)	20	350
RF power (kW)	1.4	
plasma flow rate (L min^{-1})	15	
auxiliary flow rate (L min^{-1})	1.0	
nebulization flow rate (L min^{-1}), Q$_g$	0.35	0.30
liquid flow rate (μL min^{-1}), Q$_L$	50	30
sample injected volume (μL)		5
integration time (s)	0.1	
sampling time (s)	0.4	
replicates	5	
emission lines (nm) I: atomic line II: ionic line	Ag I 328.068; Cd I 228.802; Cu I 327.393; Ni II 231.604; Pb II 220.353	

SCP SCIENCE, Clark Graha, Baie D'Urfé, Canada) was diluted in xylene (Labbox Labware S.L., Vilassar de Dalt, Barcelona, Spain) to prepare a multielemental stock solution. Additionally, 2000 mg kg^{-1} Sc and 1000 mg kg^{-1} Y standard solutions from Conostan (SCP SCIENCE, Clark Graha, Baie D'Urfé, Canada) were used as internal standards.

Citric acid, sodium citrate, acetic acid, and sodium acetate (Panreac Applichem, Barcelona, Spain) were used to prepare the buffer solutions. O,O′-Diethyldithiophosphate (DDTP) (Sigma-Aldrich, St. Louis, MO, USA) was used as a chelating agent. Ultrapure water was supplied by the three-step ion-exchange system Milli-Q, fed by the reverse osmosis system Elix 3, both from Millipore (El Paso, TX, USA).

Three seawater certified reference materials were analyzed in the present work: ERM CA 403 (European Commission, Joint Research Centre, Geel, Belgium), NASS-5 (National Research Council of Canada (NRC−CNRC), Ottawa, Canada), and CASS-6 (National Research Council of Canada (NRC−CNRC), Ottawa, Canada). The samples are certified in Ag, Cd, Cu, Ni, and Pb concentrations, together with other elements. Moreover, a total of 10 seawater samples were collected from the seashore touristic coastal areas of Alicante and San Juan (Spain). Before analysis, the samples were filtered to eliminate any suspended solid particles.

2.2. Synthetic Seawater Sample Preparation.
To optimize and validate the DLLAPE method, a synthetic seawater matrix was prepared (Table S1).[42] Sodium chloride (Scharlab, Barcelona, Spain), magnesium chloride hexahydrate (Panreac Applichem, Barcelona, Spain), calcium chloride dihydrate (Panreac Applichem, Barcelona, Spain), sodium floride (Panreac Applichem, Barcelona, Spain), potassium chloride (Sigma-Aldrich, Saint Quentin Fallavier, France), sodium bicarbonate (Panreac Applichem, Barcelona, Spain), potassium bromide (Probus, Barcelona, Spain), strontium chloride hexahydrate (Panreac Applichem, Barcelona, Spain), anhydrous sodium sulfate (Panreac Applichem, Barcelona, Spain), and boric acid (Panreac Applichem, Barcelona, Spain)

were used for the preparation. This synthetic matrix was used as a blank sample for optimization and recovery studies. Moreover, the synthetic sample was spiked by using a multielemental certified solution (Merck IV, Merck KGaA, Darmstadt, Germany).

2.3. DLLAPE Extraction. Metals were extracted using an aerosol phase extraction (DLLAPE) procedure.[38−41] 1.5 g of the seawater sample was weighed and transferred into a 5 mL polypropylene tube. Then, 0.5 g of pH buffer solution (acetic acid/acetate) and 0.5 g of a solution containing the chelating agent DDTP were added. The xylene-extracting solution was delivered to a glass pneumatic concentric nebulizer (TR-30-A2, Meinhard, USA) by means of a peristaltic pump (Perimax 16 antiplus, Spetec, Erding, Germany) using 0.25 mm i.d. solva-based material with plasticizer tubing (Solva, Ismatec SA, Glattbrugg, Switzerland). The organic solution was then aspirated and nebulized over the sample for 120 s. Then, the two liquid phases spontaneously separated, and the xylene solution was collected using a pipet for direct analysis by ICP-OES. Figure S1 shows a scheme of the DLLAPE extraction.

2.4. Instrumentation. An Optima 7300 DV PerkinElmer ICP-AES spectrometer (Uberlingen, Germany) was used, and the emission signals were axially viewed. The system was equipped with a 40.68 MHz free-running generator and a polychromator with an echelle grating. Segmented-array charge-coupled device (SCD) detectors allowed the simultaneous measurement of several lines in the UV and visible electromagnetic spectrum zones. Table 1 summarizes the instrumental conditions for the two sample introduction systems.

During the optimization of the DLLPPAE method, a PFA concentric micronebulizer (CPI International, Santa Rosa, CA, USA) attached to a cyclonic spray chamber (Glass Expansion, Australia) was used as a sample introduction system. The solutions were continuously delivered to the nebulizer by means of a peristaltic pump (Perimax 16 antiplus, Spetec, Erding, Germany) using a 0.25 mm i.d. solva-based material with plasticizer tubing (Solva, Ismatec SA, Glattbrugg, Switzerland).

To enhance the limits of detection and quantification, a high-temperature Torch Integrated Sample Introduction System (hTISIS)[43] equipped with a 9 cm^3 single-pass spray chamber was employed and operated at 350 °C. An air segmented injection methodology was employed where a peristaltic pump (Perimax 16 antiplus, Spetec, Erding, Germany) continuously aspirated air at a flow rate of 30 μL min^{-1}. A 5 μL sample volume was measured using an automatic micropipette (Eppendorf, Hamburg, Germany) and manually injected into the 0.25 mm i.d. contour flared end of the solva pump tubing. Then, the sample was driven toward the pneumatic concentric nebulizer. Transient signals were obtained according to this approach, and the peak height was taken as an analytical parameter. Metal concentration was determined according to an external calibration method without the addition of an internal standard. Calibration curves were obtained from six standards prepared by the proper dilution of the Conostan S-21 + Be + K + Li + Sb multielemental organic solution in xylene. The calibration curves ranged from 0.01 to 20 mg kg^{-1} and from 0.05 to 5 μg kg^{-1} for the recovery study and the analysis of the samples, respectively.

