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Research Article

Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples

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The use of normalized procedures designed for soil and sediment samples (like US-EPA 3051) to chemically prepare some kind of organic samples is a common practice in some laboratories. However, the performance of this method for other matrices has to be demonstrated. Three microwave-assisted digestion procedures with 0.5 g of sample and simplified reagents (10 mL HNO_3 alone and mixtures of HNO_3/HCl- and HNO_3/H_2O_2 procedures A, B, and C, resp.) were compared for quantitative determination of 25 elements (Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Pb, Th and U) in three biological reference materials provided by NIST (mussel tissue (MT), tomato leaves (TL), and milk powder (MP)) by ICP-MS. From scaling masses (from 0.1 up to 0.9 g at 0.1 g interval) in procedure A, a linear relationship among instrumental signal and mass of digested sample could be constructed at 99% CL for most of the target analytes. The slope of this linear fit provided the estimation of sample concentration, while the ordinate in origin allowed the identification of matrix interferences which were absent in the reagent blank.

1. Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) is a robust and widely used technique for multielemental and isotopic analysis of environmental materials [1–3] that has shown clear advantages when compared with other analytical techniques such as inductively coupled plasma atomic emission spectrometry (ICP-AES) [4–6], flame atomic absorption spectrometry (F-AAS), and electrothermal atomic absorption spectrometry (ET-AAS) [7, 8]. The basic setup for ICP-MS analysis requires the sample introduction as a liquid solution and thus, for solid matrices, an acid digestion procedure becomes mandatory.

Sample digestion is mainly carried out by a fusion or a wet procedure based on an acid digestion with a heated mixture of mineral acids [2, 9–13]. In general, closed digestion systems are to be preferred to minimize possible contamination of the digest, increase reproducibility, and avoid losses of volatile elements [14–17]. Wet microwave digestion equipped with temperature and pressure control assisted by common mineral acids, such as nitric, sulphuric, perchloric and hydrochloric acids, is frequently used for sample digestion [18].

In order to dissolve the silicates and eliminate the effects of silica gel in environmental samples, hydrofluoric and orthoboric acids are usually used, although they can produce unsatisfactory recoveries in volatile elements [19, 20]. A mixture of nitric acid and hydrogen peroxide is widely employed because they mineralise organic matter effectively and produce less spectral interference in ICP analyses [1]. Nitric acid has been reported to be strong enough to solubilize metals

from fly ashes [21], from soils with organic carbon content up to 38% [22], and from plant materials for environmental monitoring [23]. However, in general, plant samples require a more complete decomposition procedure due to the presence of high organic and/or silicon contents [24].

The use of normalized procedures for soil and sediment samples to prepare some kind of organic sample is a common practice. The US-EPA 3051 [25] proposes to use a representative amount of sample of 0.5 g digested with 10 mL of concentrated nitric acid. For some cases this method also proposes the use of the same amount of sample digested in a mixture of 9 mL of concentrated nitric acid and 3 mL of concentrated hydrochloric acid. In the US-EPA 3052 [26] method, a representative sample of up to 0.5 g is digested in 9 mL of concentrated nitric acid, and usually 3 mL hydrofluoric acid, although the method has provisions for scaling the sample size up to a maximum of 1.0 g and enables the analyst to select other decomposition reagents. The use of hydrofluoric acid requires strict safety procedures, and it can damage the glass components during instrumental analysis if its excess was not previously removed from the samples. Apart from the normalized US-EPA methods, other digestion methods can be extracted from the specialized literature and used with particular samples [27]. Thus, in this work, we will study the performance of methods based on the use of just nitric acid compared with those using a combination of nitric acid and hydrochloric acid or hydrogen peroxide. These options were chosen on the basis of widespread usage and seemingly minimal contribution to ICP-MS spectral interferences.

In this work, we will be concerned with the analysis of biological samples with different characteristics to show the versatility of the procedures, although this is far away from being a universal procedure due to the limited number of certified materials used. The acid digestion methods proposed in the literature are used in the preparation of soil, sediments, and other complex matrices for a limited number of elements. The US-EPA methods pursuit the acid digestion and allow the quantification of up to 26 elements (Al, Sb, As, B, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, Mo, Ni, K, Se, Ag, Na, Sr, Th, V, and Zn). The performance of this method for other analytes and/or matrices has to be demonstrated. Instrumental developments in both, ICP-MS and microwave digestion systems, and the need of limiting the use of hazardous acids lead to an increasing interest in the improvement and updating of sample digestion methods [1, 2, 28–32]. Until now, standard methods for soils and/or sediments have been directly applied to the preparation of organic samples. In this work, we will try to justify the use of those methods for organic samples and, depending on the results obtained, determine another alternative method which could give better results. For that reason, we have made a digestion procedure using only nitric acid (similar to the US-EPA 3051 method established for sediments, sludges, soils, and oils) but increasing the sample mass to acid volume ratio. Also, digestion procedures based on the use of a combination of nitric acid and hydrochloric acid or hydrogen peroxide have been checked.

The effect of microwave digestions with different reagents will be tested on 25 different elements determined through the ICP-MS technique in three different biological standard reference materials (SRMs) provided by the National Institute of Standards and Technology (NIST). The amount of sample will be scaled from 0.1 up to 0.9 g using just nitric acid. The study of the instrumental signal versus the sample mass will enable a quality test, when a good linearity is found, and will allow the determination of analyte concentration in the sample from the slope. Furthermore, an ordinate in origin different from zero at a fixed confidence level will serve to identify matrix effects noncompensated by a background subtraction based upon conventional digestion (reagents) blanks. Comparison between direct determination of concentrations based on dilution factors and the corresponding determined from the linear fit will serve to identify the mass distribution with unacceptable results. This, along with the study of relative uncertainties, will allow the construction of a figure of merit to find out the most suitable sample amount in the digest. Finally, a comparative study of three different digestion procedures applied to the SRM samples will be accomplished.

