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Development of methods for the quantification of essential and toxic elements in human biomonitoring

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Summary. - Analytical methods using sector field ICP-MS and ICP-AES were developed for the determination of Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn and Zr in cerebrospinal fluid, urine, serum and blood. Sample treatment procedures merging high sample throughput, simplicity and low contamination risk were set up. Method performances were evaluated in terms of detection limits, accuracy and precision. The limits were below 0.05 ng ml^{-1} for all the elements, except for Al (all matrices), Hg (blood), Pb (blood) and Sn (serum and blood). The accuracy varied from 86% to 110% and the precision was always below 6%.

Key words: biomonitoring, biological fluids, elements, ICP-MS, ICP-AES.

Riassunto *(Sviluppo di metodi per la quantificazione di elementi essenziali e tossici nel biomonitoraggio umano).* - Sono stati sviluppati metodi analitici che utilizzano l'ICP-MS a settore magnetico e l'ICP-AES per la determinazione di Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn e Zr nel fluido cerebrospinale, nelle urine, nel siero e nel sangue. Sono state messe a punto procedure di trattamento del campione che combinano alto numero di campioni processati, semplicità e basso rischio di contaminazione. Le prestazioni del metodo sono state valutate in termini di limiti di rivelabilità, accuratezza e precisione. I limiti erano inferiori a 0,05 ng ml^{-1} per tutti gli elementi, eccetto Al (tutte le matrici), Hg (sangue), Pb (sangue) e Sn (siero e sangue). L'accuratezza variava dall'86% al 110% e la precisione risultava sempre inferiore al 6%.

Parole chiave: biomonitoraggio, fluidi biologici, elementi, ICP-MS, ICP-AES.

Introduction

Support for a potential role of metals as environmental determinants in the aetiology of neurodegenerative diseases comes from several studies [1-6]. In particular, some elements such as Al, Cu, Fe, Hg, Mn, Pb and Zn displayed alterations in patients affected by Alzheimer's and Parkinson's diseases and multiple sclerosis [7-13]. In this framework, the assessment of the internal exposure to metals of an individual - through the analysis of fluids of healthy and diseased subjects - is central for establishing metal variations as a function of the neurodegeneration. Up to now, the knowledge of the elemental distribution in the human body is rather partial. In most cases, the extremely low levels of the elements in biological media and the risk of contamination during sample collection and treatment make this kind of evaluation hard to carry out. Further investigations including the development of contamination-free and routine analytical procedures have to be optimized and applied in the context of human biomonitoring, where the testing of a large number of specimens is generally involved.

More and more inductively coupled plasma mass spectrometry (ICP-MS) with its multi-element capability, high sensibility and wide dynamic range is being appreciated for the quantification of several pathologically interesting elements in body fluids [14-18]. However, ICP-MS suffers from several types of spectral and non-spectral interferences originating from the plasma, water and reagents used as well as matrix of the biological samples. For this reason, numerous elements cannot be determined in human fluids with instruments equipped with a quadrupole mass filter (Q-ICP-MS) without an extensive pre-treatment of the samples. Many of these interferences

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can today be overcome by sector field (SF-ICP-MS) instruments operating at higher resolution. Additional advantages of this technique over conventional Q-ICP-MS are the extremely low instrumental background and the higher ion transmission which result in a lower detection power. Another important tool to reduce some specific interferences and to improve ICP-MS sensitivity of one order of magnitude appears to be the use of a shielded torch which reduces the secondary discharges in the interface region and enhances ion transmission.

As concern the treatment of human fluids, the simple dilution of the specimen could be preferred for reducing manipulation and thus exogenous contamination and preparation time. While for serum, urine and cerebrospinal fluid (CSF) the direct analysis after dilution is possible, in the case of blood the plain dilution causes clogging of the sample introduction devices and signal instability. This problem is emphasized when dealing with routine determinations of blood. Several digestion procedures have been reported, including high-pressure ashing in quartz ampoules and microwave (MW) assisted acid digestion in open or closed vessels. This last procedure has become the most popular method because it implies efficient mineralization and reduced contamination risk. Nevertheless, very few MW digestion procedures have been applied to the processing of a large number of samples, mainly due to the low number of digestion containers (generally, max. 10) supported by the rotors and the time-consuming cleaning procedure after each MW cycle.