Seawater samples were analyzed using a 10-fold dilution factor by using an Agilent 8900 ICPQQQ instrument (Agilent

Technologies, CA, USA) (ICP-MS/MS). This instrument is equipped with an octupole collision-reaction cell (CRC) located between two quadrupole analyzers. No gas or He modes were selected. Moreover, the instrument is equipped with a HMI (High Matrix Introduction) technology. The HMI aerosol dilution technology significantly extends the matrix tolerance of the instrument, allowing for the analysis of high-matrix samples with lower dilution factors. The sample introduction system was a glass pneumatic concentric nebulizer (TR-30-A1, Meinhard Glass Products, Santa Ana, USA) and a conventional double-pass spray chamber cooled at 5 °C. The solutions were delivered in continuous sample aspiration mode to the nebulizer by means of an Agilent G3160B autosampler, using a 0.25 mm flared-end PVC tubing (Glass Expansion, Melbourne, Australia). The main operating conditions are summarized in Table S2. Calibration curves were obtained from six standards prepared by diluting a 100 mg L^{-1} multielemental stock solution (SCP33MS) purchased form SCP SCIENCE (Clark Graha, Baie D'Urfé, Canada). Internal standards were added to all solutions at the same concentration level, including blank calibration standards and samples. The final concentration was 40 μg L^{-1}. The calibration curves ranged from 0.01 to 10 μg L^{-1}.

2.5. Method Validation. The contributions of repeatability, intermediate precision, trueness, and bias were considered for the calculation of the expanded uncertainty (U) of the measurements (eq S1, Table S3). To evaluate uncertainty contributions associated with trueness, repeatability, and intermediate precision, five subsamples were measured on 4 separate days. A synthetic seawater sample was spiked with a 50 μg L^{-1} multielemental solution. Equation S2 (Table S3) was used to calculate the contribution of trueness to the uncertainty (u_t). The uncertainty of the measurement results on the spiked sample (u_{Rec}) was determined by combining the root-mean-square bias (RMS$_{Bias}$) between the experimental and theoretical added concentration values with the combined uncertainty of the spiked sample solution (u_{Conc}) and the uncertainty associated with sample preparation (u_{Prep}) (eqs S3 and S4, Table S3).

A one-way ANOVA test was used to determine the repeatability and intermediate precision as within-group and between-group standard deviations. The corresponding relative standard uncertainty contributions were determined using eqs S5 and S6 (Table S3).

In the case of Ag and Ni, where a correction factor was required (as described in Section 3.2), the variation of the correction factor should be included into the calculation of the expanded measurement uncertainty.[44,45] The contribution, u_{CF}, is the relative standard deviation of the correction factor obtained through a recovery study at three different concentration levels (0.1, 1, and 10 mg L^{-1}) by measuring six subsamples in 5 different days.

3. RESULTS AND DISCUSSION

3.1. Optimization of the Extraction Variables. The optimization of variables affecting the extraction efficiency, specifically the extraction equilibrium and aerosol characteristics, was systematically undertaken in this study. Net emission intensity was measured for 1 mg L^{-1} spiked synthetic seawater. To evaluate the impact of the parameter optimization, a relative intensity (I_{rel}) was calculated according to
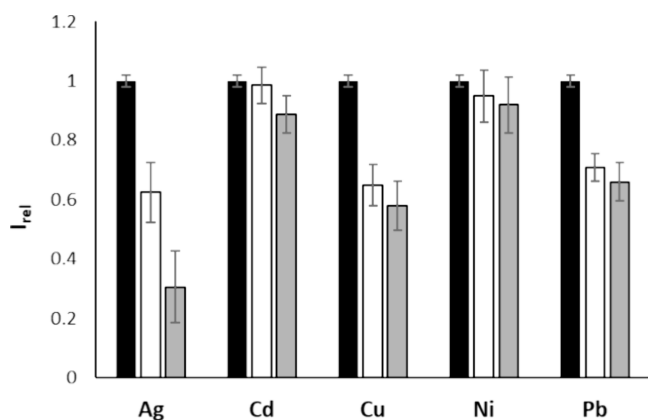
Figure 1. Effect of pH on the relative intensity. pH: 2.5 (black bars), 4.0 (white bars), and 5.5 (gray bars). Metal concentration: 1 mg L^{-1}; 1% DDTP; extraction time: 120 s; Q_g: 0.5 L min^{-1}; Q_l: 0.9 mL min^{-1}. Error bars correspond to the standard deviation (three replicates). ICP-OES experimental conditions: Table 1.
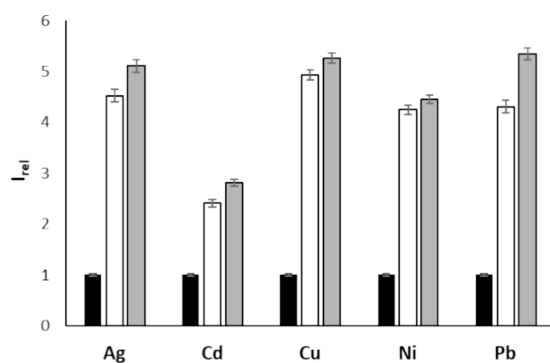

Figure 2. Effect of the nebulization gas flow rate (Q_g) on the relative intensity. Q_g (L min^{-1}): 0.4 (black bars), 0.5 (white bars), and 0.6 (gray bars). Metal concentration: 1 mg L^{-1}; pH 2.5; 1% DDTP; extraction time: 120 s; Q_l: 0.9 mL min^{-1}. Error bars correspond to the standard deviation (three replicates). ICP-OES experimental conditions: Table 1.