2. Experimental

2.1. Sample Materials and Reagents. In this work, the following biological standard reference materials (SRMs) provided by the National Institute of Standards and Technology (NIST) were used: SRM 1549 nonfat milk powder (referred hereafter as MP) [33], SRM 2976 mussel tissue (referred hereafter as MT) [34], and SRM 1573a tomato leaves (referred hereafter as TL) [35]. SRM 1549 was prepared by NIST to provide assistance in overcoming the difficulties in accurate trace and ultratrace levels analyses of food and other biological important materials (some of its certified elements have concentrations below 0.01 mg kg^{-1}), and its certified major constituents with concentrations above 1% (dry mass basis) are calcium, chlorine, phosphorus, and potassium. SRM 2976 were collected by the International Atomic Energy Agency (IAEA) from the Mediterranean coast of France as part of an effort to investigate metal speciation in the marine environment and its major constituents with concentrations above 1% (dry mass basis) are chlorine and sodium. Finally, SRM 1573a were obtained by the NIST from plants at the Horticultural Research Farm at Rock Springs, PA (USA). It was produced to evaluate the analyses of some elements in botanical materials and agricultural food products, and its certified major constituents with concentrations above 1% (dry mass basis) are calcium, nitrogen and potassium.

All reagents used for the microwave-assisted digestions, that is, hydrochloric acid (36% HCl), nitric acid (69% HNO_3) and hydrogen peroxide (30% H_2O_2), were of suprapur grade (Merck, Darmstadt, Germany). High-purity water (18 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, USA) was used for dilution of the standards, for preparing samples throughout the chemical process, and for final rinsing of the acid-cleaned vessels, glasses, and plastic utensils.

Before use, all glass and plastic utensils were thoroughly acid cleaned and then rinsed with Milli-Q water. Moreover, prior to the use of the tetrafluoroethylene (PTFE-TFM) vessels, the following cleaning procedure was carried out: 10 mL of concentrated HNO_3 was added to each vessel, and, once

Table 1: Instrumental settings and calibration for ICP-MS.

ICP-MS instrument	
Forward power	1300 W
Sampler and skimmer cones	Nickel
Argon flow rates	
Cool gas	14.5 L min^{-1}
Auxiliary	0.76 L min^{-1}
Nebuliser	0.92 L min^{-1}
Acquisition parameters	
Ion monitoring mode	
Number of sweeps	60
Channels per mass	1
Dwell time	10 ms
Number of main runs	3
Survey mode	
Cannels per mass	10
Sweeps	10
Dwell time	0.6 ms
Internal standards	^{6}Li, ^{45}Sc, ^{115}In, ^{159}Tb, ^{209}Bi

closed, the temperature was raised to 180°C within 15 min and held at this temperature for 10 min. After cooling, the content of the vessels were discarded; PTFE-TFM vessels were soaked overnight with diluted HNO_3 and then were rinsed with double deionised water.

The external calibration solutions must include known concentrations of each target analyte. They were prepared from standard certified elemental solutions (Cromlab) and Milli-Q water containing 1% HNO_3 to get a range of concentrations: 0.5, 2.5, 5.0, 25.0, 50.0, and 250 μg L^{-1} (for all elements except for Se, which were fivefold higher). A blank solution consisting in Milli-Q water containing 1% HNO_3 completed the calibration curve (counts versus μg L^{-1}) for each analyte.

The nonspectral matrix effects associated to the ICP-MS measurements were resolved by the addition of internal standards. The standard solution was prepared by diluting single-elemental stock solutions with Milli-Q water containing 1% HNO_3 up to get 50 μg L^{-1} of indium, terbium and bismuth, 500 μg L^{-1} scandium and 1000 μg L^{-1} lithium.

2.2. Analytical Instrumentation. An inductively coupled plasma mass spectrometry system Thermo Elemental ICP-MS X7 (Thermo Fisher, Cambridge, UK) with quadrupole mass analyzer, multichannel detector (Pulse Counting and Analog Methods), auto sampler ASX-500 (CETAC, Omaha, NE, USA), and software Plasma Lab version v4.5 was used for this work. The instrument, located at the Servicio de Investigación Agraria laboratory (University of Seville, Spain), was used with a concentric Meinhard type glass nebulizer, a silica impact bead spray chamber, cooled to 3°C by a Peltier cooler, and a standard silica torch. Standard nickel sample and skimmer cones were used. The internal standard solution was added online by a "Y" connection in the pipe where the sample is aspired by the peristaltic pump.

Table 2: Equations used for the correction of isobaric and polyatomic interferences[#].

Element	m/z	Correction equation
V	51	(1) ^{51}V: $-0.352\,^{52}$Cr $- 3.127\,^{53}$Cr
As	75	(2) ^{75}As: $-0.031\,^{82}$Kr $- 3.1322\,^{77}$ArCl
Se	82	(3) ^{82}Se: $-1.001\,^{83}$Kr
Cd	111	(4) ^{111}Cd: $-0.764\,^{106}$Cd $- 1.073\,^{108}$Cd
Pb	208	(5) ^{208}Pb: $1.00\,^{206}$Pb + $1.00\,^{207}$Pb

[#]With the recommended coefficients for the X-series ICP-MS instruments.

