Ingeniatrics
Nebulizers and Nozzles

Application Note

Determining Elemental Impurities in Pharmaceutical Products using ICP-MS and MultiNeb® nebulizer

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1. Introduction

Trace elements appear in pharmaceutical products as catalytic residues, impurity constituents of the active pharmaceutical ingredient (API) or excipients, and/or due to contamination from manufacturing equipment, production processes, or packaging. Low-levels of reagents or by-products may be present in the final active pharmaceutical ingredients (APIs) as residual impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity, and hence the potential impact on product quality and risk profile requires consideration and management. Consequently, the level of trace elements should be limited in the final drug product. In 2022, The International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use updated the Q3D(R2) guideline. The guideline identified 24 trace elements of potential toxicological concern and established permitted daily exposure limits accounting for the oral, parenteral, and inhalation route of administration (Li, V, Cr, Co, Ni, Cu, As, Se, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, Ba, Os, Ir, Pt, Au, Hg, Tl, and Pb). In addition, the ICH guideline highlights 10 trace elements (B, Na, Mg, Al, K, Ca, Mn, Fe, Zn, and W) that should be evaluated if present in the drug, but no permitted daily exposure limits have been established by ICH due to low inherent toxicity and/or variations in regional regulations. The concern of toxicological effects related to excessive exposure to elemental impurities is also considered in various pharmacopeias.

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For synthesis of pharmaceuticals products, some catalysts (e.g. Ir, Os, Pd, Pt, Rh, and Ru) or metals and metalloids (e.g. Ag, Au, Ba, Li, and Pt) may be incorporated in the drug intermediate reaction process. In addition, during the formulation and drug production, manufacturing equipment commonly contain metal alloys, stainless steel and glass materials because of their superior chemical resistance. Common elements found in those machines (e.g. Co, Cr, Cu, Mo, Ni, and V) under extreme/corrosive reaction conditions (as high temperature and low/high pH) may suffer leaching. In the analysis of dietary supplements (e.g. botanicals, multivitamins, creatine, and sport supplements), the elemental impurities As, Cd, Hg, and Pb are also present in low concentrations.

In this sense, elemental analysis in pharmacological products is usually performed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) or Mass Spectrometry (ICP-MS). ICP-MS offers extremely low detection limits ranging from sub part per billion (ppb) to trillion (ppt) for most elements. It has a rapid multi-element scanning capability over a wide range of masses with lower detection limits compared to GF-AAS and ICP-OES. In most of the cases, the detection limits are 100–1000 times superior to those achieved by ICP-OES. These detection limits are broadly achieved for almost all the elements across the periodic table. Thus, there is a great need for development of highly sensitive and selective techniques for determination trace metals in pharmaceutical substances not only to meet the stringent specifications but also ensure the safety and efficacy of drugs for human consumption.

Here we describe a simple, quick and robust method for elements determination in pharmacological products using the multiple inlet nebulizer MultiNeb® (Ingeniatrics Tecnologías S.L.) by a simple online dilution for small volume of sample with ICP-MS analysis, equipped with CRC was used to reduce polyatomic interferences and internal standards were used to counter the matrix effects and instrument drift. Conventionally, the internal standard is mixed with the calibration standards and samples using a Y connection, when conventional nebulizer is employed. However, the novel MultiNeb® (Ingeniatrics Tecnologías S.L.) has been developed which allows a high mixing efficiency between two liquids, miscible or immiscible, since the mixing takes place under turbulent conditions of high pressure at the tip of the nebulizer.

2. Experimental

Reagents and solutions

All reagents used were of the highest available purity. Helium was used as collision gas, respectively, in an ICP-MS system, were of high-purity grade (>99.999%). Water was purified with a Milli-Q Gradient system (Millipore, Watford, UK). The aqueous levels of calibration standards were prepared by appropriate dilution of a Multi-element calibration standard-2A, contains 10 $mg.L^{-1}$ of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Sb, Se, Sr, Tl, U, V, Zn (and Hg) in a matrix of 5% HNO_3 (Agilent Technologies, Part Number: 8500-6940). On the other hand, Ir, Os, Pd, Pt, Rh, Ru, Mo, Pt, Pd and Sn were employed by appropriated dilution of a monoelemental stock solutions 10 $mg.L^{-1}$ (SPEXcertiprep, Metuchen, NJ). All aqueous solutions are acidified using 5% (v/v) high purity nitric acid, hydrogen peroxide and hydrogen chloride (Suprapur®, Merck, Darmstadt, Germany). As internal standard, a mixture of Scandium (Sc), Indium (In) and 209Bismuth (^{209}Bi) was employed for ICP-MS analysis (Agilent Technologies, Part Number: 5190-9770).