In this context, aim of this study was: *i)* to exploit pre-treatment procedures for biological fluids combining high sample throughput, procedural simplicity and low contamination risk; *ii)* to test the capability of SF-ICP-MS to determine Al, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Tl, V, W and Zr in human CSF, urine, serum and whole blood at concentrations varying from a few ng l^{-1} to tens of µg l^{-1}; *iii)* to determine the levels of major elements such as Ca, Cu, Fe, Mg, Si and Zn in the selected body fluids by means of inductively coupled plasma atomic emission spectrometry (ICP-AES).

Materials and methods

Instrumentation

A MW oven (Milestone ETHOS MEGA II, FKV, Bergamo, Italy) equipped with a rotor (Milestone MultiPREP 80) designed for the digestion of 80 samples in one cycle was adopted for whole blood mineralization. The system used for trace and ultra-trace elements quantification was a SF-ICP-MS (ELEMENT I model, Thermo Finnigan, Bremen, Germany) operating at a radiofrequency of 1.2 kW - in the low (LR, m/Δm = 300) and in the medium (MR, m/Δm = 3000) resolution mode - and equipped with the guard electrode device, Meinhard glass type nebulizer with water-cooled Scott-type chamber and Pt interface. Argon flow rates were as follows (in l min^{-1}): plasma, 14; auxiliary, 0.9 and nebulizer, 0.85. The isotopes chosen for quantification, the major interferences on the masses and the resolution selected are reported in Table 1. The analysis of Ca, Cu, Fe, Mg, Si and Zn was performed by an ICP-AES using an Optima 3100 XL spectrometer (Perkin Elmer, Norwalk, CT, USA) with the following operational settings: radiofrequency, 1.3 kW; cross-flow nebulizer with Ryton Scott chamber; argon flow rates (in l min^{-1}): plasma, 13.0; auxiliary, 0.5 and nebulizer, 0.7; polychromator equipped with an echelle grating; simultaneous solid-state segmented-array charged-coupled device detector; maximum resolution, 0.006 nm at 200 nm. The spectral lines (nm) used for the analysis were the following: Ca, 393.3; Cu, 324.7; Fe, 259.9; Mg, 279.5; Si, 251.6; Y, 371.0 and Zn, 213.8.

Reagents and standard solutions

Deionized water (EASY-pure, PBI, Milan, Italy) was used for dilution of samples and standards and ultrapure-grade HNO_3 (Carlo Erba, Milan, Italy) for digestions. Single-element standard solutions (SPEX, Edison, NJ, USA) at the concentration of 1 mg ml^{-1} were used to prepare calibrants and internal standards (ISs). For the SF-ICP-MS analysis two ISs were used in the LR mode, namely ^{115}In (1 ng ml^{-1}) for Ba, Be, Cd, Li, Mo, Sb, Sn, Sr and Zr, and ^{195}Pt (1 ng ml^{-1}) for Bi, Hg, Pb, Tl and W. In the MR mode, ^{45}Sc (1 ng ml^{-1}) was chosen as IS for Al, Co, Cr, Mn, Ni and V. In the ICP-AES determinations, Y (500 ng ml^{-1}) was used to normalize the signals of Ca, Cu, Fe, Mg, Si and Zn.

For the accuracy study, Clinchek® Control lyophilized human urine, serum and whole blood (Recipe, Munich, Germany) were used as Certified Reference Materials (CRMs) at the lowest concentration level.

Sample collection

Four kinds of biological samples were tested, i.e., CSF, urine, serum and blood. Before the collection of specimens, subjects fasted overnight. The first millilitres of blood were used to determine haematological parameters. This also permitted to clean the drawing system from inorganic contaminants that could affect the elemental determinations.