The ion optics were tuned to optimise the sensitivity of the signal at m/z 9, 59, 115, 137, 140, and 238 for a 100 mg L^{-1} beryllium, cobalt, indium, barium, cerium, and uranium solution, respectively, which was typically 10000–60000 counts s^{-1} in standard mode. The relative standard deviation of isotopes signals was less than 5%. The oxide and double charged levels were both monitored to ensure that the $^{140}Ce^{+}/^{140}Ce^{16}O^{+}$ and $^{137}Ba^{+}/^{137}Ba^{++}$ ratios did not exceed 2% and 5%, respectively. Instrumental performance optimization, including nebulizer gas flow rate, RF power, and ion lens voltages, was performed and operational conditions are described in Table 1.

The US-EPA 200.8 method [36] is the routine analytical method implemented in our lab. In its original version, the method is applied for 21 elements, but in this work, we included B, Ti, Fe, Sr, and Cs and excluded mercury, since this last element requires a separate procedure due to its important memory effects. Thus, this was the list of 25 target analytes: Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Pb, Th, and U.

Although there are recommended targets m/z in the US-EPA 200.8 method, usually several isotopes are monitored for each element. This provides helpful information for the analyst to properly interpret and quantify the acquired data. Thus, 48 m/z have been monitored in the main run mode, and spectra were acquired in survey mode for each measured sample. These spectra will serve to check interference effects. Finally, the undesirable effects of isobaric and polyatomic interferences were corrected using the interference correction equations given in Table 2.

For closed-vessel digestions, a microwave system Multiwave 3000 (Anton Paar, Graz, Austria) with rotor HF100 and software version v1.52 was used. The system was equipped with 16 high-pressure PTFE-TFM vessels with an internal volume of 100 mL (maximum pressure and temperature of 40 bars and 240°C, resp.).

2.3. Microwave-Assisted Digestion Procedures. Three digestion procedures using different reagents were tested for digestion of standard reference biological materials: procedure A, assisted by HNO_3; procedure B, assisted by HNO_3 and HCl; and procedure C, assisted by HNO_3 and H_2O_2. For the previously mentioned procedures, approximately 0.5 g of sample were weighted directly into the PTFE-TFM vessels, to which the reagents were added (10 mL HNO_3 for procedure A, 10 mL HNO_3 + 3 mL HCl for procedure B, and 10 mL HNO_3 + 3 mL H_2O_2 for procedure C) and the vessels

were closed immediately. The operational conditions and the heating program used were carried out according to these conditions: a ramp time of 25 min to reach 200°C and a hold time of 25 min at 200°C. After cooling the vessels to room temperature, they were vented and opened. In that moment, Milli-Q water was added to the vessels and they were closed and shaken thoroughly to dilute any possible rest of colloids attached in the vessels' walls. This process was repeated three times. The resultant mixture was filtered with a 20–25 μm diameter pore filter 110 mm diameter (Whatman) and diluted to 100 mL in a volumetric flask with Milli-Q water. To accomplish the TDS requirements for sample introduction in our ICP-MS, a further dilution of 1.2 mL (1.0 mL for procedure C) of the previous digested solution to 10 mL of Milli-Q water with 1% suprapure HNO_3 was needed. Triplicate samples of the three reference materials were prepared by each digestion method.

The digestion procedure A was then modified, keeping constant the acid volume (10 mL HNO_3) and scaling the sample mass from 0.1 up to 0.9 g at 0.1 g intervals. Triplicate samples of the three reference materials were prepared by this digestion method using the same microwave conditions previously described, and being then subject to the same dilution factor.

Matrix spike samples were prepared in duplicate following the US-EPA 200.8 procedure for the three biological matrices for a final extra concentration of 5 $\mu g\,L^{-1}$ for all the analytes (25 $\mu g\,L^{-1}$ for Se). Triplicate digested reagent blank solutions for each digestion procedure were analyzed for determination of the method detection limit (MDL) [2, 37]. These reagent blank solutions were ascribed for background correction in the postexperiment analysis.

2.4. Statistical Analysis. Each individual sample was measured with three main acquisition runs during the experiment, providing mean values and standard deviation. Results reported in this work will refer to the mean value and standard deviation of the three replicates of each organic matrix and digestion method.

The general linear model procedure in Statgraphics Plus 5.1 (StatPoint 2000) was used. This software was also used for regressions analyses.

3. Results and Discussion

3.1. Recovery of Internal Standard and Quality Controls. Recovery of internal standards decreased monotonically throughout the experiment up to ~70% of their initial values for ^{115}In, ^{159}Tb, and ^{209}Bi and up to 76% for ^{45}Sc, being this an usual behaviour. The recovery of ^{6}Li increased throughout the experiment to reach approximately a 140% of its initial value (in TL sample). This isotope can be naturally present in the samples, and it can be interfered by $^{12}C^{2+}$. Accordingly, ^{6}Li was removed as internal standard in the postexperiment analysis. All target analytes were ascribed to the interpolation mode except Al, Be, B, and Ti, which were directly ascribed to ^{45}Sc.

The calibration curve for all the isotopes showed a good linearity over the whole range of concentrations, with correlation coefficients higher than 0.999 except for Fe and Al, which showed some deviations for low concentrations. Results for Al are reported, but they have to be handled carefully since some of the measured concentrations (~550 $\mu g\,L^{-1}$) were beyond the range of the calibration curve (0.5–250 $\mu g\,L^{-1}$).

Quality control (QC) samples included external calibration verification (ICV), initial and continuous blank verification, and matrix spike samples (MXP). All target analytes passed all QC, but the m/z = 66 (Zn) failed the ICV test for 5 $\mu g\,L^{-1}$, although it showed a good behaviour for higher concentrations, in the range of the certified target values.