Table 2. Optimum Operating Conditions for the Determination of Metals through the DLLAPE Method

variable	optimal value
extraction time/s	120
extractant concentration/%	1
pH	2.5
nebulizer liquid flow rate (Q_l)/mL min^{-1}	0.9
nebulizer gas flow rate (Q_g)/L min^{-1}	0.6
nebulizer tip−sample level gap, d/mm	20

$$I_{rel} = \frac{Signal_i}{Signal_m} \qquad (1)$$

where Signal$_i$ corresponds to the experimentally determined net intensity for the element "i" in the spiked sample under different conditions (pH, extractant concentration, and nebulization gas flow rate (Q_g)) and Signal$_m$ is the emission intensity at the lowest value for each studied variable. A relative intensity higher than unity indicated enhanced extraction efficiency.

Extraction equilibrium is heavily influenced by the chelating agent (extractant) nature and its concentration as well as by the pH of the sample. Different chelating agents, such as 8-hydroxyquinoline, ammonium DDTP, ammonium pyrrolidine dithiocarbamate (APDC), and sodium diethyldithiocarbamate (NaDDC), have been employed in seawater elemental analysis.[28,29,46] A preliminary study was carried out to assess the extraction capabilities of two reagents: 8-hydroxyquinoline and ammonium DDTP. The extraction efficiency found for 8-hydroxyquinoline was less than 20%, while that of DDTP was higher. Consequently, DDTP was chosen as the extractant agent for the DLLAPE method. This agent could form complexes with a variety of elements, and its selectivity for elements prevalent in seawater (Al, Ca, K, Na, and Mg) helps minimize matrix effects and reduce spectral interferences.[29,46]

The pH of the sample plays a critical role in the distribution of analytes between both phases and their interaction with the chelating agent. It is expected that using DDTP as the extractant would result in an improved extraction efficiency at acidic pH.[29] The impact of pH was evaluated by adding buffer solutions with different pH values into the samples and applying the DLLAPE method. Figure 1 shows the relative intensity values plotted against the pH of the sample. The highest relative intensity values for Ag, Cu, and Pb were observed at pH 2.5, while the relative intensity of Cd and Ni was not significantly impacted by the pH. Additionally, the effect of extractant concentration on the emission intensity was evaluated. The addition of an extractant agent improved the extraction efficiency; however, at the evaluated range, the concentration of the extractant did not notably influence the efficiency (Figure S2). Nonetheless, the extractant concentration did impact the precision of the DLLPAE method. Optimal reproducibility, as quantified by the relative standard deviation (RSD), was achieved with a 1% (w/w) DDTP concentration for all of the analyzed metals except nickel. For instance, the RSD for Cu was 10.3% at a 0.5% DDTP concentration, which was reduced to 4.8% when the DDTP concentration was increased to 1%. Similarly, the optimal RSD for lead at the 1% DDTP concentration was found to be 2.4% compared to an RSD of 5.3% at the lower DDTP concentration.

Aerosol-phase extraction employs a pneumatic nebulizer to generate an aerosol through interaction between a liquid stream and a high-velocity gas stream. The aerosol drop size distribution is influenced by the ratio between liquid flow rate (Q_l) and nebulizer gas flow rate (Q_g). The value of the liquid flow rate selected was 0.9 mL·min^{-1}, providing sufficient extractant solution for analyte extraction. As depicted in Figure 2, the impact of the liquid-to-gas flow rate ratio was evaluated by modifying the Q_g. In the DLLAPE method, the efficiency of liquid−liquid extraction was determined by the total liquid interface area, suggesting that generating finer aerosols could improve extraction efficiencies.[41] According to the data shown in Figure 2, the highest Q_g evaluated (i.e., 0.6 L·min^{-1}) was the optimum one, in terms of extraction efficiency. Although higher nebulization gas flow rates were evaluated, it was observed that a fraction of the sample was lost due to the liquid partial ejection from the sample vial. Sample loss under high gas flow rates was a critical limitation that affected the reproducibility of the measurements.

The optimization of the extraction time led to a significant improvement in extraction efficiency, with a maximum efficiency observed when the nebulization time was 120 s. It is important to note that the nebulization time was directly proportional to the amount of the extracting phase employed.
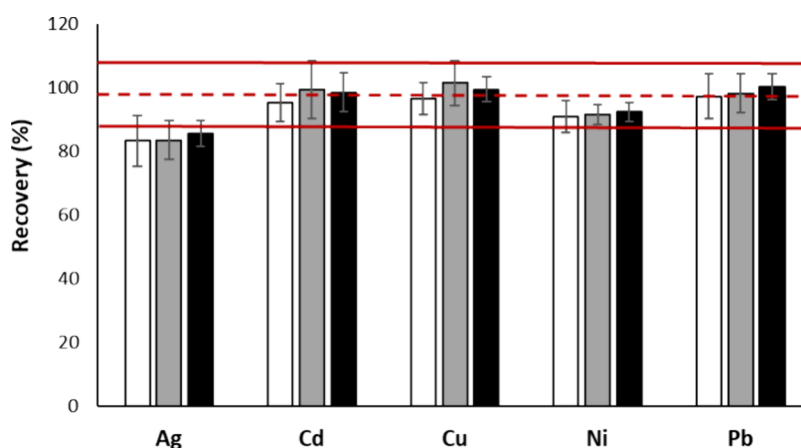
Figure 3. Mean recovery ratios obtained for three spiked samples at 0.1 (white bars), 1 (gray bars), and 10 mg L^{-1} (black bars) concentration levels ($n = 6$, 5 days). Experimental conditions DLLAPE method: Table 2. Error bars correspond to the standard deviation. ICP-OES experimental conditions: Table 1.

