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The efficient mixing between the sample and the spike solutions takes place at the inner cavity of the multinebulizer tip

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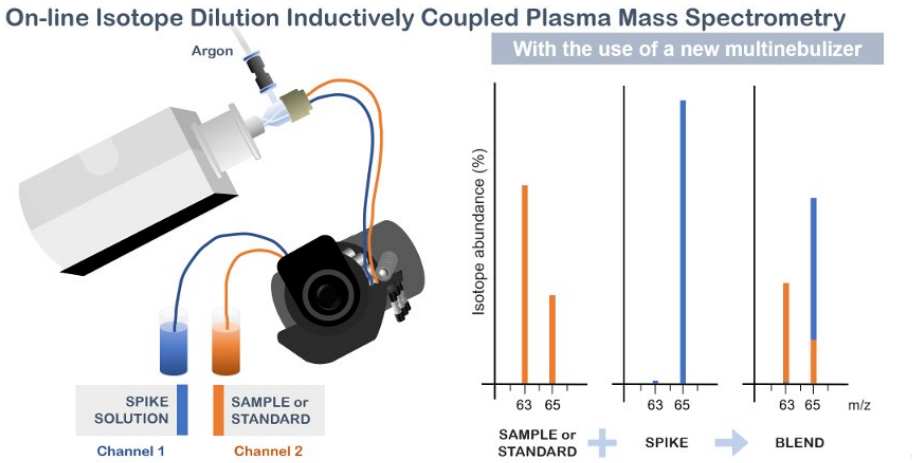

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Multinebulization technique for the determination of trace metals in marine biota sample by on-line isotope dilution inductively coupled plasma mass spectrometry (OID-ICP-MS)

Miriam García[1], Miguel Ángel Aguirre[1,2,*], Emilia Vassileva[3] and Antonio Canals[1,*]

[1]Department of Analytical Chemistry, Nutrition and Food Science and Materials Institute, University of Alicante, P.O. BOX 99, 03080 Alicante, Spain.

[2]Ingeniatrics, Camino Mozárabe 41, 41910 Camas, Sevilla, Spain.

[3]International Atomic Energy Agency, Environment Laboratories, 4 Quai Antoine 1er 98000 Monaco, Principality of Monaco.

*Corresponding authors: aguirre.pastor@ua.es (M.A. Aguirre) and a.canals@ua.es (A. Canals).

Keywords

On-line isotope dilution inductively coupled plasma mass spectrometry · Trace elements · Multinebulization · marine biota certified reference material

Abstract

A new concept of nebulizer (*i.e.*, multinebulizer) has been employed for trace elements (*e.g.*, Cd, Cu, Hg, Ni, Pb, and Zn) determination in marine biota sample (*e.g.*, fish sample). To this end, an analytical methodology based on the combination of a multinebulizer with isotope dilution (ID) calibration strategy and inductively coupled plasma mass spectrometry (ICP-MS) has been proposed. In this way, the on-line isotope dilution (OID) is accomplished by the simultaneous introduction of the liquid samples/standard and the isotopic reference solution (*i.e.*, spike) into the plasma with an efficient mixing between them, which takes place at the inner cavity of the multinebulizer tip. The proposed OID-ICP-MS analytical methodology allows the fast determination of Cd, Cu, Hg, Ni, Pb and Zn in a biota sample certified reference material (CRM) (*e.g.*, IAEA-476) with a limit of detection of 0.006, 0.4, 0.09, 30, 0.2 and 4 ng g^{-1}, respectively, and recovery values ranging from 97 to 103 %. The primary advantage offered by the proposed analytical methodology is that the time-consuming spiking step in the conventional ID analysis is avoided. This yields savings in both total time and cost of the analysis per sample. Hence, it has been proved that the OID-ICP-MS using the multinebulizer is a promising solution for the trace

element analysis of samples with high matrix content, which simplifies operation and significantly increases sample throughput and productivity.

1. Introduction

Trace element determination is constantly in high demand throughout the world for a large variety of purposes (*e.g.*, environmental pollution monitoring, human health assessment, quality control in high added value products), some of which are linked to quick-decision making and major conclusions. This fact has led to an on-going requirement to develop analytical methodologies for their accurate and reliable determination in samples of different kinds (*e.g.*, sediment, biota organisms or seawater). In this sense, analytical chemistry plays an essential role in the achievement of the United Nations' 17 sustainable development goals for 2030,[1] in particular with those related with good health and well-being, sustainable production and climate change.

Conventionally, testing laboratories employ analytical procedures that involve classical calibration techniques. Whenever larger number of samples need to be analyzed on a daily basis, the implementation of methods that do not require the establishment and periodical verification of a calibration graph may be advantageous as it involves significant savings in time and cost of analysis.

The isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) has been identified by the Consultative Committee for Amount of Substance (CCQM, *Comité Consultatif pour la Quantité de Matière*) to have the potential to be a primary method of measurement (PMM), with the highest metrological standing.[2–7] This methodology presents several advantages over other analytical methodologies conventionally employed in testing laboratories for trace elements determination, highlighting that measurements must be accepted without the use of external analytical standards (*i.e.*, the external calibration graph is avoided).

In spite of this, ID-ICP-MS is traditionally practiced only in laboratories for reference measurements and for any cases where the high accuracy criterion is of great importance to the analytical results.[8] Batch ID-ICP-MS is also regarded as being expensive and time-consuming since each sample is spiked individually. However, sample spiking can be simplified by the on-

line addition of the spike solution to the sample, a concept which was first introduced by Lásztity et al. in 1989 for elemental analysis.[9] There are many ways in which on-line ID-ICP-MS (OID-ICP-MS) has been implemented, the main ones being the mixing using a "Y" or "T" connection[2,6,10–14] and the flow-injection mode.[15,16]

Recently, a novel multinebulizer has been developed, which allows the simultaneous introduction of two or more liquids into the plasma with an efficient mixing between them, since the mixing takes place under turbulent conditions of high pressure at the inner part of the tip of the nebulizer. This mixing process inside the nebulizer creates a new concept in nebulization which has been reported to be successful in on-line standard dilution analysis requiring the simultaneous introduction of aqueous and organic solutions.[17]

Following this line of research, an OID-ICP-MS methodology based on the multinebulizer is presented. This multinebulizer incorporates two liquid inlets which enable the on-line sample spiking by means of an effective mixing between the spike (*i.e.*, isotopically enriched standard) and the sample (*i.e.*, natural isotopic composition) solutions. The target of this study is to improve the productivity of laboratories in the analysis of samples with a complex matrix by the combination of the new multinebulizer and the on-line ID-ICP-MS analysis. Thus, this conjunction gives rise to high quality results as well as the reduction of human intervention required for individual spiking, shorter total analysis time and cost savings.

In order to assess the proposed method applicability six trace elements (*i.e.*, Cd, Cu, Hg, Ni, Pb, Zn) in a certified reference material (CRM) of marine biota (IAEA-476) have been determined. This CRM is widely used as a quality control material for ecological and toxicological risk assessments (*i.e.*, marine pollution monitoring and food safety).

2. Experimental part

2.1. Reagents and Materials

High-quality Milli-Q water (18.2MΩ·cm resistivity) obtained from PureLab Flex 3 system (Lane End, United Kingdom) was used throughout this work. Ultra-pure HNO_3 (Merck, Darmstadt, Germany) and H_2O_2 (Merck) were used for sample digestion.

A multi-element isotopically-enriched standard IES-WAK (sp) (^{111}Cd, ^{65}Cu, ^{199}Hg, ^{61}Ni, ^{207}Pb and ^{67}Zn), designed for quantitative analysis by OID-ICP-MS and accredited by ENAC (*Entidad Nacional de Acreditación*, accreditation no. 3/PMR004) was obtained from ISC-Science (Oviedo, Spain). The certified abundance values and concentrations of the elements in the spike solution are shown in **Table 1**.

The fish homogenate Certified Reference Material IAEA-476 was obtained from the International Atomic Energy Agency (IAEA, Principality of Monaco).

The natural isotopic composition multielement standard (st) (C_{st} = 100 ng g^{-1} for Cu, Ni, Pb and Zn and 4 ng g^{-1} for Cd and Hg), and quality control samples (QC), QC1 and QC2, were prepared by appropriate gravimetric dilution of single-element 1000 mg L^{-1} standard solutions with natural isotopic composition from High Purity Standards (Charleston, SC, USA) with a solution containing 2 % (w w^{-1}) nitric acid. The natural isotopic abundances of the elements of interest, with the exception of lead, were taken from IUPAC tables[18] and are shown in **Table 1**. The QC1 concentration is 20 ng g^{-1} for Cu, Ni, Pb and Zn and 2 ng g^{-1} for Cd and Hg while the QC2 concentration is 10 ng g^{-1} for Cu, Ni, Pb and Zn and 2 ng g^{-1} for Cd and Hg. These solutions were used for mass discrimination correction during ICP-MS measurements.[19,20] Among all of the naturally occurring lead isotopes only ^{204}Pb is nonradiogenic, whereas ^{206}Pb, ^{207}Pb and ^{208}Pb are daughter products from the radioactive decay of ^{238}U, ^{235}U, and ^{232}Th, respectively. This fact produces small Pb isotope abundance variations in the nature.[19–22] For this reason, the isotopic composition of lead in the natural isotopic composition standard solution was determined by independent ICP-MS measurements of different pairs of Pb isotopes ratios: ^{204}Pb/^{208}Pb, ^{206}Pb/^{208}Pb, and ^{207}Pb/^{208}Pb. A certified isotopic reference material NIST SRM-981 (National Institute for Standard and Technologies, USA) was used for mass discrimination correction when Pb isotope ratios were measured. The lead isotopic composition in the natural isotopic composition standard solution is also shown in **Table 1**.

