# The use of a new MultiNeb® nebulizer for the on-line isotope dilution inductively coupled plasma mass spectrometry (OID-ICP-MS)

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## 1. Introduction

The development of analytical methodologies for the accurate and reliable determination of trace elements in samples of different kinds (*e.g.*, sediment, biota organisms, or seawater) is in continual demand. Conventionally, testing laboratories employ analytical classical calibration techniques for this purpose which require the establishment of time-consuming calibration graphs and further re-calibration procedures. Whenever larger number of samples need to be analyzed on a daily basis, the implementation of methods that do not require the establishment of a calibration graph may be advantageous as it involves significant savings in time and cost of analysis.

The isotope dilution (ID) methodology is based on the modification of the isotope composition of the element to be determined in the sample by the addition of a known amount of an isotopically enriched standard of the same element (*i.e.*, spike solution) [1]. The total amount of the element in the sample can be obtained from the isotope ratio measurement in the resulting blend. In addition, isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) has been identified to have the potential to be a primary method of measurement (PMM). Hence, the ID-ICP-MS method presents several advantages over other analytical methodologies conventionally employed in testing laboratories for trace elements determination, including: (*i*) once complete isotope equilibration between the sample and the spike has been achieved, there is no need to know the pre-concentration or dilution factor of the sample or to take into account any non-quantitative separation or evaporation process; (*ii*) any variation of the instrumental sensitivity such as signal drift has not influence on the final results; and (*iii*) the results are obtained with superior accuracy, and with a small combined uncertainty. In short, its basic advantage is that ID-ICP-MS measurements must be accepted without the use of analytical standards (*i.e.*, the calibration graph is avoided).

ID-ICP-MS is traditionally practiced only in highly sophisticated laboratories for reference measurements and for any cases where the high accuracy criterion is of great importance to the analytical results. Batch ID-ICP-MS is also regarded as being expensive and time-consuming since each sample is spiked individually. Sample

spiking is tedious, and what is more, a main error source as it is prior to isotope equilibration. However, it can be simplified by the on-line ID-ICP-MS (OID-ICP-MS), which consists of the on-line addition of the spike solution to the sample.

The new MultiNeb® enables the on-line sample spiking by means of the simultaneous introduction of the spike (*i.e.*, isotopically enriched standard) and the sample (*i.e.*, natural isotopic composition) solutions and an effective mixing between them, since the mixing takes place under turbulent conditions of high pressure at the inner part of the tip of the nebulizer.
An OID-ICP-MS methodology based on the MultiNeb® is presented. The target is to improve the productivity of the routine laboratories in the analysis of samples with a complex matrix by the implementation of the MultiNeb® in the on-line ID-ICP-MS analysis. Thus, it results in high quality results as well as the reduction of human intervention required for individual spiking, shorter total analysis time and cost savings.

## 2. Experimental

### 2.1. Instrumentation

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 1**. 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 were corrected for instrumental background, dead time, and possible spectral interferences.

**Table 1**. 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 | $^{60}Ni$, $^{61}Ni$, $^{63}Cu$, $^{65}Cu$, $^{66}Zn$, $^{67}Zn$, $^{90}Zr$, $^{204}Pb$, $^{205}Pb$, $^{206}Pb$, $^{207}Pb$ and $^{208}Pb$. |

## 2.2. 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 ($^{65}Cu$, $^{61}Ni$, $^{207}Pb$ and $^{67}Zn$), designed for quantitative analysis by OID-ICP-MS and accredited by ENAC (accreditation no. 3/PMR004) was obtained from ISC-Science (Oviedo, Spain). The certified abundance values for the elements in the standard are shown in **Table 2**.

**Table 2.** Isotopic standard composition of the IES-WAK multielement standard.

| Element | Isotope a | IES-WAK abundance (%)[a] | Natural abundance (%)[a,b] |
|---|---|---|---|
| Cu | 63 | 0.97 ± 0.06 | 69.15 ± 0.15 |
| Ni | 60 | 5.40 ± 0.02 | 26.2231 ± 0.0150 |
| Pb | 208 | 2.76 ± 0.07 | 49.69 ± 0.17[c] |
| Zn | 66 | 1.1 ± 0.1 | 27.73 ± 0.98 |
| Element | Isotope b | IES-WAK abundance (%)[a] | Natural abundance (%)[a,b] |
| Cu | 65 | 99.03 ± 0.06 | 30.85 ± 0.15 |
| Ni | 61 | 89.98 ± 0.04 | 1.1399 ± 0.0013 |
| Pb | 207 | 94.94 ± 0.09 | 33.53 ± 0.13[c] |
| Zn | 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 reference 2.
[c] Determined experimentally by ICP-MS measurements.

