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Ingeniatrics Nebulizers and Nozzles

TRENDS IN NEBULIZATION STRATEGIES FOR SPECIATION ANALYSIS. ADVANTAGES OF TWO-CHANNELS NEBULIZER FOR ONLINE INTERNAL STANDARD CORRECTION TECHNIQUE AND ISOTOPIC DILUTION ANALYSIS BY LC-ICP-MS. APPLICATION TO HUMAN BIOLOGICAL FLUIDS.

M.A. García-Sevillano[1], B. Grañán-Riesco[2], Alfonso M. Gañán-Calvo[3], J.L Gómez-Ariza[4]

[1]MG Analytical Chemistry Instruments Consulting. Av. Océano P2-10F, 21100, Punta Umbría, Huelva, Spain.
[2]Ingeniatrics Tecnologías S.L. P.I. Parque Plata, C/ Camino Mozárabe 41, 41900 Camas, Seville, Spain.
[3]Department of Aerospace Engineering and Fluid Mechanics, Higher Technical School of Engineering, University of Seville, E-41092, Seville, Spain.
[4]Department of Chemistry and Material Science, Faculty of Experimental Science, University of Huelva, Campus de El Carmen, 21007-Huelva, Spain.

Nebulizer selection is a critical but often overlooked aspect of ICP analyses. There are many different nebulizers available for ICP-OES and ICP-MS, for this reason, choosing the optimal one can be confusing and wrong selection. To achieve peak performance from your ICP analysis, it is essential to choose the optimal nebulizer based on your sample matrix, precision, sensitivity, reproducibility, and signal stability required.

Conventionally, the enriched standard required for isotopic dilution analysis or internal standard addition is mixed with the samples or chromatographic flow after LC separation using a Y connection. Recently, the novel MultiNeb® (Ingeniatrics Tecnologías S.L., Spain) 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. In addition, for speciation analysis based on LC-ICP-MS analytical approach, Ingeniatrics Tecnologias S.L. has released to allow quick, reliable and easy connection of your LC to your ICP, a one piece-high pressure connector with some advantages, such as resists blockage, fast washout, minimize dead volume and peak broadening.

In this study, we compare the performance of Micromist® and MultiNeb® nebulizers for arsenic and selenium speciation analysis based on the use of an online internal standard correction technique by LC-IS-ICP-Q-MS and simultaneous quantification of selenoproteins and selenometabolites by (SUID)-ICP-QqQ-MS online coupled to 2D/SE-AF-LC, respectively. The optimized methodology was applied to human biological fluids, such as human serum and urine samples.

The results show that the new MultiNeb® multiple nebulizer presents higher precision, sensitivity, signal stability and reproducibility in total metal determination using internal standard correction or isotopic dilution analysis and arsenic and selenium speciation in human biological fluids by LC-IS-ICP-Q-MS and two dimensional LC-SEC-AF-SUID-ICP-QqQ-MS, respectively.

The Flow Blurring® technology efficiently utilizes the available gas pressure drop across the nebulizer outlet to maximize both mixing between the nebulizing gas and the liquid formulation and the production of surface area per unit volume through its unique axisymmetric cross-flow nebulizer tip geometry. In fact, this nebulization procedure achieves the lowest ratio of number mean diameter to σ/ΔP among commercial nebulizers, where σ is the surface tension of the formulation and ΔP is the gas pressure drop. This indicates a physical maximization of the turbulent mixing efficiency between the two incoming fluid streams (gas and formulation). This energy efficiency also results in lower gas consumption for the same outlet size or a lower gas pressure for a given gas flow rate. This is because the necessary nebulization pressure can be substantially reduced. Another advantage of this efficiency is that the nebulizer outlet openings, including the nebulizer discharge orifice and liquid capillary, can be larger than those of other commercial nebulizers with the same gas and liquid sample flow rates. This fact has demonstrated superiority in minimizing handling, cleaning, clogging, and stability issues.