2.2. Instrumentation

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Acid digestion of the sample was carried out in a closed vessel device using a Milestone Start D microwave acid digestion system (Sorisole, Italy), equipped with 10 polytetrafluoroethylene vessels. The samples were digested at conditions recommended by the manufacturer (Application note HPR-FO-17 for dried fish).

All isotope ratio measurements were performed with an inductively coupled plasma mass spectrometer (model 7700x, Agilent Technologies, Santa Clara, CA, United States), working in helium collision mode with the 3rd generation Octopole Reaction System (ORS^3) for better polyatomic interferences removal. A standard quartz torch (*i.e.*, 2.5 mm internal diameter) and standard nickel cones (*i.e.*, sampler and skimmer) were used, while no instrument's autosampler was used throughout the work. The ICP-MS operating conditions, which were auto-tuned using the 7700 MassHunter software, are listed in **Table 2**. Total analysis time per sample, including wash-in and wash-out, was 3 minutes.

The selection of the isotopes to be measured in the ICP-MS was done with respect to the availability in the spike material, abundance of the isotopes, and possibility of spectral interferences. The signal intensities used for isotope ratio measurements (**Table 3**) were corrected for instrumental background, dead time,[23] and possible spectral interferences. Dual rinsing followed by a check of the instrumental background was performed to monitor sample memory effects and correct for them, if necessary. The dead time value was determined according to a method described by Nelms et al.[24]

Cd measurements by ICP-MS can easily suffer from isobaric (^{114}Sn) and polyatomic (MoO^+ and ZrO formation) spectral interferences. These spectral interferences were partially removed using the helium collision mode. In order to take into account the potential interferences, together with Cd isotopes, the intensities of ^{90}Zr, ^{95}Mo and ^{117}Sn were also monitored. Cd interferences were overcome by the application of correction equations.[23,25] The rest of selected isotopes were free of spectral interferences.

2.3. Sample pretreatment

A portion of ~0.2 g of the biota sample was weighed directly into a polytetrafluoroethylene vessel and 7 mL of HNO_3 65 % (w w^{-1}) and 1 mL of H_2O_2 30 % (w w^{-1}) were subsequently added. The heating program was applied in two steps: (1) 15 min to reach 200 °C and (2) 15 min at 200 °C, and an additional 15-min cooling step. A maximum 1.5 kW of microwave power was applied. After completing the digestion and cooling down steps, the final digests were diluted to 50 g of final solution with Milli-Q water, filtered through syringe with 0.45 µm pore size polyvinylidene fluoride filters (Millipore, Madrid, Spain) and then stored in polyethylene tubes at 4 ºC until the analysis.

In order to avoid memory effects from previous experiments, the set of polytetrafluoroethylene vessels employed for sample digestion were first cleansed using a microwave procedure, consisting of the addition of 8 g of concentrated HNO_3 to each vessel, followed by a microwave treatment at 350 W during 10 min to reach 100 ºC and 10 min at 100 ºC (*i.e.*, 20 min in total). This procedure was performed twice before sample digestion.

Four procedural blanks (*i.e.*, digestion vessels with no sample) were also prepared and subjected to the entire analytical methodology together with the fish homogenate replicates in order to evaluate the contribution of the blank contamination. Six replicates were prepared for the analysis.

2.4. On-line isotope dilution

The nebulizer used in this study was a multinebulizer (MultiNeb®, Ingeniatrics, Seville, Spain) (**Figure 1**) which incorporates two independent liquid inlets into a single nebulization body with a common nebulization gas inlet and a unique outlet orifice. The liquid streams are mixed at the tip inside the nebulizer in the aerosol phase at high pressure and the aerosol resulting from the mixture of the liquids exits by the unique hole. As a result, there is an increase in the mixing efficiency and in chemical reaction speed. This nebulization device significantly improves earlier prototypes described elsewhere[26] and it has been already applied to other calibration methodologies with successful results.[17]

The liquid sample introduction system was composed by a multinebulizer coupled to a quartz double pass Peltier-cooled (2 ºC) spray chamber without any additional modification required, as

the multinebulizer is built on the right dimensions to allow the easy connection to any commercial spray chamber conventionally used in ICP-based techniques. Two Tygon® peristaltic tubes for aqueous solutions (R-3607, white-white code color, i.d. 1.02 mm, Ismatec Cole-Parmer GmbH, Wertheim, Germany) were used for the sample/standard and spike solutions.