Table 1. - Isotopes and resolution (m/Δm) selected in the SF-ICP-MS analysis

Element	m/z	Resolution	Main interferences	IS[(a)]
Al	27	3000	$^{11}B^{16}O$, $^{13}C^{14}N$, $^{54}Fe^{++}$	Sc
Ba	138	300	(b)	In
Be	9	300	(b)	In
Bi	209	300	(b)	Pt
Cd	114	300 and MC[(c)]	$^{98}Mo^{16}O$, ^{114}Sn	In
Co	59	3000	$^{40}Ar^{19}F$, $^{43}Ca^{16}O$, $^{41}K^{18}O$, $^{42}Ca^{16}O^{1}H$	Sc
Cr	52	3000	$^{40}Ar^{12}C$, $^{36}Ar^{16}O$, $^{38}Ar^{14}N$, $^{35}Cl^{17}O$, $^{37}Cl^{15}N$, $^{35}Cl^{16}O^{1}H$	Sc
Hg	202	300	(b)	Pt
Li	7	300	(b)	In
Mn	55	3000	$^{37}Cl^{18}O$, $^{40}Ar^{15}N$, $^{39}K^{16}O$, $^{40}Ar^{14}N^{1}H$	Sc
Mo	100	300	(b)	In
Ni	60	3000	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{36}Ar^{24}Mg$ $^{120}Sn^{++}$	Sc
Pb	208	300	(b)	Pt
Sb	121	300	(b)	In
Sn	120	300	(b)	In
Sr	88	300	(b)	In
Tl	205	300	(b)	Pt
V	51	3000	$^{35}Cl^{16}O$, $^{37}Cl^{14}N$, $^{40}Ar^{11}B$, $^{36}Ar^{14}N^{1}H$	Sc
W	184	300	(b)	Pt
Zr	90	300	(b)	In

(a): IS, internal standard; *(b)*: no relevant interference; *(c)*: MC, use of mathematical corrections; SF-ICP-MS: sector field inductively coupled plasma mass spectrometry.

Polystyrene tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) were used to collect 15 ml of whole blood and aliquots of 1 ml were immediately stored at -20 °C until they were mineralised. The remaining blood was centrifuged at 1500 rpm for 15 min to obtain serum which was transferred in polystyrene tubes and kept at -20 °C until analysis. Morning spot urine were collected in high-density polyethylene bottles (Kartell, Milan, Italy) and added with ultrapure HNO_3 (1%, v/v) for stabilization purposes. In order to minimize the contamination from collection devices, all containers were thoroughly cleaned with a mixture of 10% (v/v) of HNO_3 followed by final rinses with deionized water. Another possible source of exogenous metals was avoided by eliminating the use of anticoagulants.

Sample preparation and calibration

Blood samples were added of 2 ml of HNO_3 in the same polystyrene tubes in which they have been previously collected and then MW digested at atmospheric pressure in accordance with the following procedure: *i)* overnight pre-digestion at room temperature; *ii)* enhancement of temperature up to 80 °C within 1 hr; *iii)* digestion at 80 °C for 5 hrs. To control the digestion conditions an optical fiber temperature probe was placed in one of the tubes filled with the same reaction mixture. After cooling, the digested solutions of blood were water-diluted up to 15 ml in the same reaction tubes [19].

Aliquots of 1 ml of CSF, urine and serum were made up to 5 ml or to 30 ml (Ca and Mg in all matrices; Cu, Fe and Zn in serum; Si in urine and serum) with water including the addition of the ISs. All treatment procedures were performed in a Class 100 clean room (Tamco, Rome, Italy). Blanks and CRMs were subjected to the same treatment as the samples. For the elements not certified in the CRMs, the accuracy was evaluated by recovery tests spiking adequate amounts of the elements on samples. Method precision was checked by measurements of 10 independent real samples, i.e., water-diluted CSF, urine and serum as well as digested solutions of blood. Standard addition procedure was used for calibration by adding standard solutions in pooled sample of each specimen.

Results and discussion

Pre-treatment procedures

In the analysis of trace elements in biological matrices the following crucial requirements should be fulfill: *i)* the reduction of sample preparation time; *ii)* the lowering of blanks values; *iii)* the control of the deposition of solids in the sample introduction devices; *iv)* the minimization of saline matrix influence on the analytical signals; *v)* the capability of detection of elements present at ultra-trace level.