Total arsenic and selenium determination in Human Biological Fluids

Conventionally, for online internal standard or enriched element solution addition required for internal calibration or isotopic dilution analysis, respectively, the solution is mixed with the digested samples using a Y connection when Micromist® nebulizer is employed. However, the novel MultiNeb® nebulizer allows a high mixing efficiency between two liquid inlets, miscible or immiscible, under turbulent conditions caused by high pressure at the tip of the nebulizer (Figure 1). In this work, Re is employed as internal standard in arsenic determination by ICP-MS using internal calibration correction, and 74Se is used as enriched standard for selenium determination by IDA-ICP-QqQ-MS. Operational conditions are shown in Table 1 and Table 2, respectively.

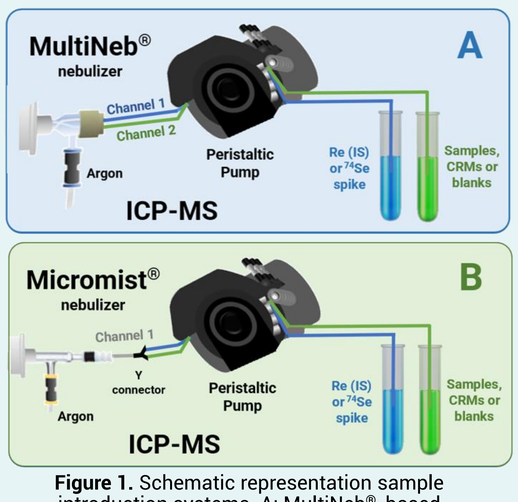


Figure 1. Schematic representation sample introduction systems. A: MultiNeb®-based configuration. B: Micromist®-based configuration for total arsenic and selenium determination.

Arsenic speciation in human biological fluids using LC-ICP-MS by online internal standard correction technique

In speciation analysis based on the use of LC-ICP-MS, signal stability and plasma drift, as well as the perturbations in the pressure pump, nebulization process, nebulizer blockage and changes in the mobile phase composition during gradient elution, might produce some plasma perturbations. As a result, the detection response and the baseline signals often fluctuate during analysis, limiting precision and accuracy of quantifications. In this sense, in order to reduce the previously mentioned effects, an analytical methodology has been proposed in the present work for arsenic speciation analysis using LC-ICP-MS, with online internal standard correction technique based on the instrumental configuration including the MultiNeb® nebulizer, that is schematically represented in Figure 2.

Table 1. Operational conditions for 7900 ICP-MS and 1260 HPLC optimized for arsenic speciation by online internal calibration technique (Agilent Technologies).

LC-ICP-MS Operational Conditions	
7900 ICP-MS Parameters (Agilent Technologies)	
RF Power (W)	1550
Plasma gas flow (L min^{-1})	15
Auxiliary gas flow (L min^{-1})	0.5
Carrier gas flow (L min^{-1})	0.6
Sampling Depth (mm)	8.0
Cell gas flow (mL min^{-1})	4.0 (He Collision Gas)
KED (V)	3.0
Isotopes monitored	^{75}As, ^{35}Cl, ^{185}Re
Sampling and skimmer cones	Nickel
Dwell time (s)	0.3 per isotope
1260 HPLC Parameters (Agilent Technologies)	
Column	Hamilton PRP-X100 anion-exchange column (250 × 2.1 mm × 10 µm particle size) with an associated guard column.
Temperature	30 ºC
Mobile phase	Gradient Elution Mode. Chanel A: 5 mM $(NH_4)_2CO_3$, pH 9.0, 0.05% Na_2EDTA; Chanel B: 50 mM $(NH_4)_2CO_3$, pH 9.0, 0.05% Na_2EDTA and 5% of MeOH.
Flow rate	700 µL min^{-1}
Injection volume	100 µL