Table 3. Analyte Concentration (μg L^{-1}) Measured for Each Certified Reference Seawater Sample[a]

	CAAS−6		NASS−5		ERM CA403	
analyte	DLLAPE method	certified value	DLLAPE method	certified value	DLLAPE method	certified value
Cu	0.504 ± 0.014	0.52 ± 0.03	0.308 ± 0.008	0.30 ± 0.05	0.86 ± 0.02	0.87 ± 0.13
Ni[b]	0.393 ± 0.008	0.41 ± 0.04	0.251 ± 0.008	0.26 ± 0.03	1.07 ± 0.04	1.04 ± 0.16
Pb	<0.09	0.014 ± 0.004	<0.09	0.008 ± 0.005	0.101 ± 0.005	0.098 ± 0.010

[a]Experimental conditions DLLAPE method: Table 2. ICP-OES experimental conditions: Table 1. [b]Correction factor applied (1.089)

Table 4. Relative Standard Uncertainty Contributions and Expanded Relative Uncertainty for Measurement of the Ag, Cd, Cu, Ni, and Pb Contents in Seawater Samples[a]

uncertainty contributions	Ag	Cd	Cu	Ni	Pb
intermediate precision (u_{ip})	2.1	1.2	1.3	0.4	0.7
repeatability (u_{rep})	1.2	1.9	1.0	0.5	0.9
trueness (u_t)	2.0	1.1	1.3	0.5	0.7
correction factor (u_{CF})	7.7			2.8	
expanded relative uncertainty (U, k = 2)	*16.6*	*5.1*	*4.1*	*5.9*	*2.8*

[a]Experimental conditions DLLAPE method: Table 2. ICP-OES experimental conditions: Table 1. Equations: Table S3.

Longer extraction times resulted in higher extraction solution quantities and consequently greater dilution factors. Taking into account the nebulizer liquid flow rate (Table 2) and the nebulization time, the estimated amount of xylene used was 1.8 mL (1.5 g). However, after the aerosol was generated at the nebulizer nozzle, the solvent evaporation from the droplet surface had begun. Consequently, a fraction of the extracting solution did not contact with the sample. Approximately 25% of the xylene aerosol evaporated before it reached the sample surface and was not available for extracting metals. To compensate for this loss of extracting solution, the extraction tubes were weighed with their contents before and after the extraction step. Given that the seawater sample amount was 1.5 g, this sample preparation method achieved a preconcentration factor of approximately 1.4. While this factor is not particularly high, it is crucial to emphasize that the main benefit of the DLLAPE technique compared to conventional methods is its ability to analyze samples without dilution. Conventional seawater analysis techniques, such as those using ICP-based methods, typically require dilution factors between 10 and 100, which can greatly impair analytical detection capabilities. The

optimal experimental conditions used for the extraction are included in Table 2.

3.2. Accuracy and Bias of the Method. To assess the method accuracy, an interday recovery study was conducted using synthetic seawater samples spiked with 0.1, 1, and 10 mg L^{-1} multielemental solution, with six subsamples analyzed over 5 different days. A recovery rate was calculated by applying eq 2:

$$R(\%) = \frac{C_S - C_{NS}}{C_A} \times 100 \tag{2}$$

where C_S corresponded to the experimentally determined analyte concentration in the spiked synthetic seawater sample, C_{NS} was the analyte concentration in the blank synthetic seawater, and C_A was the added concentration for a given element.

For the DLLAPE method, the mean values of the recoveries were approximately 100%, except for that of Ag (Figure 3). The minimum and maximum values were in the ±10% recovery range. Furthermore, the relative standard deviation values were consistently below 10% for all cases. The recovery values obtained for the studied concentration levels were statistically evaluated to determine whether these values could be considered statistically equivalent to 100%. Equations S7, S8, and S9 were applied.[47] Calculated biases were below the bias uncertainty, indicating accurate measurements (Table S4). Therefore, no statistically significant differences were found between the measured and real concentrations except for silver at 1 and 10 mg L^{-1} spiked levels and nickel with the highest concentration. The observed recovery values below 100% for Ag may be associated with its partial precipitation as AgCl. A correction factor (eq S10, Table S3) based on the recovery value could be employed to obtain the actual concentration of Ag and Ni. It is important to note that the Guide to the

Table 5. Analyte Concentration (μg L^{-1}) for Seawater Obtained by the DLLAPE Extraction Method Together with ICP-OES and the Dilution and ICP-MS/MS Determination (Concentration ± Standard Deviation)[a]