As concern CSF, urine and serum treatment, the selected dilution factor 1+5 (v+v) appeared to be an adequate compromise to satisfy the aforesaid necessities. On the other hand, in the whole blood analysis the rapid blockage of the torch injector and pneumatic nebulizer and the subsequent signal instability were overcome by the MW destruction of the organic constituents. The MW field applied allowed to not exceed a decomposition temperature of 80 °C, i.e., below both the boiling point of the reaction mixture and the crack temperature of the plastic tubes. Moreover, the initial ramp to reach 80 °C was set rather slow to prevent overheating of the mixture as a consequence of the massive exothermic oxidation reactions. For this reason, an overnight pre-digestion step was also performed before the absorption of the MW energy. With this approach, homogeneous and low viscous blood solutions were produced, combining, in the same time, low contamination risk, high sample throughput and ease of operation. The analysis of samples directly in the same collection and digestion containers minimized the handling and allowed to obtain lower blanks. Moreover, the high sample throughput was achieved by the use of a multiple-batch rotor that made possible to digest a large number of samples simultaneously. Simplicity of operation was attained by the use of disposable and screw-cap tubes for sample digestion instead of commonly used teflon vessels.

Anyway, the subtraction of the blank signal and the use of ISs covering all the mass range and standard addition approach were sufficient for controlling reagents impurities, instrumental drifts and matrix effects. Moreover, memory effects that may led to positive errors during the analytical sequence were reduced by deeply rinsing the system between consecutive samples.

Interferences study

In the SF-ICP-MS analysis the quantification of the elements under study could be hampered by spectral interferences as listed in Table 1. The determination of Ba, Be, Bi, Hg, Li, Pb, Sb, Sn, Sr, Tl, W and Zr was not troubled by any significant spectral interference at the mass of their most abundant isotope and their determination was accomplished in LR mode without any correction. Only the Mo was determined at mass 100 (less abundant isotope) instead of mass 98, because the signal of this isotope was affected by the not-negligible contribution of $^{40}Ar^{58}Fe$ due to the high concentration of Fe in the matrices, i.e., about 1,500 and 500,000 ng ml^{-1} in serum and blood, respectively. On the other hand, the isotopes of Al, Co, Cr, Mn, Ni and V - affected by isobaric interfering species and double charged or polyatomic ions formed in the plasma - were quantified in MR mode that allowed a physical shift of the interfering peak. For these elements again the most abundant isotope was selected with the single exception for Ni, for which the quantification at the mass 58 was hampered by the isobaric overlapping of ^{58}Fe. Moreover, the mathematical corrections to the outcomes of the LR measurement were employed in the determination of ^{114}Cd (the most abundant isotope). In fact, the interference species ^{114}Sn and $^{98}Mo^{16}O$ could not be separated from Cd even in the high resolution mode. Moreover, during the daily instrument optimization, special attention was paid to reduce the formation of oxide species and to maintain them lower than 0.001, as BaO^+/Ba^+ ratio.

Table 2. - Limits of detection (ng ml^{-1}) for SF-ICP-MS determination of elements in different matrices

Element	CSF	Urine	Serum	Blood
Al	0.10	0.25	0.50	0.90
Ba	0.01	0.02	0.03	0.02
Be	0.01	0.02	0.02	0.03
Bi	0.001	0.001	0.002	0.002
Cd	0.001	0.007	0.01	0.03
Co	0.002	0.008	0.01	0.01
Cr	0.01	0.02	0.02	0.07
Hg	0.01	0.04	0.04	0.15
Li	0.007	0.008	0.008	0.02
Mn	0.004	0.01	0.03	0.05
Mo	0.005	0.01	0.04	0.05
Ni	0.03	0.03	0.05	0.04
Pb	0.01	0.01	0.01	0.32
Sb	0.002	0.003	0.003	0.007
Sn	0.03	0.04	0.06	0.08
Sr	0.01	0.02	0.05	0.06
Tl	0.001	0.002	0.003	0.003
V	0.001	0.002	0.003	0.003
W	0.001	0.002	0.002	0.003
Zr	0.002	0.002	0.004	0.006

CSF: cerebrospinal fluid; SF-ICP-MS: sector field inductively coupled plasma mass spectrometry.