Instrumentation

A heating block system type DigiPREP MS (SCP Science, Courtaboeuf, France) was employed for sample preparation. All measurements were carried out using an Agilent 7900 ICP-MS (Agilent Technologies) equipped with a SPS4 autosampler (Agilent Technologies) with 0.5 mm ID sampling probe (Agilent Technologies, Part No.: G8410-80101). The instruments operating conditions are shown in Table I.

The MultiNeb® nebulizer used in this study consists of two independent liquid inlets and a common gas inlet in a single nebulizer body of polytetrafluoroethylene (Figure 1). Peristaltic pump tubings employed were of 0.25 mm i.d., used for ISTD solution (blue/orange taps) and 0.5 mm i.d. (white/white taps) for digested sample, calibration levels solutions, blanks and quality controls.

Figure 1. MultiNeb® nebulizer.

ICP-MS Operational Conditions		
7900 ICP-MS Parameters (Agilent Technologies)		
RF Power (W)	1550	
Plasma gas flow (L min^{-1})	15.0	
Nebulization gas flow (L min^{-1})	MultiNeb®	0.7
Sampling Depth (mm)	8.0	
Cell gas flow (mL min^{-1})	4.0 (He)	
KED (V)	5.0	
Cones	Nickel	
Integration time (s)	0.7 per isotope	
Extract 2 (V)	-240	
Omega Bias (V)	-80	
Omega Lens (V)	7.2	
OctP RF (V)	200	
Sample introduction rate (rps)	0.1	
Isotopes monitored	^{7}Li, ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{75}As, ^{78}Se, ^{95}Mo, ^{101}Ru, ^{103}Rh, ^{105}Pd, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{189}Os, ^{193}Ir, ^{195}Pt, ^{197}Au, ^{202}Hg, ^{205}Tl, ^{208}Pb ^{45}Sc, ^{115}In, ^{209}Bi (as Internal Standards)	

Table I. Operational conditions for 7900 ICP-MS using online internal calibration.

Additionally, it was demonstrated that the spectral interferences caused by rich-organic matrices can be eliminated by MultiNeb® nebulizer. Traditionally, the carbon deposition in cones, can be avoided by adding a small amount of oxygen to the intermediate gas flow. However, the use of oxygen to support complete combustion of carbon further increases the complexity of the experimental setup and the cost per analysis.

Related to this, adding aqueous solution in the aerosol, through MultiNeb's second channel, will help the complete combustion of carbon, avoiding its byproducts and the subsequent clogging of the torch's injector, cones and will, therefore prevent the spectral interferences, providing a better sensitivity, reproducibility and precision.

In this sense, in this study a solution with internal standards in 10 % (v/v) of hydrogen peroxide was employed to obtain better background in analytes interfered by carbon matrix, by increasing the contribution of oxygen in the aerosol compared to water, favoring the formation of CO_2 and reducing carbon deposition in torch's injector and cones, and reducing carbon interferences in plasma. In addition, this solution contains the internal standards with order to evaluate the long-term stability of the signal and matrix effects.

The MultiNeb®-based configuration is composed by the MultiNeb® nebulizer associated with a spray chamber without any additional modification required, as the MultiNeb® is built on the right dimensions to allow easy connection to any commercial spray chamber conventionally used in ICP-based (Figure 2). This implies an important advantage over conventional systems since it does not require the continuous cleaning of ICP components or the use of expensive additional components such as cooled spray chambers or an auxiliary oxygen supply. This simple and powerful alternative to remove spectral interference caused by organic component from matrix enables to analyze samples with accuracy.