sample	Ag		Cd		Cu		Ni		Pb	
	DLLAPE + ICP-OES	dilution + ICP-MS/MS	DLLAPE + ICP-OES	dilution + ICP-MS/MS	DLLAPE + ICP-OES	dilution + ICP-MS/MS	DLLAPE + ICP-OES	dilution + ICP-MS/MS	DLLAPE + ICP-OES	dilution + ICP-MS/MS
M1	0.47 ± 0.08	0.47 ± 0.05	0.245 ± 0.012	0.227 ± 0.013	0.66 ± 0.03	0.70 ± 0.04	1.16 ± 0.07	1.25 ± 0.07	<0.2	<0.06
M2	0.55 ± 0.08	0.50 ± 0.05	<0.14	0.194 ± 0.016	0.317 ± 0.013	0.34 ± 0.02	0.93 ± 0.05	0.96 ± 0.06	0.84 ± 0.02	0.83 ± 0.07
M3	1.6 ± 0.3	1.71 ± 0.14	<0.14	0.155 ± 0.011	2.00 ± 0.08	2.65 ± 0.10	0.77 ± 0.04	0.75 ± 0.06	0.252 ± 0.007	0.216 ± 0.005
M4	<0.4	0.95 ± 0.03	0.366 ± 0.018	0.35 ± 0.03	0.72 ± 0.03	0.68 ± 0.05	0.87 ± 0.05	0.92 ± 0.07	0.72 ± 0.3	0.745 ± 0.017
M5	0.96 ± 0.15	0.93 ± 0.09	0.278 ± 0.013	0.260 ± 0.016	0.434 ± 0.018	0.409 ± 0.017	1.06 ± 0.06	1.01 ± 0.06	2.06 ± 0.06	1.99 ± 0.09
M6	0.67 ± 0.11	0.60 ± 0.06	0.315 ± 0.016	0.297 ± 0.018	<0.2	0.193 ± 0.011	0.276 ± 0.016	0.94 ± 0.04	0.55 ± 0.02	0.53 ± 0.05
M7	0.48 ± 0.08	0.40 ± 0.04	0.292 ± 0.015	0.42 ± 0.03	<0.2	0.185 ± 0.011	0.39 ± 0.02	0.37 ± 0.03	<0.2	<0.06
M8	<0.4	0.28 ± 0.03	0.40 ± 0.02	0.38 ± 0.03	1.12 ± 0.05	1.20 ± 0.08	0.96 ± 0.06	0.92 ± 0.04	1.04 ± 0.03	1.10 ± 0.10
M9	<0.4	<0.10	<0.14	<0.09	0.73 ± 0.03	0.69 ± 0.04	0.62 ± 0.04	0.65 ± 0.05	<0.2	<0.06
M10	<0.4	0.23 ± 0.02	<0.14	0.165 ± 0.012	0.446 ± 0.018	0.46 ± 0.02	1.01 ± 0.06	0.93 ± 0.05	<0.2	0.09 ± 0.04

[a]Experimental conditions DLLAPE method: Table 1. ICP-MS/MS experimental conditions: Table S2. ICP-OES experimental conditions: Table 2. Statistically equivalent concentrations (95%-confidence level) are highlighted in bold.

expression of uncertainty in measurement (GUM) recommends the correction of known biases. The variation of the correction factor must be included in the expanded measurement uncertainty.[44,45] It is crucial to consider that this correction factor, used to compensate for bias, increases the expanded measurement uncertainty. The correction factor took a value of 1.184 for Ag, while for Ni, it was equal to 1.089.

The procedural limit of quantification (pLOQ) was calculated by taking into account the limit of quantification and the preconcentration factor (Table S5):

$$\text{pLOQ} = \frac{10 \times s_b}{\text{slope}} \times \frac{1}{\text{preconcentration factor}} \quad (3)$$

The obtained pLOQ values were lower than the concentration limits established by the European Commission[7] and the EPA.[17] Moreover, it is important to note that the pLOQs obtained by applying the DLLAPE method in combination with the ICP-OES analysis (Table S5) met the minimum performance criteria established by Directive 2009/90/EC,[48] recommending that the LOQ of the methods should be equal or below a value of 30% of the EQS.

Furthermore, the accuracy of the method was evaluated through the analysis of three certified samples of seawater. Five replicate samples were analyzed. Table 3 shows the concentration and certified values for the analyzed samples. No data are shown for Ag, as its concentration is not certified in the analyzed reference samples, while in the case of Ni, the results are not shown because the certified concentrations are below the pLOQ (Table S5). To evaluate the results, the difference (Δ_m) between the certified and measured values was compared to the expanded uncertainty (U_Δ) of both values. Equation S11 (Table S3) was used to calculate the absolute difference between the mean measured value and the certified value. The uncertainty u_Δ was calculated from the uncertainty of the certified value and the standard deviation of the measurement result (eq S12 and Table S3). The expanded uncertainty U_Δ was calculated by multiplying u_Δ by a coverage factor (k), which is usually 2 and represents a confidence level of approximately 95%.[49] Since Δ_m was lower than U_Δ, it was concluded that there were no statistically significant differences between the concentrations obtained by applying the DLLAPE method and the certified concentrations (Table S6).

3.3. In-House Validation Method. According to the results previously shown, the combination of the developed DLLAPE method with ICP-OES allows for the accurate analysis of seawater samples. Under optimum extraction conditions, an in-house validation and uncertainty estimation was carried out.[50,51] The expanded uncertainty values for Cd, Cu, Ni, and Pb were lower than 6% for all elements, whereas for Ag, this parameter was 16.6% (Table 4). In the case of Ag and Ni, where a correction factor must be applied to compensate for the bias, the assessment of this correction factor represents the highest uncertainty contribution.

3.4. Analysis of Real Samples. Based on the above-discussed results, 10 seawater samples were analyzed by applying the DLLAPE method under optimal conditions, followed by ICP-OES measurement. The concentrations of metals detected in these real-world samples were all found to be within legal limits (Table 5).[7,17]

The presence of Ag and Cu in seawater could be attributed to a variety of sources including industrial activities, maritime transport, and the natural erosion of coastal rocks and soils. Cu is an essential trace element for aquatic life. High