Table 3. - Accuracy and recovery data (mean ± SD, in ng ml^{-1}) for SF-ICP-MS determination of elements

	CSF		Urine		Serum		Blood	
Element	**Certified**	**Found**	**Certified**	**Found**	**Certified**	**Found**	**Certified**	**Found**
Al	2.5*(a)*	2.46 ± 0.12	129.5 ± 36.1	122 ± 5.0	20 ± 5	19.8 ± 0.4	10*(a)*	9.20 ± 0.31
Ba	1.0*(a)*	0.86 ± 0.08	2.5*(a)*	2.43 ± 0.15	0.5*(a)*	0.46 ± 0.01	1.0*(a)*	0.87 ± 0.04
Be	0.2*(a)*	0.19 ± 0.01	0.5*(a)*	0.45 ± 0.12	0.5*(a)*	0.47 ± 0.02	0.5*(a)*	0.48 ± 0.03
Bi	0.1*(a)*	0.095 ± 0.005	1.0*(a)*	0.94 ± 0.14	1.2 ± 0.3	1.14 ± 0.09	0.05*(a)*	0.044 ± 0.003
Cd	0.1*(a)*	0.098 ± 0.008	11.8 ± 2.5	11.3 ± 0.6	0.22 ± 0.06	0.21 ± 0.02	1.2 ± 0.4	1.32 ± 0.09
Co	0.1*(a)*	0.094 ± 0.009	6.7 ± 1.6	6.4 ± 0.5	1.0 ± 0.3	0.96 ± 0.05	1.6 ± 0.5	1.68 ± 0.11
Cr	0.5*(a)*	0.48 ± 0.02	14.6 ± 3.0	14.5 ± 0.5	1.2 ± 0.3	1.11 ± 0.08	2.0 ± 0.5	1.98 ± 0.15
Hg	0.5*(a)*	0.55 ± 0.02	8.2 ± 2.5	8.6 ± 1.3	0.27 ± 0.07	0.25 ± 0.03	2.9 ± 1.0	2.92 ± 0.11
Li	1.0*(a)*	1.06 ± 0.09	1.0*(a)*	1.07 ± 0.14	1.6 ± 0.4	1.56 ± 0.11	1.0*(a)*	1.10 ± 0.08
Mn	0.5*(a)*	0.45 ± 0.08	7.1 ± 1.7	6.9 ± 0.2	1.4 ± 0.3	1.46 ± 0.11	23.8 ± 4.7	23.7 ± 1.5
Mo	1.0*(a)*	0.98 ± 0.05	1.0*(a)*	0.93 ± 0.12	3.3 ± 0.8	3.12 ± 0.18	1.0*(a)*	0.98 ± 0.04
Ni	2.5*(a)*	2.60 ± 0.15	11.9 ± 3.0	11.0 ± 0.8	1.4 ± 0.3	1.50 ± 0.10	7.5 ± 1.8	8.1 ± 0.6
Pb	0.5*(a)*	0.49 ± 0.02	41.0 ± 9.9	39.8 ± 1.0	0.5*(a)*	0.46 ± 0.04	105 ± 24	107 ± 5
Sb	0.5*(a)*	0.43 ± 0.09	10.9 ± 2.5	10.4 ± 1.4	0.5*(a)*	0.53 ± 0.03	0.5*(a)*	0.52 ± 0.01
Sn	1.0*(a)*	0.92 ± 0.11	1.0*(a)*	0.87 ± 0.15	0.5*(a)*	0.47 ± 0.02	1.0*(a)*	0.95 ± 0.04
Sr	10*(a)*	9.75 ± 0.80	10*(a)*	9.85 ± 1.50	10*(a)*	9.70 ± 0.31	10*(a)*	9.82 ± 0.50
Tl	0.05*(a)*	0.043 ± 0.002	3.5 ± 1.3	3.2 ± 0.5	0.83 ± 0.21	0.81 ± 0.07	0.05*(a)*	0.046 ± 0.003
V	0.1*(a)*	0.096 ± 0.002	1.0*(a)*	0.91 ± 0.11	0.05*(a)*	0.047 ± 0.002	0.05*(a)*	0.053 ± 0.003
W	0.05*(a)*	0.048 ± 0.005	0.05*(a)*	0.043 ± 0.005	0.05*(a)*	0.052 ± 0.003	0.05*(a)*	0.044 ± 0.002
Zr	0.5*(a)*	0.47 ± 0.08	0.5*(a)*	0.48 ± 0.06	0.5*(a)*	0.51 ± 0.03	0.5*(a)*	0.54 ± 0.02

(a): amount spiked on real samples; CSF: cerebrospinal fluid; SF-ICP-MS: sector field inductively coupled plasma mass spectrometry.