Quality control (QC) solutions, 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 QC1 concentration is 20 ng $g^{-1}$ for Cu, Ni, Pb and Zn and the QC2 concentration is 10 ng $g^{-1}$ for Cu, Ni, Pb and Zn. These solutions were used for mass discrimination correction during ICP-MS measurements. The natural isotopic abundances of the elements of interest, with the exception of lead, were taken from IUPAC tables [2] and are shown in **Table 2**. Lead natural isotopic composition instead, was analyzed prior to isotope ratios measurements in the ICP-MS [3] and is also shown in **Table 2**. Finally, the fish homogenate Certified Reference Material IAEA-476 was obtained from the International Atomic Energy Agency (IAEA, Principality of Monaco).

### 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 4ºC 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 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 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.

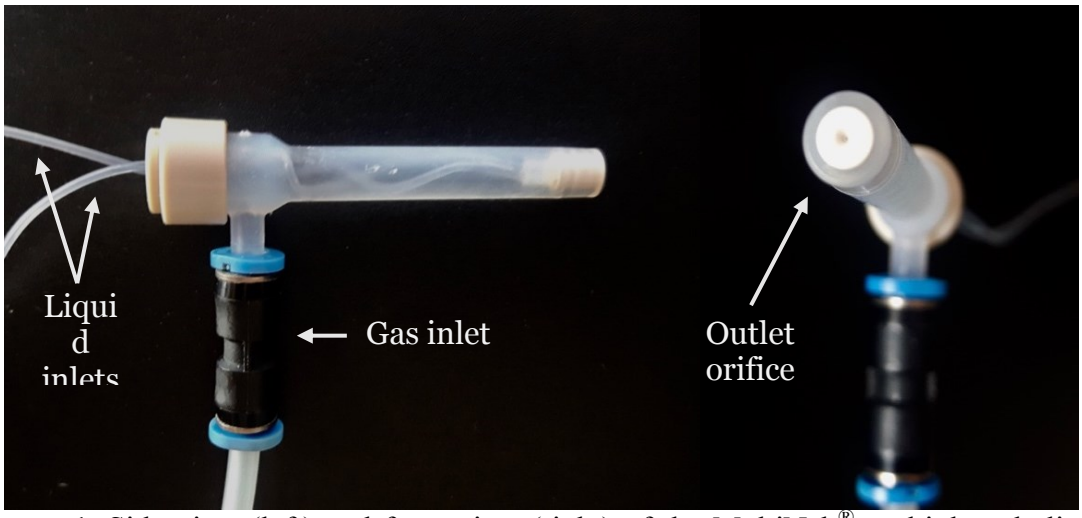


**Figure 1**. Side view (left) and front view (right) of the MultiNeb® multiple nebulizer prototype.

The liquid sample introduction system was composed by a MultiNeb® nebulizer coupled to a quartz double pass Peltier-cooled (2ºC) spray chamber without any additional modification required, as the MultiNeb® 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/QC solutions and spike solutions.

A scheme of the sample introduction system for OID-ICP-MS is shown in **Figure 2**. The on-line sample spiking was achieved by using both liquid inlets of the MultiNeb®: one inlet (Channel 1 in **Figure 2**, flow rate: 0.3 mL $min^{-1}$) for the continuous multi-spike solution addition and the other one (Channel 2 in **Figure 2**, flow rate: 0.3 mL $min^{-1}$) for the sequential introduction of the procedural blanks, the liquid samples and the QC solutions. Thus, the two solution streams were mixed inside of the tip of the nebulizer, leading to a primary aerosol of the resulting blend.