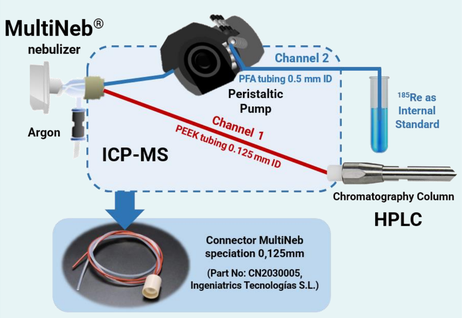


Figure 2. Schematic representation of MultiNeb®-based configuration for Speciation Analysis using a high-pressure connector for LC-ICP using MultiNeb® nebulizer (Two Liquid Inlets).

Speciation of selenium containing biomolecules in human serum by 2D/SEC-AF-LC-SUID-ICP-QqQ-MS

Speciation of selenium in human serum was carried out by 2D/SEC-AF-LC-SUID-ICP-QqQ-MS following a procedure described by the author elsewhere with some modifications. Briefly, separation of the analytes was performed by in series stacking of two 5 mL HiTrap® Desalting columns in series connected with a dual affinity column arrangement comprising a 1 mL heparin-sepharose column (HEP-HP) and a 1 mL blue-sepharose column (BLU-HP) interconnected by a six-way switching column valve. The HiTrap column is based on size exclusion chromatography, and it is normally used to separate low molecular mass components (MW<1000Da) from high molecular mass molecules, such as DNA, proteins or peptides (MW>5000Da), the combination of two columns increases the resolution of the chromatographic separation. On the other hand, HEP-HP column is able to retain selectively SeP whereas BLUE-HP column retains both SeP and SeAlb which has been previously described.

In this study, high pressure connector for LC-ICP using MultiNeb® Nebulizer (Two Liquid Inlets) was employed (Part No: CN2030075, Ingeniatrics Tecnologías S.L.)). Optimized operating conditions for selenium biomolecules speciation analysis by 2D/SEC-AF-LC-SUID-ICP-QqQ-MS are shown in Table 2 using the schematic arrangement shown in the Figure 3.

Table 2. Operational conditions for 8800 ICP-QqQ-MS and 1260 HPLC optimized for arsenic speciation by online internal calibration technique (Agilent Technologies).

2D/SEC-AF-LC-ICP-QqQ-MS Operational Conditions		
8800 ICP-QqQ-MS (Agilent Technologies)		
RF Power (W)	1550	
Plasma gas flow (L min^{-1})	15	
Auxiliary gas flow (L min^{-1})	0.8	
Nebulization gas flow (L min^{-1})	Micromist®	0.80
	MultiNeb®	0.65
Sampling Depth (mm)	7.0	
Cell gas flow (mL min^{-1})	1.35 O_2/H_2 gas mode	
KED (V)	2.0	
Isotopes monitored	^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se, ^{79}Br, ^{81}Br, ^{83}Kr (detected as SeO^+ using O_2+H_2 mode in Q2)	
Dead Time Detector	47 ns	
Dwell time (s)	0.3 per isotope	
1260 HPLC (Agilent Technologies)		
Sample loop	100 µL	
Flow rate (mL min^{-1})	1.3	
Mobile phase A	0.05 M ammonium acetate pH 7.4	
Mobile phase B	1.5 M ammonium acetate pH 7.4	
Gradient	0-7 min 100% A, 6-18 min 100% B, 18-20 min 100% A	
Valve position	1-9 min Inject (Position A)	
	9-16 min Load (Position B)	
	16-20 min Inject (Position A)	