3.2. Reagent Blanks and Method Detection Limit (MDL). Detection limits for each digestion procedure (MDL) were determined from reagent blanks by using the US-EPA 200.8 definitions. Each reagent blank was prepared by using the same volume and acid combinations, and following the same experimental procedure used to prepare the real samples. Results are shown in Table 3. They are reported for the three digestion procedures which used 0.5 g of sample and referred to concentration in original sample (mg kg^{-1}). For the variations made in procedure A with different sample amount, m_i (g), the corresponding MDLs can be obtained by correcting those from procedure A by a factor $0.5/m_i$. The use of correction equations may affect the MDL, as shown in Table 3. The MDLs reported are about one order of magnitude higher than those reported by Sucharová and Suchara [1] because the dilution factor was also one order of magnitude higher in our case. For most of the elements, the choice of the digestion procedure had little influence on the MDL, in agreement with previous results [2].

When reagent blanks were treated as unknown samples with subtraction of the calibration blank, only Al (2.4–5.5 $\mu g\,L^{-1}$) and Cd (~0.03 $\mu g\,L^{-1}$) could be quantified over the instrument detection limits. The first one is an airborne pollutant, and the second one is likely linked to some cross-contamination coming from previous tracing studies carried out in our lab.

3.3. Procedure A with Scaling Masses: Linear Regression Method. Concentrations in the digests were determined for each m/z ratio in each target analyte by using the corresponding calibration curve from calibration standards and the measured signal (after subtracting the corresponding reagent blank). Then, a mean value and a standard deviation of mean value were obtained from the three analytical replicates. Results were then plotted against the respective digested masses. Figures 1(a) and 1(b) show examples for a selected group of elements measured in MT. Data followed a linear relationship. Linear regression analysis provided the slope and ordinate in the origin (with their corresponding uncertainties) and the correlation coefficient. Concentrations below the detection limit in the digest were excluded from the analysis, applying the regression fit to the remaining data point.

Only in few cases the linear relationship did not hold (a confidence level below 90% was used as criteria for rejection),

and the corresponding elements were discarded for quantification (see Tables 4(a), 4(b), and 4(c)). This usually happened for those analytes whose concentrations were below the detection limit for all or most of the masses (e.g., Ag or Cs in MT). The element ^{47}Ti was interfered by ^{40}Ar + ^{7}Li, being this last added as internal standard.

For most of the m/z ratios, the linear relationship held at 99% CL (Table 4), with correlation coefficients over 0.97. Results are summarised in Tables 4(a), 4(b) and 4(c), where only one isotope (the one showing the best behaviour) has been selected for each target element.

There were two groups of elements regarding the ordinate in origin: those elements with an ordinate in origin compatible with zero value within 99% CL and those ones with a value being different from zero at the same CL. Figures 1(a) and 1(b) show some examples for MT (noncompatible with zero and compatible with zero at 99% CL, resp.). The physical meaning of the slope is just the contribution to the instrumental signal (provided as μg L^{-1} in the aspired solution) per unit mass of original organic sample. Thus, in both cases, the concentration of the target analyte in the organic sample can be obtained from the slope and the common dilution factor used for all samples (results are shown in Tables 4(a), 4(b), and 4(c), with associated uncertainties arising from the error in the slope). The comparison of these values with certified/reference ones is also provided in Tables 4(a), 4(b), and 4(c), and it will be discussed further.

The physical meaning of the ordinate in origin is just a contribution to the signal coming from matrix effects which are not present in the reagent blanks. Thus, these last include contributions from impurities in the reagent acids and water, as well as contamination throughout the analytical procedure. When a certain mass of organic sample is digested, even if the target analyte was absent, isobaric, polyatomic, and physical interferences could account to the background signal. It is worth to note that a direct quantification of the analyte using the total registered signal (corrected by reagent blank) and the dilution factor could misestimate the concentration value. A nonzero ordinate in origin could be likely lessened by using interference correction equations. Tables 4(a), 4(b), and 4(c) incorporate information of those elements showing nonzero ordinate in origin, and a brief discussion of the most relevant cases is presented in what follows.

Isotope ^{51}V has a negative ordinate in origin when using the correction equation (Table 2) which becomes positive when omitting it. This is related with the nonfully adapted (to instrument and matrix) values of the parameters in the correction equation. The slope was not affected by the use of the correction equation. A similar situation was found for ^{75}As. Isotope ^{52}Cr was used for element quantification (as recommended in the US-EPA 200.8 method), and its background was likely contributed by C and Ca in the matrix. Similarly, polyatomic interferences of ^{1}H + ^{59}Co and ^{1}H + ^{64}Zn could contribute to the observed background in the ^{60}Ni and ^{65}Cu signals, respectively. Isotopes ^{106}Cd and ^{108}Cd showed background contribution likely linked to Zn in the sample, but ^{111}Cd was used for element quantification. Quantification of ^{133}Cs was difficult due to the low concentration and the interferences contributed by In, added as internal standard.

Table 3: Method detection limits (concentrations in mg kg^{-1}) determined for laboratory blank solutions.