The on-line sample spiking was achieved by using both liquid inlets of the multinebulizer: one inlet (Channel 1, flow rate: 0.3 mL min^{-1}) for the continuous multielement spike solution addition and the other one (Channel 2, flow rate: 0.3 mL min^{-1}) for the sequential introduction of the natural isotopic composition reference standard, the procedural blanks and the liquid samples solutions. Thus, the two solution streams were mixed inside of the tip of the nebulizer, leading to a primary aerosol of the resulting blend.

In this work, analyte concentrations in the sample digests, C_s, were calculated using the following simplified equation, obtained from the double IDMS (*i.e.*, combination of direct and reverse IDMS), in which a natural abundance standard is measured with the samples to eliminate the concentration of the spike solution from the equation:[23,27]

$$C_s = C_{st}\frac{1 - R_{st}R_nR_m - R_{sp}}{1 - R_mR_nR_{st} - R_{sp}} \quad (1)$$

where C_s is the concentration of the analyte in the sample digest solution (s); C_{st}, the concentration of the analyte in the reference standard with natural isotopic abundance (st); R_{sp}, the isotope ratio of the spike solution (sp); R_n, the natural isotope ratio of the analyte (IUPAC value[18]); R_m, the measured isotope ratio of the mixed sample and spike solution (s + sp); and R_{st}, the measured isotope ratio of the mixed reference standard and spike solution (st + sp).

From the equation above, it seems clear that the only parameters that needed to be experimentally determined for the calculation of C_s were R_m and R_{st}. Both isotope ratios were determined by OID-ICP-MS measurements and then incorporated into the equation.

The individual uncertainty components of the parameters in the model **Equation 1** were combined according to the ISO guidelines.[28] The uncertainty for the isotope abundances in the multielement spike solution were given on its corresponding certificate. The reference standard and quality control solutions were assumed to contain natural isotopic composition of Cd, Cu, Hg, Ni and Zn

and their isotope abundancy uncertainties were taken from IUPAC.[18] Lead isotopic composition instead, was determined experimentally by ID-ICP-MS measurements within the same analytical run (**Table 1**).

Combined standard uncertainties of the results were obtained according to the uncertainty propagation law. The values for the isotope ratios selected for the analysis in the multi-spike solution and naturally present in the sample are shown in **Table 3**.

2.5. Analysis procedure

In this work, the Agilent's Application Note for the on-line isotope dilution analysis with the 7700 Series ICP-MS[29] was used as a guide.

In ICP-MS the transmission of the different isotopes of the same element into the mass spectrometer is mass dependent. Some physical processes that take place in the extraction interphase produce an enrichment in the ion beam of the heavier isotopes with respect to the light isotopes of the element, resulting in a preferential loss of the light isotopes (*i.e.*, heavier isotopes are transmitted more efficiently than lighter isotopes). This physical effect is called mass discrimination and causes a measurable mass bias in the obtained isotope ratios that needs to be corrected for.[27,30–32] The standard-sample bracketing method[33] was employed in the proposed analytical procedure (OID-ICP-MS) for calibration and an effective mass discrimination correction. For this method, before and after each batch a standard solution with known isotopic composition was analyzed. The mass bias correction factor was obtained by comparing the measured isotope ratios with their theoretical values, according to IUPAC abundances.[18]

A scheme of the analysis procedure is shown in **Figure 2**. Channel 1 remained exclusively for the continuous introduction of the spike solution. The rest of solutions (*e.g.*, standards, procedural blanks, samples) were introduced through Channel 2. In the first place, a reference standard solution (C_{st}: 100 ng g^{-1} for Cu, Ni, Pb and Zn, 4 ng g^{-1} for Cd and Hg) with known isotopic composition was analyzed at the beginning of the analysis. R_{st} value in **Equation 1** was obtained from this measurement. Then, after careful check of the instrumental background, procedural blank solutions were analyzed. The sample was later analyzed three times per replicate.

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Procedural blank corrections were performed by subtracting the concentration obtained in the procedural blank solutions using **Equation 1** to the concentrations observed in the sample replicates. The average final concentration as well as standard deviation was calculated for each element. Further Quality Control (QC) standards with natural isotopic composition but lower concentration values (QC1: 20 ng g^{-1} for trace elements, 2 ng g^{-1} for Cd and Hg; QC2: 10 ng g^{-1} for trace elements, 2 ng g^{-1} for Cd and Hg) were also analyzed every three sample replicates in order to check for mass drift and to verify that the obtained recovery values were close to 100 %.