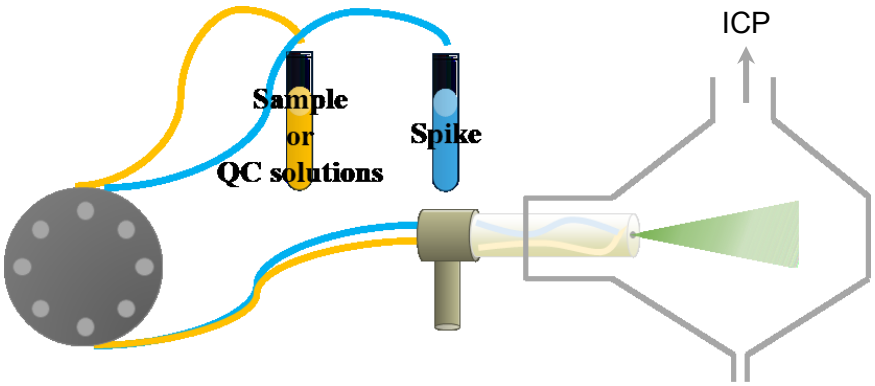


**Figure 2**. Scheme of the on-line isotope dilution system using the MultiNeb®.

Analyte concentrations in the sample digests, $C_s$, were calculated using the following simplified equation, obtained from the isotope dilution theory [1]:

$$C_s = C_{sp} \frac{F_{sp}}{F_s} \frac{R_m A^b_{sp} - A^a_{sp}}{A^a_s - R_m A^b_s} \quad (1)$$

where $C_s$ is the concentration of the analyte in the sample digest solution (s); $C_{sp}$, the concentration of the analyte in the spike solution (sp); $F_{st}$, molar flow of the spike solution (Channel 1); $F_s$, molar flow of the sample solution (Channel 2); $R_m$, the measured isotope ratio of the mixed sample and spike solution (s + sp); $A^b_{sp}$, abundance of isotope b (**Table 2**) of the spike solution (sp); $A^a_{sp}$, abundance of isotope a (**Table 2**) of the spike solution (sp); $A^a_s$, abundance of isotope a (**Table 2**) of the sample solution (s); $A^b_s$, abundance of isotope b (**Table 2**) of the sample solution (s).

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.
Quality control solutions were assumed to contain natural isotopic composition of Cu, Ni and Zn and their associated uncertainties were taken from IUPAC [2]. Lead isotopic composition instead, was determined experimentally by ICP-MS measurements within the same analytical run (**Table 2**).

### 2.5. Analysis procedure

A scheme of the analysis procedure is shown in **Figure 3**. The standard-sample bracketing method [4] was employed for calibration and an effective mass discrimination correction. Quality control solution (QC1: 20 ng $g^{-1}$ for trace elements) with known isotopic composition was analyzed at the beginning of the analysis. Then, procedural blank solutions were analyzed. The sample was later analyzed. Finally, QC2 solution with natural isotopic composition but lower concentration values (QC2: 10 ng $g^{-1}$ for trace elements) was also analyzed every three sample replicates in order to correct for mass bias and to verify that the obtained recovery values were close to 100 %. Each sample replicate was measured three times and average final concentration as well as standard deviation was calculated for each element.