Analytical performances of SF-ICP-MS

In Table 2 the Limits of Detection (LoDs) in the four matrices are reported, calculated on the 3σ criterion and considering the dilution factors. For most of the elements these limits were below 0.05 ng ml^{-1}, with the exception of Al (0.1, 0.25, 0.5 and 0.9 ng ml^{-1} in CSF, urine, serum and blood, respectively), Hg (0.15 ng ml^{-1} in blood), Pb (0.32 ng ml^{-1} in blood) and Sn (0.06 and 0.08 ng ml^{-1} in serum and blood, respectively). In general, these values resulted to be from 5 to 10 times lower than the minimum concentration encountered in the real samples, with the exception of Ni in serum and Co in blood for which the limits were relatively close to the minimum of the experimental range in the samples. Comparing LoDs of Al, Cd, Co, Cr, Mn, Mo and Pb in serum with those obtained by Muñiz *et al*. [20] in the same matrix 1+4 water-diluted, they completely agreed. On the contrary, values obtained by Schramel and Wendler [21] for Al, Co, Cr and Mn in urine and serum digested by ultraviolet radiation and blood by pressure ashing were higher than those found in this work. As regards Ni, the use of Pt-cones permitted a 25-fold improvement of the LoDs when compared with those reported by other authors obtained with Ni-cones [21, 22]. For elements such as Ba, Be, Bi, Hg, Sb, Sn, Sr, Tl and V the LoDs found in serum, blood and urine were fully comparable with those reported by Krachler *et al.* in MW digested body fluids [17]. No literature data were reported for LoDs in the CSF elements determination. The assessment of the accuracy (Table 3) was carried out by using the CRMs for those elements with available certified values - only eight elements in urine, eleven in serum and seven in blood - and by recovery tests for not-certified analytes. As concerns CSF, due to the lack of CRMs only the recovery tests were carried out. As follows from these data, the accuracy ranged from 86% (Ba, Sb and Tl) to 110% (Hg) in CSF; from 87% (Sn and W) to 107% (Li) in urine; from 92.5% (Ba, Cr, Hg and Pb) to 107% (Ni) in serum, and from 87% (Ba) to 110% (Cd and Li) in blood. The precision of the method covered the interval from 2.3% for Mn to 4.8% for Al in urine, from 1.9% for Cr to 3.8% for Mn in serum, from 1.2% for Pb to 4.3% for Ni in blood and from 2.1% for Hg to 5.2% for V in CSF.

Analytical performances of ICP-AES

The spectroscopy emission technique applied to the determination of Ca, Cu, Fe, Mg, Si and Zn in biological samples was fully satisfactory for the purposes of the present investigation. In particular: *i)* the LoDs were in general several orders of magnitude lower than the levels found in the actual samples; *ii)* the accuracy in urine CRM ranged from 92% for Zn to 102% for Cu; in serum CRM it ranged from 98% for Zn to 107% for Cu; in blood CRM it was from 91% to 105% for Cu and Zn; *iii)* recoveries on spiked CSF were between 95% (Cu) and 101% (Ca); *iv)* the precision of the method varied from 2.9% (Cu, in urine) to 5.3% (Si, in serum).

Conclusions

The use of SF-ICP-MS combined with ICP-AES allowed to accurately and precisely determine twenty-six major, trace and ultra trace elements in CSF, urine, serum and whole blood. A great number of interferences usually encountered in the ICP-MS analysis of biological matrices was successfully eliminated through the improvement of resolution or the application of mathematical corrections. The clean, high-throughput and easy pre-treatment procedures here optimized appeared to be fully adequate for biological monitoring studies. These results make confident about the ability of the developed analytical procedures to monitor neurological diseases groups and ascertain their eventual metal unbalances.

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