3. Results and discussion

3.1. CRM analysis and uncertainty estimation

In conventional isotope dilution analysis, the amount of spike added to the sample is usually optimized by calculating the ideal ratio using the error magnification factor, f_R. This factor depends on the isotopic abundances of the enriched element spike, as well as of the natural isotopic abundances of that element in the samples. For the on-line isotope dilution analysis, the spike amount is constant, but it is preferable to "over-spike" the samples to yield better counting statistics and therefore less uncertainty in the isotope ratio measurements.[6,29] The effective quantitative range of OID-ICP-MS depends on the concentration of the reference standard for each analyte. The concentration of each element in the reference standard should ideally be midway between the lower and upper quantification limit, becoming into a quantification range of at least 4 orders of magnitude. Therefore the concentration levels of analytes in the multielement isotopic standard are matched to the reference standards used.[29] In this work, the optimization of the concentration of the selected elements in the spike solution was based on the spike data from their respective certificates, and the data from a preliminary semi-quantitative analysis of the analyzed sample. The optimum values for the blend isotope ratios for the respective elements were calculated as a compromise between lowest error magnification factor[34] (*i.e.*, concentration levels that would provide a minimum error propagation from the measurement of the isotope ratios in the blends), sufficient counting rate and the detector range of the instrument, taking into account the expected concentration of the corresponding elements in the sample. Since

the elements of interest were present in the multielement spike solution at the appropriate level (**Table 1**), the on-line spike standard addition was feasible.

The analytical performance of the proposed OID-ICP-MS method was evaluated. As it can be seen in **Table 4**, the concentrations found in the fish homogenate reference material were in good agreement with the certified[35] and information values for all the analytes. Trueness of results obtained with the proposed method was verified by evaluating recovery values of each analyte. Recovery values were found within the range of 97-103 %.

Regarding the uncertainty estimation, the uncertainty of each component in **Equation 1** and other possible biases were carefully evaluated and the relative expanded uncertainties associated with each element (k = 2) were as follow: on the Cd (U′ = 1.8 %), Cu (U′ = 1.2 %), Hg (U′ = 5.4 %), Ni (U′ = 0.7 %), Pb (U′ = 0.8 %) and Zn (U′ = 0.7 %). The uncertainty estimation was fit for purpose for all the analytes as the relative expanded uncertainty values were within 1-5 %. Only a high uncertainty value was noted in the recovery value for Ni (31 % RSD), mainly arising from the high relative expanded uncertainty value given from the certification report (U′ = 31 %, 14 participant laboratories). These low uncertainty values offer a great advantage over other conventional analytical methodologies commonly employed for the same purpose, as the analysis of trace metals in environmental samples often generates data with large uncertainty values. The term "environmental samples" includes samples of different nature, with variable matrix composition and components that may produce interferences. This fact justifies large uncertainty values usually obtained in the analysis of this type of samples, mainly due to polyatomic interferences coming from the matrix. The lower uncertainty values obtained with the proposed OID-ICP-MS methodology demonstrate that it was less susceptible to matrix effect that other calibration methodologies (*e.g.*, external calibration). Even though it is true that this fact had been previously proven with other on-line isotope dilution analysis methodologies,[6,29] advantages from a practical point of view should be also highlighted.

There are several practical benefits of implementing the proposed OID-ICP-MS methodology using the multinebulizer. Firstly, the individual spiking step is avoided, which is often a main

error source of the expanded uncertainty estimation as it is prior to isotope equilibrium, thereby also reducing sample handling and analysis time per sample. Considering the sample pretreatment, a throughput of 10 samples per hour could be analyzed using OID-ICP-MS. The same analysis was performed by batch procedure,[25] yielding a throughput of 1 sample per hour. This fact has a direct beneficial impact on the costs of the analysis. The only sample pretreatment required in this method was sample digestion, that could even be omitted in the case of liquid samples (*e.g.*, wastewater). Moreover, it should be noted that the analysis of the fish sample using OID-ICP-MS just required the preparation of three standard solutions (*i.e.*, Reference standard, QC1 and QC2) for all the analytes and that they could be used for the analysis of more samples. The method has been implemented in the IAEA-476 CRM simply for the convenience, but several high-matrix samples, which are commonly employed in toxicological and ecological risk monitoring (*e.g.*, wastewater, sediment, seawater) could have been used instead.

3.2. Limits of detection and quantification (LOD and LOQ)

According to the Eurachem guidelines,[36] both LOD (*i.e.*, the level at which detection of the analyte becomes problematic) and LOQ (*i.e.*, the lowest level of analyte that can be determined with acceptable performance) are normally calculated by multiplying the obtained standard deviation at low levels (expressed in concentration units) by a suitable factor, k_Q. The number of blank replicates measurements should be sufficient to obtain a reliable estimate of the standard deviation, being 10 replicates often recommended. The LOD in elemental ID-ICP-MS is commonly estimated following the '3s' approach (*i.e.*, $k_Q = 3$),[23] while the IUPAC default value for LOQ estimation is $k_Q = 10$.

Hence, 10 replicate measurements of the procedural blanks were performed and their corresponding concentrations were calculated using **Equation 1**. The standard deviation of the resulting concentrations was used for calculating both the LOD and the LOQ.