Table 6. Comparison of the Different Applications of the DLLAPE Procedure as Sample Preparation Treatment for Metal Determination

	developed method	ref 38	ref 39	ref 40	ref 41
analytical technique	ICP-OES	ICP-OES	ICP-MS	ICP-OES, ICP-MS, ICP-MS/MS	ICP-OES
sample	seawater	seawater	olive oil	oils and animal fat	biodiesel
extracting solution	xylene	hexane	2% HNO_3	choline chloride and ethylene glycol (1:2 mass ratio)	$0.1\ mol \cdot L^{-1}$ HNO_3
analyte	Ag, Cd, Cu, Ni, Pb	Mo	Al, Ca, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn	Ag, Al, Ba, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Ni, Pb	Ca, K, Mg, Na
dilution factor			2	2	
preconcentration factor	1.4	10			3
pLOQ	$0.14-0.4\ \mu g\ L^{-1}$	$7\ \mu g\ L^{-1}$	not reported	$13-530\ \mu g\ kg^{-1}$ (ICP-OES); $12-130\ \mu g\ kg^{-1}$ (ICP-MS); $1-17\ \mu g\ kg^{-1}$ (ICP-MS/MS)	$24-51\ \mu g\ kg^{-1}$

concentrations of this element could negatively impact water quality and degrade marine ecosystems. Copper concentration found in all the analyzed samples was below 2 μg L^{-1} (Table 5), demonstrating compliance with environmental standards. Despite being a less common element in seawater, the concentration values obtained for Ag were similar to those for Cu (Table 5).

Moreover, samples were analyzed by applying a dilution factor of 10 and their subsequent analysis by ICP-MS/MS. The latter methodology was taken as a reference method to compare the concentration results (Table 5). Statistical comparison was performed to evaluate any differences between the concentration results obtained by both methods. F test was applied to assess statistical differences in terms of variance between both methodologies. When variances were statistically comparable, the concentration results were evaluated using the Student t test. However, If the variances were not statistically comparable, t value and the degree of freedom were calculated by applying eqs S13 and S14 (Table S3). Only in four out of the 36 evaluated cases, statistically different results were provided by the two methods (see the figures in bold characters in Table 5 and Tables S7 and S8).

4. CONCLUSIONS

The DLLAPE method combined with ICP-OES analysis yielded an improvement in the analytical performance for the determination of Ag, Cd, Cu, Ni, and Pb in seawater. This improvement underscores the importance of selecting an appropriate chelating agent, specifically DDTP, and adjusting the sample's pH to optimize extraction equilibrium and efficiency. Additionally, the nebulizer gas flow rate and extraction time play important roles in achieving an optimal extraction efficiency. Under optimized conditions, the DLLAPE method combined with ICP-OES offers several advantages: (i) mitigation of matrix effects for seawater sample analysis, (ii) minimization of reagent consumption and waste generation in the sample preparation step, (iii) decreased extraction times, (iv) low pLOQs that comply with regulatory standards, and (v) low expanded uncertainty values, ensuring the reliability and suitability of the method for determining metal content in seawater samples.

Moreover, it is important to note that the DLLAPE method has been applied for the first time to perform multielemental analyses of aqueous samples. Previously, the DLLAPE method was applied for the extraction of Mo using organophosphorus chelating agents in hexane for seawater analysis. However, this application was limited to one analyte (Table 6). On the other side, the extraction method has been applied to the analysis of organic samples by using aqueous solvents as extracting solutions. Furthermore, the experimental conditions applied in this recent application allowed the sample to be preconcentrated, which has a positive impact on the pLOQ values. Consequently, this methodology has demonstrated enhanced sensitivity relative to other methods, which have reported higher pLOQ values (Table 6).

Regarding the analysis of Valencian Community seawater samples, the determined concentrations of Ag, Cd, Cu, Ni, and Pb were below the legal limits established by the European Commission and the United States EPA.[7,17]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c01615.

Experimental setup of the DLLAPE; effect of the extractant concentration; composition of synthetic seawater (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Raquel Sánchez − Department of Analytical Chemistry, Nutrition and Food Sciences University of Alicante, 03690 San Vicente del Raspeig, Alicante, Spain; Ⓞ orcid.org/0000-0003-0283-6103; Email: r.sanchez@ua.es

Authors

Nuria Guijarro-Ramírez − Department of Analytical Chemistry, Nutrition and Food Sciences University of Alicante, 03690 San Vicente del Raspeig, Alicante, Spain; Ⓞ orcid.org/0000-0003-2867-0340

José-Luis Todolí − Department of Analytical Chemistry, Nutrition and Food Sciences University of Alicante, 03690 San Vicente del Raspeig, Alicante, Spain; Ⓞ orcid.org/0000-0002-9151-7050

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.4c01615

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Matthai, C.; Birch, G. F. Effect of coastal cities on surficial sediments mantling an adjacent high-energy continental margin in central New South Wales. *Aust. J. Mar. Freshwater Res.* **2000**, *51*, 565−576.

(2) Mayer-Pinto, M.; Underwood, A. J.; Tolhurst, T.; Coleman, R. A. Effects of metals on aquatic assemblages: What do we really know? *J. Exp. Mar. Biol. Ecol.* **2010**, *391*, 1−9.

(3) Bargagli, R. Environmental contamination in Antarctic ecosystems. *Sci. Total Environ.* **2008**, *400*, 212−226.

(4) Maulvault, A. L.; Anacleto, P.; Barbosa, V.; Sloth, J. J.; Rasmussen, R. R.; Tediosi, A.; Fernandez-Tejedor, M.; van den Heuvel, F. H. M.; Kotterman, M.; Marques, A. Toxic elements and speciation in seafood samples from different contaminated sites in Europe. *Environ. Res.* **2015**, *143*, 72−81.

(5) Langston, W.J., Toxic effects of metals and the incidence of metal pollution in marine ecosystems, in *Heavy Metals in the Marine Environment*; CRC Press (2018) 101−122.

(6) European Commission. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. *Off. J. Eur. Comm.* **2000**, *L 327*, 1.