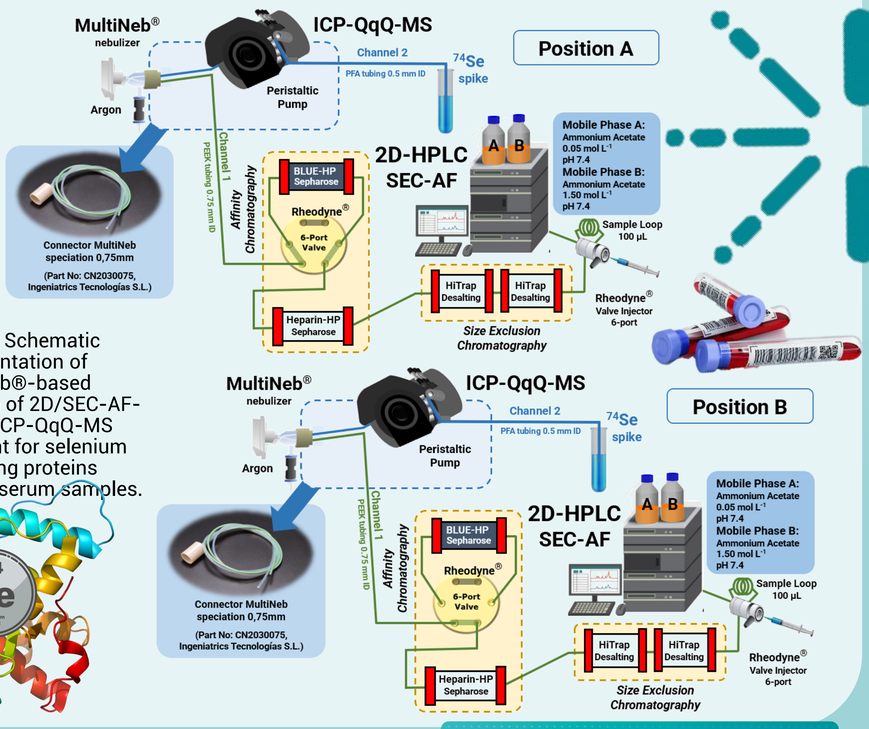
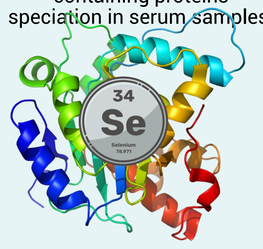


Figure 3. Schematic representation of MultiNeb®-based configuration of 2D/SEC-AF-LC-SUID-ICP-QqQ-MS arrangement for selenium containing proteins speciation in serum samples.



Results and discussion

The development of accurate and precise arsenic speciation procedures, for biological and environmental materials, is a current trend in analytical chemistry. In this sense, the hyphenation of high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) is a very versatile and powerful tool for arsenic speciation analysis.

Precision values were evaluated using different certified reference materials (CRMs) following the procedures for sample preparation previously described. The results obtained are shown in Table 3. In order to validate the method performance in real samples, a spike recovery test was performed using the mixed As species standard solution. The recoveries of AsB, iAsIII, MMA, DMA and iAsV obtained in all cases were in the range of 91-107 %. To evaluate the signal stability along the analysis sequence, a monitoring standard solution containing 5 µg g^{-1} of arsenic was prepared. This solution was analyzed once every five samples, in order to evaluate the stability of the signal. The recoveries must fall within the limits of 96-104 %. Additionally, the stability of retention time was studied. The stability of retention time was achieved in the range of 0.1-2.0 % (short term, n = 5) and 0.4-6.0 % (long term, n= 20) for all the species AsB, iAsIII, MMA, DMA and AsV.

Table 3. Experimental and certified mean values for total arsenic and arsenic species concentrations in the different human biological fluids analyzed using online internal standard calibration. SD obtained for 2 replicates using HPLC-ICP-MS and 5 replicates for ICP-MS (In grey certified values, in green Experimental results).