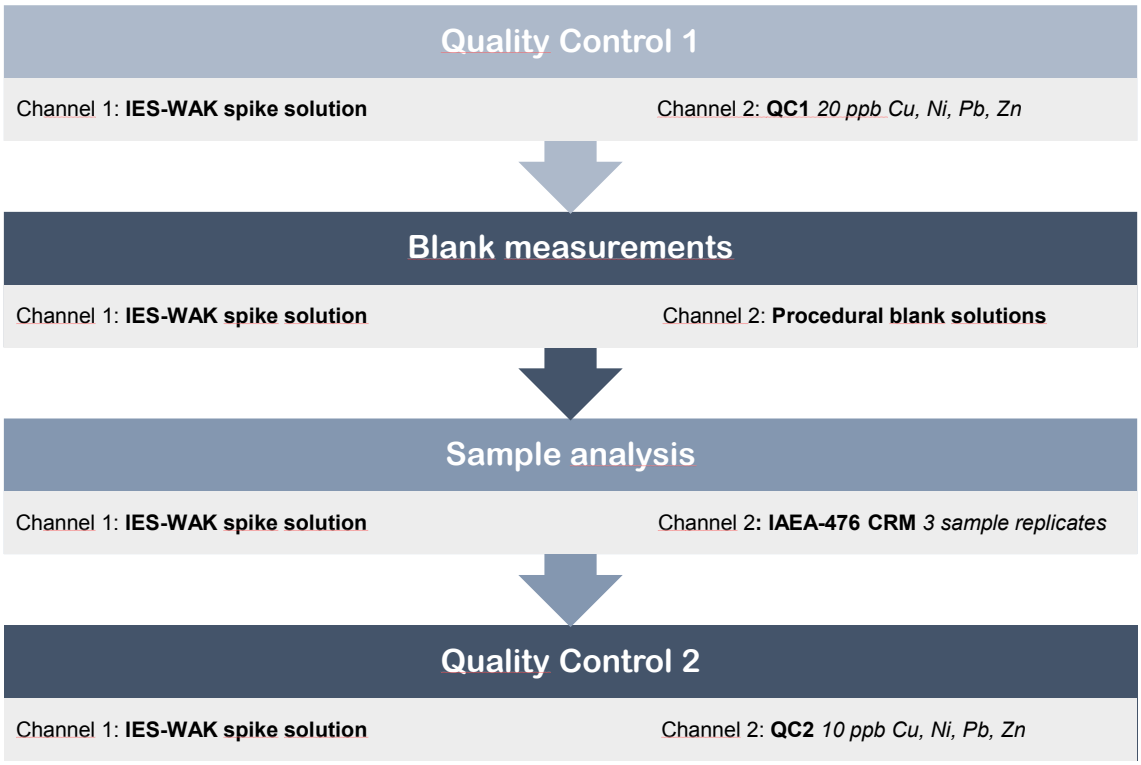


**Figure 3**. Analytical procedure for the on-line isotope dilution analysis using the MultiNeb® nebulizer with two independent liquid inlets (Channel 1 and 2).

## 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". 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 [5].

The analytical performance of the proposed OID-ICP-MS method was evaluated. As it can be seen in **Table 3**, the concentrations found in the fish homogenate reference material were in good agreement with the certified 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 94-109 %.

**Table 3**. 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 $g^{-1}$) (n=14) | Recovery (%) |
|---|---|---|---|
| Cu | 2.28 ± 0.08 | 2.4 ± 0.3 | 95 ± 12 |
| Ni | 4.56 ± 0.12 | 4.2 ± 1.3[c] | 109 ± 34 |
| Pb | 0.605 ± 0.016 | 0.64 ± 0.05 | 95 ± 8 |
| Zn | 51.0 ± 1.2 | 54 ± 3 | 94 ± 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 candidate CRM.
[c] Information value.

Regarding the uncertainty estimation, the relative expanded uncertainties (k=2) for the obtained values of the investigated elements were as follow: on the Cu (U′ =3.5 %), Ni (U′=2.6 %), Pb (U′=2.6 %) and Zn (U′=2.4 %). 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. It was demonstrated that OID-ICP-MS was less susceptible to matrix effect that other calibration methodologies (*e.g.*, external calibration).

There are several practical benefits of implementing the OID-ICP-MS methodology using the MultiNeb®. 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. 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 two QC solutions 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 samples with high matrix, which are commonly employed in toxicological and ecological risk monitoring (*e.g.*, wastewater, sediment, seawater) could have been used instead.

## 4. Conclusions

It has been demonstrated that the new MultiNeb® is valid for on-line isotope dilution analysis of trace elements in biota sample by ICP-MS. Its implementation for the on-line isotope dilution provides significant advantages: (*i*) it simultaneously corrects matrix effects (*i.e.*, matrix-based polyatomic interferences) and any variation of the instrumental sensitivity (*i.e.*, signal drift); (*ii*) it significantly reduces the total time of the analysis as the time-consuming individual spiking step of conventional IDA 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 reliable information with lower uncertainty values (*i.e*., within 1-5 % relative combined uncertainty for the evaluated elements) compared to conventional quantification methods and excellent agreement with IAEA-476 CRM certified values; and (*v*) it could be applied to the analysis of many analytes and with different samples.

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.

## References


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