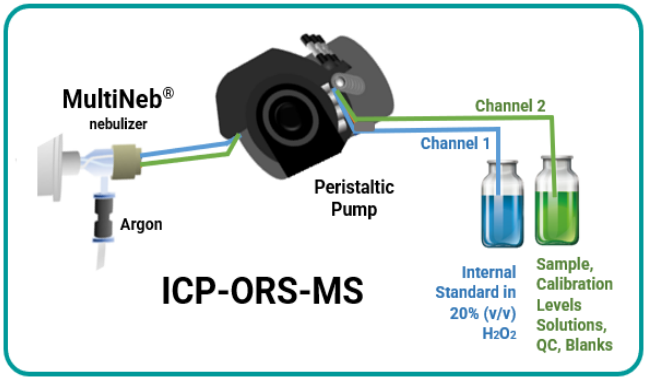

Figure 2. Schematic representation of MultiNeb®-based configuration employed in this work.

A total of three internal standards have been used depending on the mass of the isotope in ICP-MS. The Table II summarizes the internal standard employed for each analyte throughout this work.

Internal Standard Calibration ICP-ORS-MS	
Isotopes monitored (m/z)	**IS**
^{7}Li, ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{75}As, ^{78}Se	^{45}Sc
^{95}Mo, ^{101}Ru, ^{103}Rh, ^{105}Pd, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba	^{115}In
^{189}Os, ^{193}Ir, ^{195}Pt, ^{197}Au, ^{202}Hg, ^{205}Tl, ^{208}Pb	^{209}Bi

Table II. Internal standard used for each analyte throughout this work using ICP-ORS-MS.

Sample preparation

Sample preparation is a critical step prior to accurate instrumental analysis. Generally, pharmaceutical samples present a wide range of different compositions, such as excipients, flavoring agents, stabilizers, and active ingredients. Consequently, these complex matrices make sample preparation a challenging step. Three most common pharmacological products were analyzed in this Application Note, amoxicillin, paracetamol and ibuprofen. From each sample were ground and homogenized using a porcelain mortar and pestle. Then, 100 mg was weighed using an analytical balance and transferred directly into 10 mL glass capped vials employing in DigiPrep digestor. After that, 1500 µL concentrated 65% nitric acid (HNO_3) and 500 µL concentrated 37% HCl were added to each digestion vial. The vials were gently whirled by hand and allowed to pre-digest for 5 min. Following, 500 µL concentrated 30% H_2O_2 was added and pre-digested for 15 min. The digestion vials were capped and digested in triplicates. The digestion program is shown in Table III. After the digestion procedure, the vials cooled for additional 24 h in a fume hood for the convenience of the following sample analysis.

Step	Procedure	Time (min)	Temperature (°C)
1	Ramp	20	120
2	Hold	20	120
3	Ramp	20	180
4	Hold	20	180

Table III. Operational parameters used during acid digestion using DigiPrep heating block.

The digests were transferred to 2 mL polypropylene vials and centrifuged for 10 min at 14,000 rpm (Centrifuge 5420, Eppendorf, Hamburg, Germany). All digested sample contained residual solids. Before ICP-ORS-MS analysis, 1 mL of the digested sample solutions were transferred to a 15 mL polypropylene autosampler tubes and ultrapure water (>18,2 MΩ cm) was added to a final volume of 10.00 mL. The same procedure was applied for blank digestions and QC samples. The diluted solutions were analyzed using ICP-ORS-MS. An overview of the total sample preparation procedure is presented in Figure 3.

When high matrix samples are measured using ICP source, the matrix elements can affect analyte signals in several distinct ways. Probably the most widely recognized is the gradual downward drift that typically occurs due to the build-up of matrix deposits on the ICP-MS interface components (sampling cone, skimmer cone and torch injector) when high matrix samples are measured over an extended analytical batch. After sample preparation, resulting digested solutions are directly aspirated for one channel of MultiNeb® nebulizer, using peristaltic pump tubings with 0.5 mm i.d., normally employed for ISTD solutions, which in turn is diluted with the solution aspirated through the second channel of the nebulizer containing internal standards solutions in 20 % H_2O_2 (v/v) to correct instrument drift. In this sense, the resulting aerosol is diluted two times prior to plasma, reducing the carbon content and benefiting the formation of carbon dioxide.