(7) European Commission. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 on Amendment Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy. *Off. J. Eur. Union* **2013**, *L 226*, 1.

(8) Albarico, F.P.J. B.; Chen, C. W.; Lim, Y. C.; Wang, M. H.; Chen, C. F.; Dong, C. D. Non-proportional distribution and bioaccumulation of metals between phytoplankton and zooplankton in coastal waters. *Mar. Pollut. Bull.* **2022**, *184*, No. 114168.

(9) Yeh, G.; Hoang, H. G.; Lin, C.; Bui, X. T.; Tran, H. T.; Shern, C. C.; Vu, C. T. Assessment of heavy metal contamination and adverse biological effects of an industrially affected river Environ. *Sci. Pollut. Res.* **2020**, *27*, 34770−34780.

(10) Robards, K.; Worsfold, P. Cadmium: toxicology and analysis. A review. *Analyst* **1991**, *116*, 549−568.

(11) McGeer, J. C.; Brix, K. V.; Skeaff, J. M.; DeForest, D. K.; Brigham, S. I.; Adams, W. J.; Green, A. Inverse relationship between bioconcentration factor and exposure concentration for metals: Implications for hazard assessment of metals in the aquatic environment. *Environ. Toxicol. Chem.* **2003**, *22*, 1017−1037.

(12) Camizuli, E.; Monna, F.; Scheifler, R.; Amiotte-Suchet, P.; Losno, R.; Beis, P.; Bohard, B.; Chateau, C.; Alibert, P. Impact of trace metals from past mining on the aquatic ecosystem: A multiproxy approach in the Morvan (France). *Environ. Res.* **2014**, *134*, 410−419.

(13) Jitar, O.; Teodosiu, C.; Oros, A.; Plavan, G.; Nicoara, M. Bioaccumulation of heavy metals in marine organisms from the Romaniansector of the Black Sea. *N Biotechnol.* **2015**, *32*, 369−378.

(14) Baltas, H.; Sirin, M.; Dalgic, G.; Bayrak, E. Y.; Akdeniz, A. Assessment of metal concentrations (Cu, Zn, and Pb) in seawater, sediment and biota samples in the coastal area of Eastern Black Sea, Turkey. *Mar. Pollut. Bull.* **2017**, *122*, 475−482.

(15) Makedonski, L.; Peycheva, K.; Stancheva, M. Determination of heavy metals in selected black sea fish species. *Food Control* **2017**, *72*, 313−318.

(16) Gledhill, M.; Nimmo, M.; Hill, S.; Brown, M. The toxicity of copper (II) species to marine with particular reference to macroalgae. *J. Phycol.* **1997**, *33*, 2−11.

(17) https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table. Latest access: 16th June 2023.

(18) Cano, J. M.; Todolí, J. L.; Hernandis, V.; Mora, J. The role of the nebulizer on the sodium interferent effects in inductively coupled plasma - atomic emission spectrometry. *J. Anal. At. Spectrom.* **2002**, *17*, 57−63.

(19) Todolí, J. L.; Gras, L.; Hernandis, V.; Mora, J. Elemental matrix effects in ICP-AES. *J. Anal. At. Spectrom.* **2002**, *17*, 142−169.

(20) Stepan, M.; Musil, P.; Poussel, E.; Mermet, J. M. Matrix-induced shift effects in axially viewed inductively coupled plasma atomic emission spectrometry, Spectrochim Acta Part B At. *Spectrosc* **2001**, *56*, 443−453.

(21) De Klerk, A.; Rademeyer, C. J. effect of sodium on the determination of some noble metals with inductively coupled plasma optical emission spectrometry. *J. Anal. At. Spectrom.* **1997**, *12*, 1221−1224.

(22) Xu, J. Q.; Balik, D.; Agnes, G. R. Aerosol static electrification and its effects in inductively coupled plasma spectroscopy. *J. Anal. At. Spectrom.* **2001**, *16*, 715−723.

(23) Maestre, S.; Mora, J.; Todolí, J. L. Studies about the origin of the non-spectroscopic interferences caused by sodium and calcium in inductively coupled plasma atomic emission spectrometry. Influence of the spray chamber design. *Spectrochim Acta Part B At Spectrosc* **2002**, *57*, 1753−1770.

(24) Chan, G. C. Y.; Hieftje, G. M. Spatial Emission Profiles for Flagging Matrix Interferences in Axial-Viewing Inductively Coupled Plasma-Atomic Emission Spectrometry: 1. Profile Characteristics and Flagging Efficiency. *Anal. Chem.* **2013**, *85*, 50−57.

(25) Leonhard, P.; Pepelnik, R.; Prange, A.; Yamada, N.; Yamada, T. Analysis of diluted sea-water at the ng L^{-1} level using an ICP-MS with an octopole reaction cell. *J. Anal. At. Spectrom.* **2002**, *17*, 189−196.

(26) Louie, H.; Wu, M.; Di, P.; Snitch, P.; Chapple, G. Direct determination of trace elements in sea-water using reaction cell inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2002**, *17*, 587−591.

(27) Bagherian, G.; Arab Chamjangali, M.; Shariati Evari, H.; Ashrafi, M. Determination of copper(II) by flame atomic absorption spectrometry after its perconcentration by a highly selective and environmentally friendly dispersive liquid−liquid microextraction technique. *J. Anal. Sci. Technol.* **2019**, *10*, 3.

(28) Carasek, E. A low-cost flame atomic absorption spectrometry method for determination of trace metals in aqueous samples. *Talanta* **2000**, *51*, 173−178.