Element	m/z	Equation[#]	Procedure		
			A	B	C
Be	9		0.010	0.010	0.009
B	11		0.76	0.85	0.69
Al	27		2.9	4.7	2.0
Ti	47		2.5	1.4	3.5
V	51	(1)	0.12	0.69	0.44
V	51		0.12	N.R.	0.91
Cr	52		0.055	1.9	0.047
Mn	55		0.033	0.11	0.039
Fe	56		10.2	9.8	8.6
Co	59		0.012	0.013	0.024
Ni	60		0.15	0.38	0.12
Cu	63		0.33	0.71	0.44
Cu	65		0.50	0.27	0.17
Zn	66		1.4	0.61	0.36
As	75		0.020	N.R.	0.20
As	75	(2)	0.27	0.86	0.20
Se	82	(3)	0.87	1.4	0.89
Se	82		0.88	0.89	0.97
Sr	88		0.045	0.014	0.028
Mo	95		0.26	1.13	0.32
Ag	107		0.081	0.013	0.009
Cd	111	(4)	0.083	0.055	0.081
Sb	123		0.005	0.004	0.007
Cs	133		0.014	0.028	0.023
Ba	137		0.059	0.063	0.20
Tl	205		0.003	0.006	0.006
Pb	208	(5)	0.024	0.017	0.090
Th	232		0.005	0.003	0.007
U	238		0.002	N.Q.	0.003

Procedures: A (HNO_3) and B (HNO_3 + HCl) use dilution factors of 0.6 g L^{-1}, while 0.5 g L^{-1} is applied for procedure C (HNO_3 + H_2O_2). In the three cases, 0.5 g of dry sample was digested. For the variations of method A with different amount of sample, m_i (g), the corresponding MDLs have to be corrected by a factor of $0.5/m_i$.

[#]Correction equations from Table 2.

N.R.: not recommended; N.Q.: not quantified.

Concerning the determination of analyte concentrations from the slope, the values reported in Tables 4(a), 4(b) and 4(c), were in reasonable good agreement with the certified/reference ones, but in some cases significant statistical differences were found. When plotting the determined slope versus certificated/reference values of concentrations for all the analytes (not shown), a linear relationship holds at 99% CL ($R^2 = 0.991$, $n = 38$) with slope 0.886 ± 0.014. This last provides a gross estimation of the digestion yield, but elemental yields depend on the element geochemistry. Thus, incomplete recoveries in the digestions were found

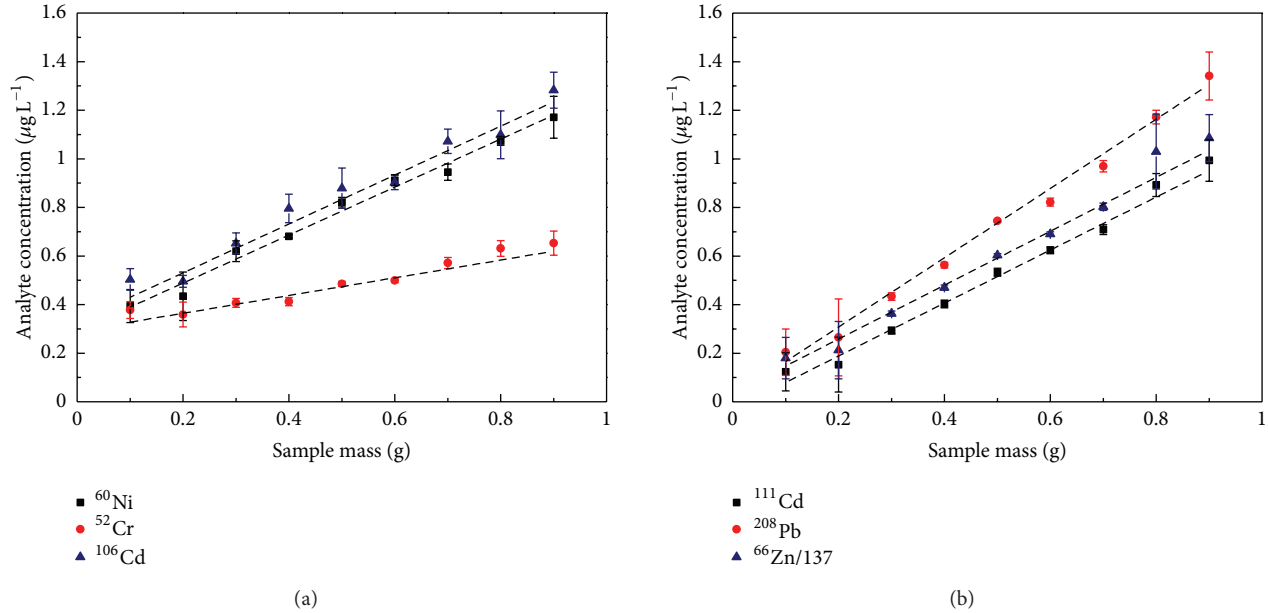

Figure 1: Examples of linear regression for a selected group of elements measured in MT matrix: (a) elements with noncompatible with zero ordinate in the origin at 99% CL and (b) elements with compatible with zero ordinate in origin at 99% CL. See text for explanation. Analyte concentrations are reported as a mean value and standard deviation from the three analytical replicates.

for Fe and Sr in MT and for Al, V, Cr, Fe, Cu, and Sb in TL. Overestimations were identified for Se in MT (likely due to poor signal calibration in the low concentration range) and Co and Ni in TL. ^{82}Se recoveries could also be affected by ^{81}Br^{1}H interference (MT has a Br concentration of 329 mg kg^{-1}). ^{59}Co is also affected by an interference with Ca (formation of species ^{43}Ca^{16}O and ^{42}Ca^{17}OH), present in TL in a percentage of 5.05%. Arunachalam et al. [38] have also reported this overestimation in materials BCR CRM-141 and BCR CRM-142 digested by HNO_3 (118% and 110%, resp.) and by a mixture of HNO_3 + HCl + HF (127% and 106%, resp.).