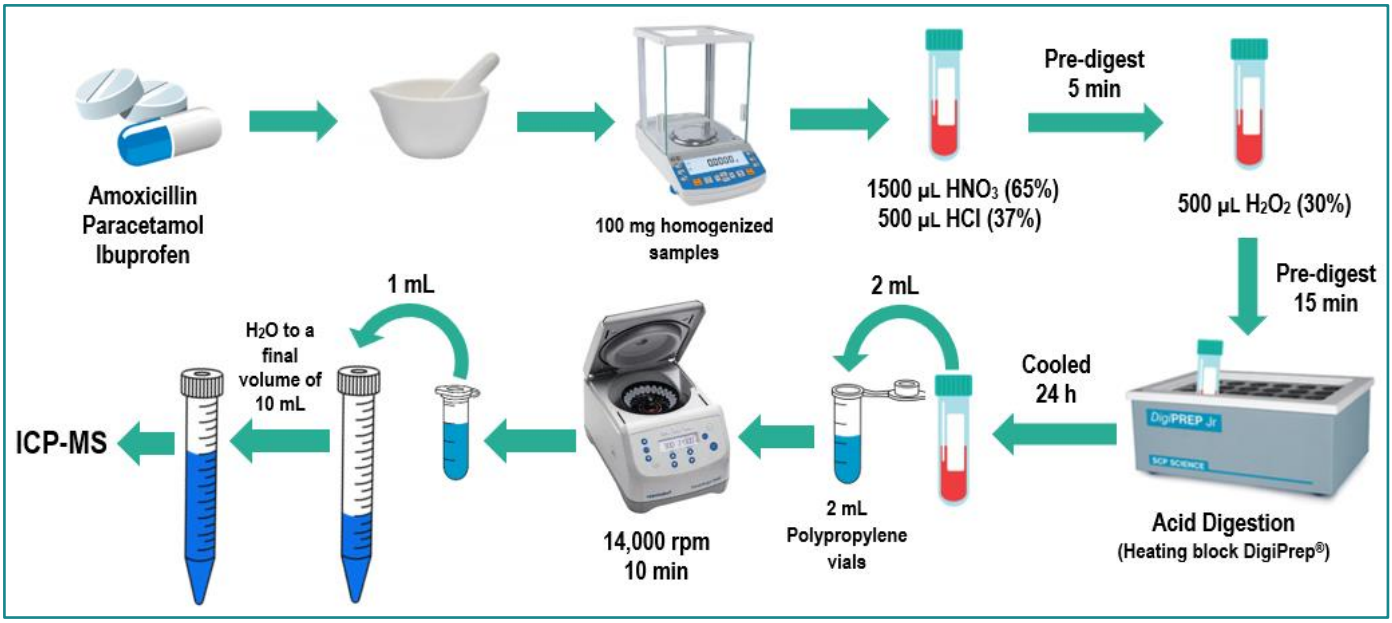

Figure 3. Workflow used for sample preparation.

3. Results and Discussion

Sensitivity and signal stability

Nebulizers designed by Ingeniatrics Tecnologías SL, such as MultiNeb®, use *Flow Blurring* nebulization technology instead of the traditional Venturi effect, as conventional concentric nebulizer. This allows the generation of a very fine droplet aerosol with a narrow size distribution (most droplets are smaller than 10 µm), which improves efficiency over a wide range of nebulization gas flow rates, especially 0.60-0.75 L min^{-1} (150-250 kPa nebulization pressure).

The detection limits (LD) were established by analyzing five of the calibration blank and multiplying the obtained standard deviation by three. The results obtained are show in Table IV.

For the study of signal stability and plasma drift, a monitoring standard solution as quality control (QC) was analyzed once every 5 samples, in order to evaluate the stability of the signal. The recoveries must fall within the limits of 94-105 % (Table V). The concentration of the different elements in QC sample using as a monitoring control solution is shown in Table V.