The limits of detection (LOD) and quantification (LOQ) for the determination of Cd, Cu, Hg, Ni, Pb and Zn with the proposed OID-ICP-MS methodology using the multinebulizer are shown in Table 5. The relatively high LOD and LOQ values reported for Ni and Zn are presumably due to contamination arising from rather unexpected sources, particularly with Zn, which is present in the laboratory environment, despite the extensive cleaning of the labware and the high purity of chemicals used in the present study. The elevated level of Ni is most probably coming from the ICP-MS nickel cones used in this study.

3.3. Comparison with different OID-ICP-MS approaches

To the best of our knowledge, the only authors reporting LOD values by OID-ICP-MS and following the 3s criterion are Etxeandia and Raposo.[2] The LOD values reported by the authors were 0.01 μg L^{-1} for Cd and Ni, 0.03 μg L^{-1} for Pb and 0.17 μg L^{-1} for Zn. Althought LOD values are lower in comparison with those obtained in this work (with the exception of Cd), the LOD values obtained for all the analytes were sufficient as the concentration of the elements of interest in biota samples are normally well above these values. Multinebulizer is a promising solution since it allows the efficient mixing between two liquids, even if they are inmiscible[17] (*e.g.*, aqueous spike solution and organic sample), due to its high-pressure conditions, leading to a primary aerosol of the blend. The use of multinebulizer presents a new step forward in the applicability of OID-ICP-MS to the analysis of liquid samples. Particularly, the analysis of organic matrix samples using aqueous multi-spike solutions and the development of possible automation strategies for the whole analytical process are all possible ways for further improving the method performance, which are currently under study in our laboratory.

4. Conclusions

In this study, it has been demonstrated that the new multinebulizer (MultiNeb®) is valid for on-line isotope dilution analysis of trace elements in biota sample by ICP-MS. The implementation

of the multinebulizer for the on-line isotope dilution provides significant advantages: (*i*) the high speed and pressure conditions of the multinebulizer allows an intensive mixing of the liquid flows which promotes the establishment of isotopic equilibrium in on-line conditions; (ii) it significantly reduces the total time of the analysis as the time-consuming individual spiking step of conventional ID analysis is avoided, no external calibration graph is needed (making unnecessary any recalibration and re-analysis procedures), and only the preparation of one standard solution per sample is required (*i.e.*, reference standard solution); (*iii*) it provides ease of operation, since the system is simple (*i.e.*, the use of valves and specific components is not required), robust, easy to handle, the multinebulizer perfectly couples to commercial spray chambers (without any additional modification), and multi-element isotope-enriched spike solutions are commercially available; (*iv*) it provides high-quality measurement results with low uncertainty values (*i.e.*, within 1-5 % relative expanded uncertainty for the evaluated elements) compared to conventional quantification methods, based on external calibration approach and an excellent agreement with IAEA-476 CRM certified values was obtained.

All these features result in a promising solution for the trace element analysis of real-world samples which simplifies operation and significantly increases sample throughput and, thus, enhances productivity, with associated economic benefits. It yields savings in total cost of the analysis per sample, in spite of the relatively high cost of the enriched isotope standard.

The proposed multinebulizer-based OID-ICP-MS methodology fulfills some essential requirements for being suitable as a routine method: minimal sample pretreatment, ease of use and it is not affected by matrix effects, good time and cost efficiency, sufficient robustness and accuracy.

Conflict of interest

There are no conflicts to declare.

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TABLES

Table 1. Isotopic composition of the IES-WAK multielement standard and the reference standard.

Element	Concentration (ng g^{-1}) [a]	Isotope	IES-WAK abundance (%)[a]	Natural abundance (%)[a,b]
Cd	21 ± 3	111	96.09 ± 0.06	12.795 ± 0.012
		114	0.90 ± 0.03	28.754 ± 0.081
Cu	309 ± 13	63	0.97 ± 0.06	69.15 ± 0.15
		65	99.03 ± 0.06	30.85 ± 0.15
Hg	19 ± 1	199	91.15 ± 0.09	16.94 ± 0.12
		202	1.90 ± 0.07	29.74 ± 0.13
Ni	302 ± 12	60	5.40 ± 0.02	26.2231 ± 0.0150
		61	89.98 ± 0.04	1.1399 ± 0.0013
Pb	201 ± 8	207	94.94 ± 0.09	33.53 ± 0.13[c]
		208	2.76 ± 0.07	49.69 ± 0.17[c]
Zn	608 ± 54	66	1.1 ± 0.1	27.73 ± 0.98
		67	97.2 ± 0.2	4.04 ± 0.16

[a] Expanded uncertainty for each value was calculated as U = 2·u, where k = 2 is the coverage factor for a 95 % confidence interval and u is the combined standard uncertainty.

[b] From IUPAC.[18]

[c] Determined experimentally by ICP-MS measurements.