3.4. Procedure A with Scaling Masses: Direct Quantification through the Dilution Factor. Concentrations of target analytes can be directly quantified for each amount of digested organic sample by using the corresponding dilution factors. This procedure has been applied in its standard version, that is, the recorded signal is corrected only by subtraction of the reagent blank and applying, when appropriate, the interference correction equations. Detailed results will not be reported here, but a general discussion is presented below.

The good linear relationship reported previously already ensures consistent results for the group of elements which are free of background contribution from matrix effect (those reported in Tables 4(a), 4(b), and 4(c) with compatible zero ordinate in origin and which will be referred hereafter as group "a"). For the complementary group (group "b", with background contribution), it is expected that a direct quantification will misestimate the values of the concentrations.

It has been found that digestion of low amount of sample leads to results with higher statistical dispersion. Results with very high associated uncertainties can pass a test of comparison against certified or reference values, but precision might be a target objective for the analyst. Thus, relative uncertainties have been estimated for each quantifiable analyte, and after, their averaged value and standard deviations were found for each digested mass. The resulting magnitude can be compared for the different masses ranging from 100 up to 900 mg. Results are shown in Figure 2 for MT and TL matrices. The smaller mass amounts (100 and 200 mg) produced overall higher relative uncertainties.

A statistical test to compare the mean values has been conducted to detect significant differences among the direct determination and those previously found from the slope. In this way, the effect of incomplete recovery during the digestion procedure can be discarded. The total number of failures found in MT and TL matrices has been quantified for each digested mass and separately for "a" and "b" class analytes. Results are reported in Figure 3, given as a percentage of the total number of analyte quantifications involved in each case. As expected, most of the direct determinations for class "b" analytes failed, with a trend of decreasing for increasing amounts of digested samples. The percentage of failures for "a" class analytes is small and concentrated around the low masses range. Actually, they are likely linked to the statistical variability previously reported in Figure 2.

Class "b" elements should not be directly quantified without an appropriate treatment of physical and isobaric interferences due to matrix effects. Thus, the selection of the most suitable amount of mass to be digested should fulfil two criteria: precision and accuracy. The first one can be quantified by the associated relative uncertainties; meanwhile, for the second criteria, the percentage of failures in comparison

Table 4: (a) Measured (from slope) and certified/reference concentrations (mg kg^{-1}) for the target analytes in the SRM 2976 mussel tissue. (b) Measured (from slope) and certified/reference concentrations (mg kg^{-1}) for the target analytes in the SRM 1549 nonfat milk powder. (c) Measured (from slope) and certified/reference concentrations (mg kg^{-1}) for the target analytes in the SRM 1573a tomato leaves.

(a)

Element	m/z	SRM 2976 mussel tissue			
		From slope		Certified/reference	Stat.
Be	9	N.L.			
B	11	22.5 ± 1.0			Aa
Al	27	140 ± 11	R	134 ± 4	Ab-Ca
Ti	49	15.02 ± 0.57			Aa
V	51	1.39 ± 0.10			Ab
Cr	52	0.324 ± 0.029	R	0.50 ± 0.16	Ab-Ca
Mn	55	37.1 ± 1.2	R	33.0 ± 2.0	Aa-Ca
Fe	56	112.3 ± 7.5	C	171.0 ± 4.9	Aa-Cb
Co	59	0.644 ± 0.021	R	0.61 ± 0.02	Aa-Ca
Ni	60	0.82 ± 0.04	R	0.93 ± 0.12	Ab-Ca
Cu	65	3.35 ± 0.17	C	4.02 ± 0.33	Ab-Ca
Zn	66	137 ± 6	C	137 ± 13	Aa-Ca
As	75	14.6 ± 0.5	C	13.3 ± 1.8	Aa-Ca
Se	82	2.75 ± 0.23	C	1.80 ± 0.15	Aa-Cb
Sr	88	67.0 ± 2.4	R	93 ± 2	Aa-Cb
Mo	98	0.412 ± 0.025			Ab
Ag	107	N.L.; <MDL	R	0.011 ± 0.005	
Cd	111	0.938 ± 0.03	C	0.82 ± 0.16	Aa-Ca
Sb	123	N.L.			
Cs	133	N.L.; <MDL	R	0.027 ± 0.001	
Ba	137	0.65			
Tl	205	N.L.			
Pb	208	1.19 ± 0.04	C	1.19 ± 1.18	Aa-Ca
Th	232	0.0123 ± 0.0035	R	0.011 ± 0.002	Bb-Ca
U	238	0.244 ± 0.008			Aa

Statistical analysis: first (capital) refers to linear fit: A > 99% CL, B > 95% CL; C > 90% CL. Second (lower case) refers to the ordinate in origin: not significantly different from zero at 99% CL; b, else. Third refers to the comparison between measured and certificated/reference values: Ca not statistically significant difference at 95% CL, Cb, else. For those reference values without associated uncertainties, relative errors of 20% have been assumed.
N.L: nonpositive linear relationship; <MDL: below method detection limit;
R: reference value; C: certified value.

(b)

Element	m/z	SRM 1549 nonfat milk powder			
		From slope		Certified/reference	Stat.
Be	9	N.L.			
B	11	2.8 ± 0.7			Ba
Al	27	N.L.; <MDL	R	2.0	
Ti	49	2.63 ± 0.17			Aa
V	51	N.L.			
Cr	52	N.L.; <MDL	C	0.0026 ± 0.0007	
Mn	55	0.254 ± 0.010	C	0.26 ± 0.06	Ab-Ca
Fe	56	N.L.; <MDL	C	1.78 ± 0.1	
Co	59	<MDL	R	0.0041	
Ni	60	0.308 ± 0.027			Ab
Cu	65	0.71 ± 0.29	C	0.70 ± 0.1	Ca-Ca
Zn	66	43.9 ± 1.4	C	46.10 ± 2.2	Aa-Ca
As	75	<MDL	R	0.0019	
Se	82	<MDL	C	0.110 ± 0.010	

(b) Continued.