Experimental Values							
Analyte	Detection Limit (DL) ($\mu g.L^{-1}$)	Amoxicillin		Ibuprofen		Paracetamol	
		Mean ($\mu g.g^{-1}$)	RSD (%)	Mean ($\mu g.g^{-1}$)	RSD (%)	Mean ($\mu g.g^{-1}$)	RSD (%)
As	0.016	**0.108**	1.36	**0.014**	2.26	**0.021**	1.82
Ag	0.001	**0.006**	1.84	**< DL**	---	**0.002**	2.37
Au	0.001	**0.113**	1.11	**< DL**	---	**< DL**	---
Ba	0.014	**2.364**	1.64	**0.804**	2.78	**1.647**	2.03
Cd	0.002	**0.021**	2.11	**< DL**	---	**0.002**	3.26
Co	0.001	**0.534**	1.16	**0.065**	1.82	**0.031**	1.48
Cr	0.009	**0.066**	2.08	**0.161**	1.66	**0.027**	3.13
Cu	0.026	**3.241**	0.78	**0.782**	1.09	**2.224**	1.66
Hg	0.012	**0.014**	2.21	**0.006**	2.16	**0.001**	4.04
Ir	0.001	**0.231**	1.21	**0.018**	3.26	**0.072**	2.08
Li	0.136	**2.314**	3.16	**0.064**	3.66	**1.181**	2.79
Mo	0.007	**0.074**	2.22	**0.112**	1.34	**0.260**	0.87
Ni	0.009	**0.062**	2.04	**0.636**	0.95	**0.181**	1.36
Os	0.008	**0.103**	1.61	**0.031**	3.22	**0.026**	3.18
Pb	0.004	**0.014**	2.62	**0.083**	1.91	**0.032**	2.14
Pt	0.002	**0.141**	1.24	**< DL**	---	**< DL**	---
Pd	0.013	**< DL**	---	**0.008**	3.16	**< DL**	---
Rh	0.001	**0.112**	1.14	**< DL**	---	**0.024**	0.89
Ru	0.003	**< DL**	---	**< DL**	---	**< DL**	---
Sb	0.002	**0.018**	2.51	**< DL**	---	**0.165**	1.55
Se	0.015	**0.721**	1.74	**< DL**	---	**0.167**	2.39
Sn	0.013	**0.063**	1.98	**0.086**	1.77	**0.012**	2.41
Tl	0.001	**0.009**	1.41	**0.021**	0.99	**0.014**	0.97
V	0.005	**0.214**	0.98	**0.154**	1.12	**0.073**	2.14

Table IV. Experimental values for each analyte monitored in the different pharmaceutical products studied, LD as well as the RSD (%) using MultiNeb® nebulizer employed in this work by ICP-ORS-MS detection.

Element	QC sample Composition ($\mu g.L^{-1}$)	Recoveries ICP-ORS-MS (%)
As	100	98-102
Ag	10	96-104
Au	100	94-103
Ba	2000	95-102
Cd	25	98-104
Co	500	99-102
Cr	100	95-103
Cu	2500	98-101
Hg	10	95-105
Ir	200	96-101
Li	1000	97-103
Mo	100	98-101
Ni	100	96-104
Os	50	99-103
Pb	25	98-102
Pt	100	99-103
Pd	25	96-104
Rh	50	99-102
Sb	50	98-103
Se	100	98-101
Sn	50	96-104
Tl	25	98-102
V	100	99-103

Table V. Experimental values for recoveries results each analyte in QC sample analyzed for 5 replicates of the different samples using MultiNeb® nebulizer employed in this work by ICP-MS detection in a sequence of 50 samples in the same analytical batch.

Based on the results shown in Table V, the use of the multiple inlet nebulizer, MultiNeb®, which allows the simultaneously nebulization of two liquid flows, it demonstrates the long signal stability along the sequence in the same analytical batch. Because of a supplementary addition of oxygen in plasma, as a hydrogen peroxide solution, a resulting aerosol is prior to plasma, reducing the carbon content and benefiting the formation of carbon dioxide is minimized.

Precision and reproducibility

As certified reference materials were not available, precision was evaluated based on recovery assays, spiked twice time the concentration of each analyte in each pharmacological products employed in this study. The results are represented in Figure 4.

Additionally, each sample was analyzed using 5 replicates, and the results obtained, expressed as relative standard destination (RSD, %) are shown in Table IV. The results obtained demonstrate the remarkable reproducibility demonstrates the notable reproducibility obtained using the proposed methodology in this technical application note for multielemental quantification of 24 elements in pharmacological products studied.