(29) Sánchez, R.; Horstkotte, B.; Fikarová, K.; Sklenářová, H.; Maestre, S. E.; Miró, M.; Todolí, J. L. Fully automatic in-syringe magnetic stirring-assisted dispersive liquid−liquid microextraction hyphenated to high-temperature torch integrated sample introduction system-inductively coupled plasma spectrometer with direct injection of the organic phase. *Anal. Chem.* **2017**, *89*, 3787−3794.

(30) Sun, C.; Zhang, Y.; Gong, Z.; Wang, X.; Yang, Y.; Wang, Y. Determination of trace elements in samples with high salt content by inductively coupled plasma mass spectrometry after solid-phase preconcentration. *Int. J. Mass Spectrom.* **2018**, *431*, 22−26.

(31) Li, H.; Zheng, B.; Li, C.; Zheng, J.; Zhang, Y. Trace element determination in high-salinity water using an online ion imprinted helical tube demineralizer combined with ICP-MS. *J. Anal. At. Spectrom.* **2020**, *35*, 1306−1315.

(32) Jackson, S. L.; Spence, J.; Janssen, D. J.; Ross, A. R. S.; Cullen, J. T. Determination of Mn, Fe, Ni, Cu, Zn, Cd and Pb in seawater using offline extraction and triple quadrupole ICP-MS/MS. *J. Anal. At. Spectrom.* **2018**, *33*, 304−313.

(33) Bowles, K. C.; Apte, S. C.; Batley, G. E.; Hales, L. T.; Rogers, N. J. A rapid chelex column method for the determination of metal speciation in natural waters. *Anal. Chim. Acta* **2006**, *558*, 237−245.

(34) Fischer, L.; Smith, G.; Hann, S.; Bruland, K. W. Ultra-trace analysis of silver and platinum in seawater by ICP-SFMS after off line matrix separation and pre-concentration. *Mar Chem* **2018**, *199*, 44−52.

(35) Soriano, E.; Pastor, A.; de la Guardia, M. Multielemental determination of trace mineral elements in seawater by dynamic reaction cell inductively coupled plasma- mass spectrometry after $Al(OH)_3$ coprecipitation. *Microchem. J.* **2020**, *157*, No. 104864.

(36) Freslon, N.; Bayon, G.; Birot, D.; Bollinger, C.; Barrat, J. A. Determination of rare earth elements and other trace elements (Y, Mn, Co, Cr) in seawater using Tm addition and $Mg(OH)_2$ co-precipitation. *Talanta* **2011**, *85*, 582−587.

(37) Sarafraz-Yazdi, A.; Amiri, A. Liquid-Phase microextraction, TrAC. *Trends Anal. Chem.* **2010**, *29*, 1−14.

(38) Boucherit, A.; Khalaf, H.; Paredes, E.; Todolí, J. L. Development of a new aerosol phase extraction method for metal determination through inductively coupled plasma atomic emission spectrometry. *Talanta* **2012**, *99*, 330−334.

(39) Mirón, C.; Sánchez, R.; Prats, S.; Todolí, J. L. Total polyphenol content and metals determination in Spanish virgin olive oils by means of a dispersive liquid-liquid aerosol phase extraction method and ICP-MS. *Anal. Chem.* **2020**, *1094*, 34−46.

(40) Torres, A.; Romero, I.; Sánchez, R.; Beltrán, A.; Guillena, G.; Todolí, J. L. Multielemental analysis of oils and animal fat by using deep eutectic solvents assisted by an aerosol phase extraction procedure. *Talanta Open* **2023**, *7*, No. 100234.

(41) Sánchez, R.; Maestre, S. E.; Prats, M. S.; Todolí, J. L. Novel aerosol phase extraction method for the determination of Ca, K, Mg and Na in biodiesel through inductively coupled plasma atomic emission spectrometry. *Anal. Chem.* **2017**, *89*, 13618−13625.

(42) *Water quality. Determination of the toxicity of water samples on the embryo-larval development of Japanese oyster (Crassostrea gigas) and mussel (Mytilus edulis or Mytilus galloprovincialis)*, ISO: Geneva (Switzerland) 17244:2015; 2015.

(43) Sanchez, R.; Todolí, J. L.; Lienemann, C. P.; Mermet, J. M. Effect of solvent dilution on the ICP-AES based silicon sensitivity, the aerosol characteristics and the resulting organic solution properties in the analysis of petroleum products. *J. Anal. At. Spectrom.* **2010**, *25*, 178−185.

(44) EUROLAB *Measurement Uncertainty in Testing, Technical Report 1/2002*, June 2002 (http://www.eurolab.org/docs/el_11-01_02_7871.pdf).

(45) Linsinger, T. P. J. Use of recovery and bias information in analytical chemistry and estimation of its uncertainty contribution, TrAC. *Trends Anal. Chem.* **2008**, *27*, 916−923.

(46) Ramos, J. C.; Curtius, A. J.; Borges, D. L. G. Diethyldithio-phosphate (DDTP): A review on properties, general applications, and use in analytical spectrometry. *Appl. Spectrosc. Rev.* **2012**, *47*, 583−619.

(47) Hund, E.; Massart, D. L.; Smeyers-Verbeke, J. Operational definitions of uncertainty, TrAC. *Trends Anal. Chem.* **2001**, *20*, 394−406.

(48) European Commission (2009) Directive 2009/90/EC of the European Parliament and of the Council of 31 July 2009 on technical specifications for chemical analysis and monitoring of water status. *Off J. Eur. Union L* 201:36.

(49) Linsinger, T.. Application Note 1 "Comparison of a measurement result with the certified value". *European Commission - Joint Research Centre Institute for Reference Materials and Measurements (IRMM)*, 2010.

(50) Thompson, M.; Ellison, S. L.; Wood, R. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). *Pure Appl. Chem.* **2002**, *74*, 835−855.

(51) EURACHEM Guide (1998) *The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics.* LGC Teddington.