Element	m/z	SRM 1549 nonfat milk powder			
		From slope		Certified/reference	Stat.
Sr	88	3.68 ± 0.12			Aa
Mo	98	0.350 ± 0.017	R	0.34	Aa-Ca
Ag	107	<MDL	R	<0.0003	
Cd	111	<MDL	C	0.0005 ± 0.0002	
Sb	123	<MDL	R	0.00027	
Cs	133	N.L.			
Ba	137	0.87 ± 0.04			Aa
Tl	205	N.L.; <MDL			
Pb	208	<MDL	C	0.019 ± 0.003	
Th	232	N.L.			
U	238	N.L.			

Statistical analysis: first (capital) refers to linear fit: A > 99% CL; B > 95% CL; C > 90% CL. Second (lower case) refers to the ordinate in origin: not significantly different from zero at 99% CL; b, else. Third refers to the comparison between measured and certificated/reference values: Ca not statistically significant difference at 95% CL, Cb, else. For those reference values without associated uncertainties, relative errors of 20% have been assumed.
N.L: nonpositive linear relationship; <MDL: below method detection limit;
R: reference value; C: certified value.

(c)

Element	m/z	SRM 1573a tomato leaves			
		From slope		Certified/reference	Stat.
Be	9	0.0274 ± 0.0016			Ab
B	11	30.5 ± 1.1			Aa
Al	27	543 ± 12	C	598 ± 12	Aa-Cb
Ti	49	22.6 ± 1.6			Aa
V	51	0.56 ± 0.05	C	0.835 ± 0.010	Ab-Cb
Cr	52	1.51 ± 0.06	C	1.99 ± 0.06	Ab-Cb
Mn	55	236 ± 9	C	246 ± 8	Aa-Ca
Fe	56	301 ± 8	C	368 ± 7	Ab-Cb
Co	59	0.756 ± 0.022	C	0.57 ± 0.02	Aa-Cb
Ni	60	1.93 ± 0.10	C	1.590 ± 0.07	Ab-Cb
Cu	65	3.85 ± 0.23	C	4.70 ± 0.14	Aa-Cb
Zn	66	29.0 ± 1.8	C	30.90 ± 0.7	Aa-Ca
As	75	0.063 ± 0.008	C	0.112 ± 0.004	Ab-Cb
Se	82	<MDL	C	0.054 ± 0.003	
Sr	88	78.2 ± 1.8	R	85	Aa-Ca
Mo	98	0.400 ± 0.010			Aa
Ag	107	0.016 ± 0.004	R	0.017	Aa-Ca
Cd	111	1.56 ± 0.04	C	1.52 ± 0.04	Aa-Ca
Sb	123	0.035 ± 0.002	C	0.063 ± 0.006	Aa-Cb
Cs	133	0.036 ± 0.005	R	0.053	Ab-Ca
Ba	137	57.6 ± 1.6	R	63	Aa-Ca
Tl	205	0.0444 ± 0.0018			Aa
Pb	208	0.594 ± 0.016			Aa

(c) Continued.

Element	m/z	SRM 1573a tomato leaves			
		From slope	Certified/reference		Stat.
Th	232	0.101 ± 0.003	R	0.120	Aa-Ca
U	238	0.0298 ± 0.0015	R	0.035	Aa-Ca

Statistical analysis: first (capital) refers to linear fit: A > 99% CL; B > 95% CL; C > 90% CL. Second (lower case) refers to the ordinate in origin: not significantly different from zero at 99% CL; b, else. Third refers to the comparison between measured and certificated/reference values: Ca not statistically significant difference at 95% CL, Cb, else. For those reference values without associated uncertainties, relative errors of 20% have been assumed.
N.L: nonpositive linear relationship; <MDL: below method detection limit;
R: reference value; C: certified value.

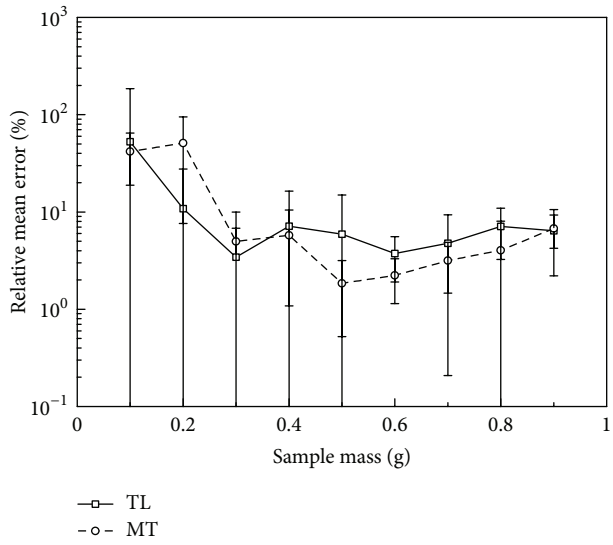

Figure 2: Statistical variability estimated for each quantifiable analyte in matrices TL and MT. The resulting magnitude can be compared for the different masses ranging from 100 up to 900 mg (the smaller mass amounts produced overall higher relative uncertainties).