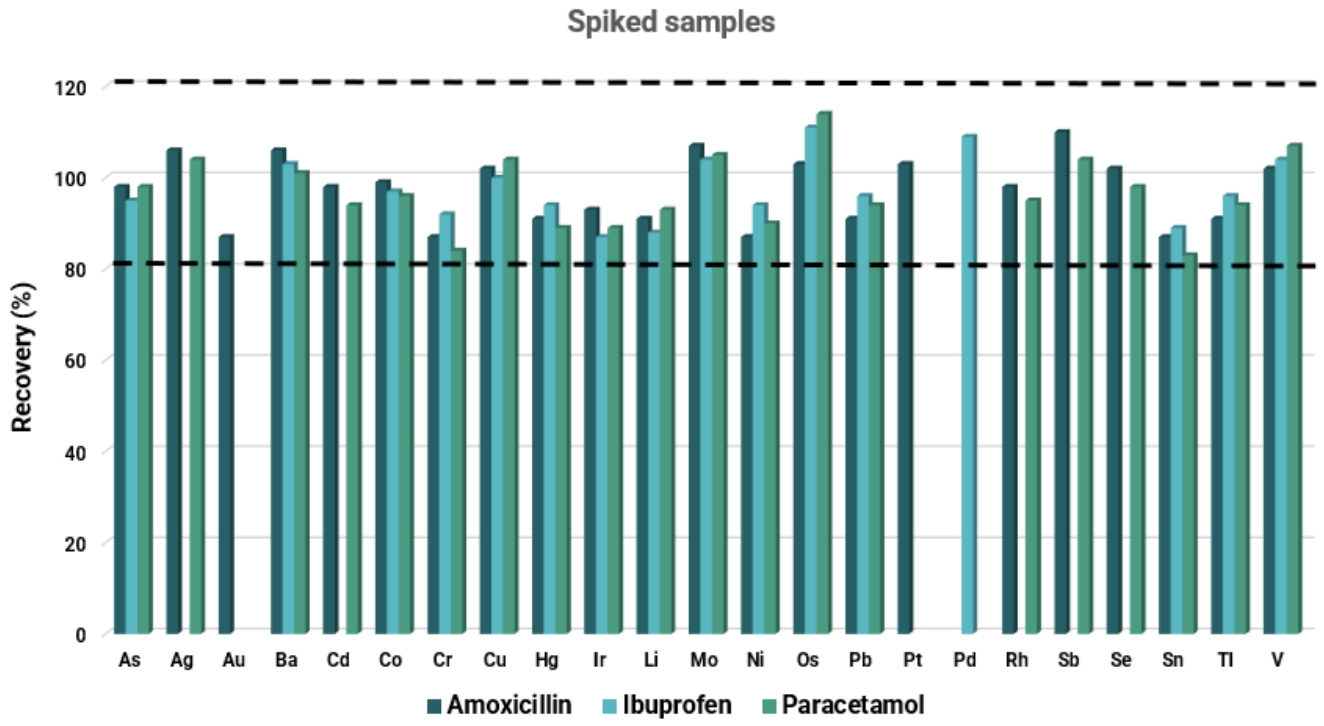

Figure 4. Experimental values for recoveries assays spiked twice time the concentration of each analyte in each pharmacological products employed in this study.

4. Conclusions

The aim of this study was to establish an ICP-ORS-MS based multi trace elemental analysis method for solid pharmaceuticals in relation to the ICH Q3D(R1) guideline to investigate the possibility of linking trace element content to tablet drug formulation and to distinguish between drug products from the trace elemental profile.

The results obtained in this study using MultiNeb® nebulizer provides higher precision, sensitivity and reproducibility for multielement determination in wine samples by ICP-ORS-MS.

On the other hand, the enhanced precision results obtained with the MultiNeb® nebulizer are related to the higher sensitivity and reproducibility obtained in comparison with the Y connection normally employed for this purpose, what demonstrates that the mixing of the internal standard dissolved in hydrogen peroxide solution, as a supplementary oxygen supply when carbon matrices are analyzer using plasma source, minimizes the effects on the nebulization process and therefore improves the analytical operation and results, , favorizing the complete combustion of carbon, avoiding its byproducts and the subsequent clogging of the torch's injector, cones and will, therefore prevent the spectral interferences, providing a better sensitivity, reproducibility and precision.

Additionally, the proposed analytical methodology prolongs the lifetime of torches compared to the direct supply of oxygen to the plasma.

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