As Species	LOD	Clinchek Urine Level I Mean ± SD		SRM2670 Level II Mean ± SD		Human Urine Volunteers Mean ± SD
		Experimental values	Certified value[a]	Experimental values	Certified value[b]	Experimental values
Total As (µg L^{-1})	0.12	19.2±0.8	17.0 (13.6-20.3)	504±21	480±100	53.2±4.1
iAs^{III} (µg L^{-1})	0.88	ND	4.55 (2.73-6.37)	ND	ND	ND
iAs^{V} (µg L^{-1})	0.42	3.22±0.26		436±18	443±20	4.22±0.26
AsB (µg L^{-1})	0.29	11.8±0.38	16.8 (12.6-21.0)	16.3±0.41	15±3	38.1±2.6
MMA (µg L^{-1})	0.36	1.10±0.08	2.5 (1.5-3.5)	7.4±0.53	7±1.3	4.12±0.8
DMA (µg L^{-1})	0.21	5.02±0.22	9.8 (5-88-13.7)	43±2.1	49±3	11.3±1.1
Sum As Species (µg L^{-1})		21.1	33.6	503	514	57.7

The proposed speciation method has been validated using some CRM of human serum (BCR-637 and Clinchek Human Serum Level I and II). These materials were additionally spiked with 25 µg kg-1 of inorganic selenium (sodium selenate) to evaluate the recovery and precision of method. Interconversion among selenium species was not observed under detailed experimental condition.

For evaluate the signal stability along the sequence of analysis, a monitoring standard solution (BCR-637) spiked with 25 µg kg-1 of inorganic selenium (sodium selenate) was prepared. This solution was analyzed once every five samples, in order to evaluate the stability of the signal. The recoveries must fall within the limits of 97-106 %. Additionally, the stability of the retention time was studied. The stability of retention time was achieved in the range of 0.2–2.2% (short term, n = 5) and 0.5–4.5% (long term, n=20) for all selenium containing biomolecules.

Se species		Total Se (µg g^{-1})	Sum Species (µg kg^{-1})	eGPx (µg kg^{-1})	Semetabolites (µg kg^{-1})	SeP (µg kg^{-1})	SeAlb (µg kg^{-1})
LOD MultiNeb®-based configuration		0.05	---	0.15	0.37	0.45	0.41
LOD Micromist®-based configuration		0.11	---	0.22	0.61	0.68	0.66
Sample or CRM Description		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Human serum BCR-637	Certified Values	81±7	79±3	15±4	---	60±7	13±4
	MultiNeb®	80±3	79±2	14±2	<LOD	61±3	12±2
	Micromist®	83±6	80±4	16±4	<LOD	63±5	13±4
Human Serum Clinchek II	Reported Values*	105±11	94±4	14±4	---	69±3	9±4
	MultiNeb®	103±3	101±4	13±3	3.0±0.4	73±3	10±2
	Micromist®	101±5	99±4	12±5	2.1±0.4	72±5	11±4
Human Serum volunteers	MultiNeb®	87±2	87±5	11±4	4.2±1	57±4	18±3
	Micromist®	85±6	87±6	12±5	3.9±1	58±6	16±5

Table 4. Experimental and certified mean values for total selenium and selenium species concentrations in the different certified reference materials and human serum samples, as well as the SD obtained for 2 replicates using 2D/SEC-AF-LC-SUID-ICP-QqQ-MS and 3 replicates for IDA-ICP-QqQ-MS using MultiNeb® and Micromist® nebulizers.

Conclusions

In this study, it has been demonstrated that the new MultiNeb® multiple nebulizer presents higher precision, sensitivity, signal stability and reproducibility in comparison with Micromist® nebulizer. The implementation of the multinebulizer for the on-line isotope dilution and internal standard correction provides a significant advantage, 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.

On the other hand, the high-pressure connector designed by Ingeniatrics Tecnologias S.L. present some advantages, such as resists blockage, fast washout, minimize dead volume and peak broadening, principally. In addition, this connector is simple to use, to allow quick, reliable and easy connection of your HPLC to your ICP instrument.

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P.I. Parque Plata · C/ Camino Mozárabe, 41
41900 Camas, Seville (Spain)
(+34)954081214
info@ingeniatrics.com
www.ingeniatrics.com

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Contact:
magarciasevillano@outlook.es