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Table 2. ICP-MS operating conditions for the on-line isotope ratio determinations.

Parameter	Value
Radiofrequency power (W)	1550
He collision gas flow (mL min^{-1})	5
Kinetic energy discrimination (V)	3
Plasma gas flow rate (L min^{-1})	15
Auxiliary gas flow rate (L min^{-1})	0.90
Nebulizing gas flow rate (L min^{-1})	1.09
Number of replicates	3
Sweeps/replicate	100
Integration time (s)	0.3
Total liquid uptake rate (mL min^{-1})	0.6
Channel 1 liquid uptake rate (mL min^{-1})	0.3
Channel 2 liquid uptake rate (mL min^{-1})	0.3
Nebulizer type	MultiNeb®
Spray chamber	Double pass Peltier-cooled
Spray chamber temperature (ºC)	2
ICP torch	Quartz with 2.5 mm i.d. injector
Measured isotopes	^{60}Ni, ^{61}Ni, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{67}Zn, ^{90}Zr, ^{95}Mo, ^{111}Cd, ^{114}Cd, ^{117}Sn, ^{199}Hg, ^{202}Hg, ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb.

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Table 3. Certified isotope ratios in the multielement spike solution (R_{sp}) and natural isotope ratios in the reference standard (R_n).

Element	Isotope a	Isotope b	R_{sp}[a]	R_n[a]
Cd	111	114	0.0094 ± 0.0003	2.247 ± 0.007
Cu	63	65	1.03 ± 0.06	0.446 ± 0.002
Hg	202	199	48 ± 2	1.578 ± 0.008
Ni	60	61	16.66 ± 0.06	0.04347 ± 0.00006
Pb	208	207	34.4 ± 0.9	0.675 ± 0.005[b]
Zn	66	67	88 ± 8	1.50 ± 0.08

[a] Isotope abundances ratio was calculated as abundance isotope b/abundance isotope a.

[b] Determined experimentally by ICP-MS measurements

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Table 4. Obtained mass fractions (μg g^{-1}) and expanded uncertainty (k = 2) using the OID-ICP-MS for the IAEA-476 CRM.

Element	Obtained value[a] (μg g^{-1})	Certified value for IAEA-476[a.b] (μg g^{-1})[35] (n = 14)	Recovery (%)
Cd	0.0279 ± 0.0005	0.028 ± 0.003	100 ± 11
Cu	2.41 ± 0.03	2.4 ± 0.3	101 ± 13
Hg	0.56 ± 0.03	0.58 ± 0.02	97 ± 6
Ni	4.19 ± 0.03	4.2 ± 1.3[c]	100 ± 31
Pb	0.633 ± 0.005	0.64 ± 0.05	99 ± 8
Zn	55.8 ± 0.4	54 ± 3	103 ± 6

[a] Mean value ± expanded uncertainty with a coverage factor k = 2.

[b] n is the number of laboratories participating in the characterization of the IAEA-476 CRM.

[c] Information value.

Table 5. Limits of detection (LOD) and quantification (LOQ) of the multinebulizer-based QID-ICP-MS method for trace elements determination in fish samples.

Element	LOD (ng g^{-1})	LOQ (ng g^{-1})
Cd	0.006	0.0019
Cu	0.4	1.4
Hg	0.09	0.3
Ni	30	100
Pb	0.2	0.7
Zn	4	12

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FIGURES

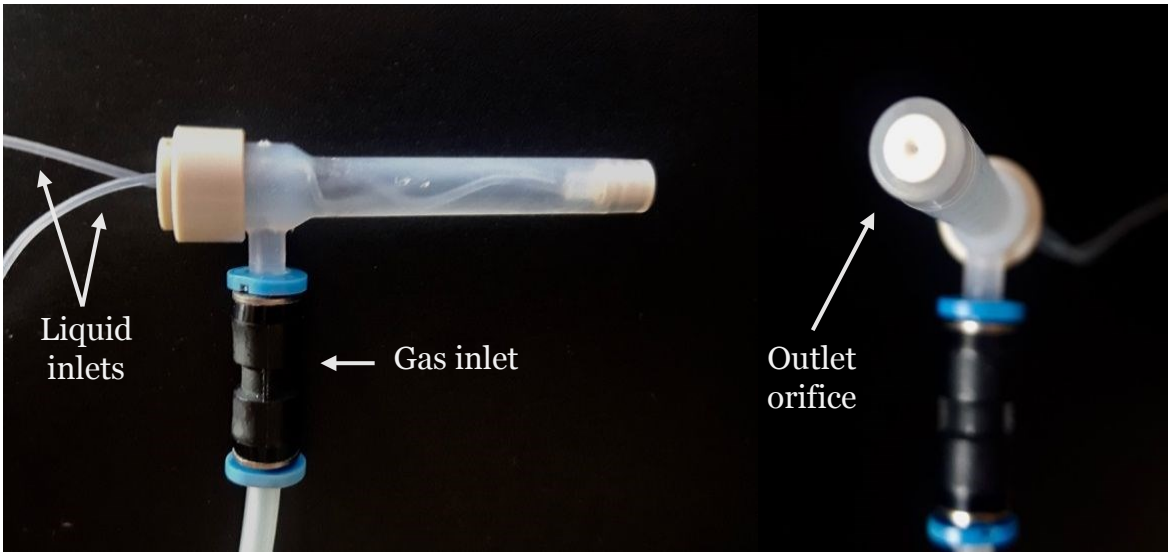

Figure 1. Side view (left) and front view (right) of the multinebulizer prototype.[17]

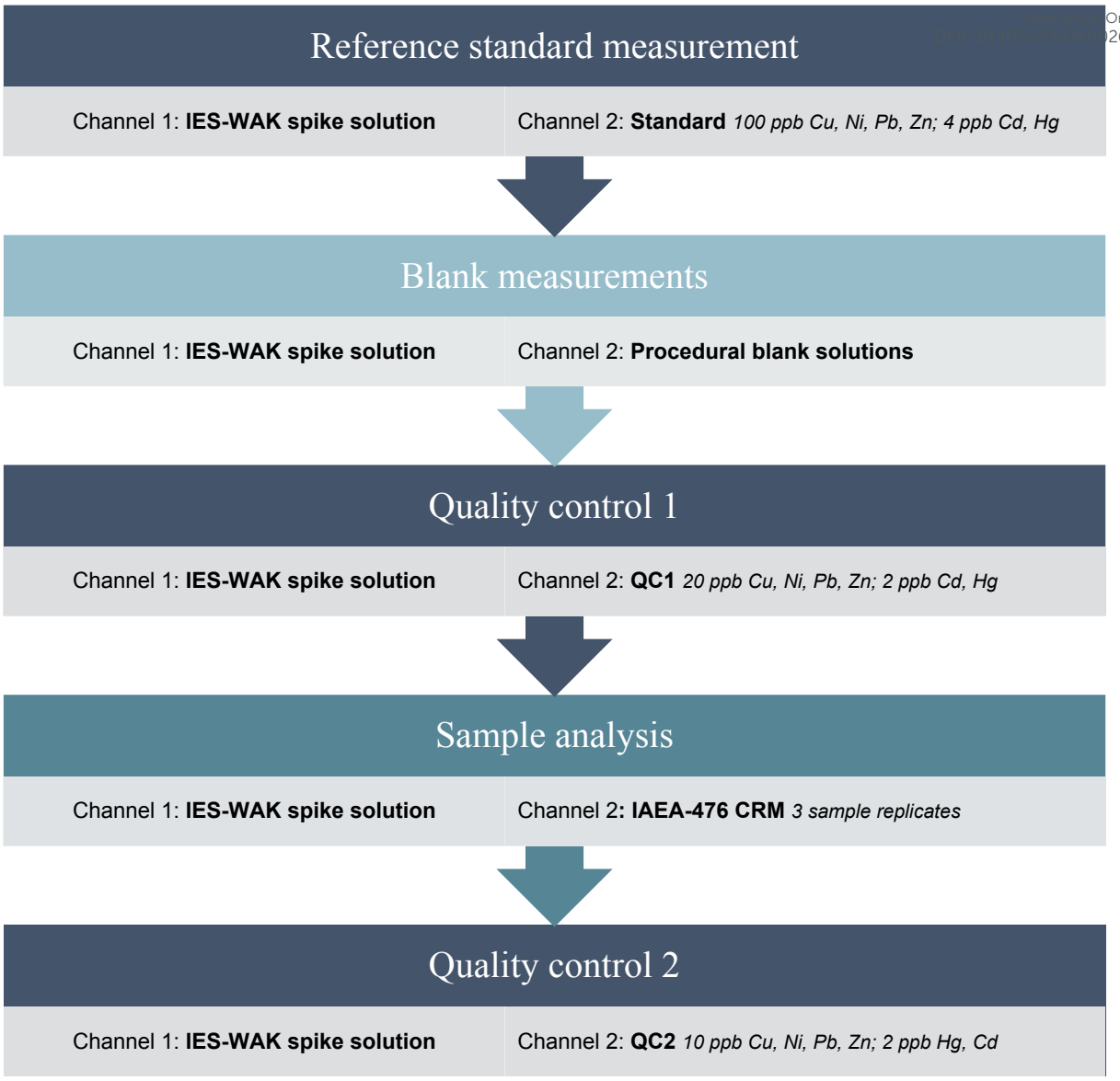

Figure 2. Analytical procedure for the on-line isotope dilution analysis using the multinebulizer with two independent liquid inlets (Channels 1 and 2).