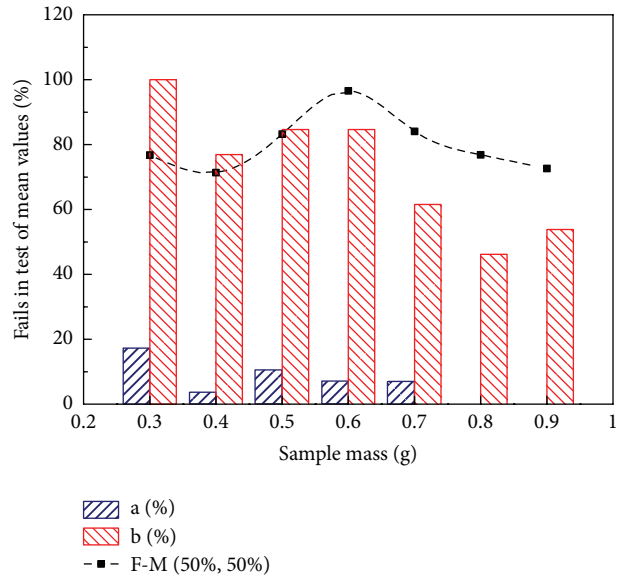

Figure 3: Percentages of direct determinations from the dilution factor with statistically significant differences (at 95% CL) with respect to the value found from the slope (see Table 4 and Figures 1(a) and 1(b)) in MT and TL matrices). Classes "a" and "b" refer to isotopes with an ordinate in origin statistically nondifferent and statistically different from zero at 99% CL, respectively (see text for explanation). The "figure of merit" (as defined in the text) pursuits the digested mass with the best compromise between precision and accuracy.

against reference values can serve as a reasonable quantification. Thus, the following "figure of merit" has been defined:

$$\mathrm{FM} = f_1\,(100 - \%\mathrm{failures}) + f_2 \frac{\langle \varepsilon_r \rangle}{\varepsilon_r\,(m_i)}, \quad (1)$$

where f_1 and f_2 are user-defined weighting factors, $\varepsilon_r(m_i)$ is the averaged relative uncertainty found for mass m_i (from Figure 2), and $\langle \varepsilon_r \rangle$ is the averaged value for all sample masses. Figure 3 shows this "figure of merit" for $f_1 = f_2 = 50$. The best mass amount to be used in the digestion procedure is found to be around 600 mg, which corresponds with a sample mass to acid volume ratio of 60 mg mL^{-1}. Slight changes in the values of the weighting factors f_1 and f_2 do not affect to the final result. This sample mass to acid volume ratio is similar to those reported elsewhere. For example, by Sucharová and Suchara [1] use a relation mass sample to acid ratio of 36 and 72 mg mL^{-1} (depending on the concentration of Si present in the samples) to determine the concentration of 36 elements in different plant reference materials by ICP-MS after microwave digestions assisted by three different types of mixtures. González et al. [39] use a ratio of 67 to determine by ICP-OES the concentration of 7 elements in plant and animal samples after a microwave-assisted digestion with also nitric acid and hydrogen peroxide. Rodushkin et al. [40] reported a ratio of 50 for a microwave digestion with nitric acid and hydrogen peroxide prior to the determination of 16 elements in the certified reference material SRM 1547 (peach leaves) by ICP-AES, and ICP-SMS is reported. Finally, Sastre et al. [15] used a ratio of 66 in an acid digestion with nitric acid for the determination of Cd, Cu, Pb, and Zn in a wide range of environmental samples (covering sediment, soil, sewage sludge, and plant matrices) by ICP-MS.

3.5. Comparison among Digestion Procedures A, B, and C. Attending to previous results, the best procedure is the one using 600 mg of sample and 10 mL of nitric acid. However, the US-EPA methods recommend 500 mg of sample mass, so this amount was used to compare the digestion procedures with different mixtures of acids. 500 mg of sample and dilution factors provided in the experimental section were used in all digestion procedures. Each target element was directly quantified from the measured signal after subtracting the corresponding reagent blank signal. Results are shown in Tables 5(a), 5(b), and 5(c), where the mean values and the standard deviation from three analytical replicates of each of the three matrices are reported. These data can be compared with certified/reference values and those obtained from the linear regression method (Tables 4(a), 4(b), and 4(c)). A Fisher's Least Significant Difference (LSD) test at 95% CL has been conducted to discriminate the reported values for each analyte. Results are also shown in Tables 5(a), 5(b), and 5(c) and they will serve for the present discussion. It is worth to note that this statistical test is mediated by the effect of nonuniform uncertainties and by spurious background contributions by matrix effects which are not properly detected and treated by the quantification method.

Total recovery can be understood as the absence of statistically significant differences between digestion procedures and certified/reference values. As seen in Tables 5(a), 5(b) and 5(c), this was found to be dependent on the matrix (the effect of the element concentration within the same matrix cannot be tested with our data).

There were not statistically significant differences among different digestion procedures at 95% CL (including determinations from the slope) and between procedures and certified/reference values (when available) for the following set of elements (matrices are indicated as superindexes): Mn^{MP}, Co^{MT}, Cu^{MP}, $Zn^{MT,TL}$, As^{MT}, $Sr^{MP,TL}$, Mo^{MP}, $Ag^{MT,TL}$, $Cd^{MT,TL}$, $Ba^{MT,TL}$, Th^{MT}, and Pb^{MT}. Nevertheless, most of these elements exhibited statistically significant differences in other matrices, and some of them were affected by spurious background contributions (see Tables 4(a), 4(b), and 4(c)). Thus, Mn showed apparently excellent results in MP matrix (in which it is affected by matrix background); meanwhile, the three digestion procedures overestimated its concentration in MT and underestimated it in TL matrix. Concentrations for the following set of elements were underestimated (when compared against certified/reference values) by all digestion procedures: $Fe^{MT,TL}$, $Cu^{MT,TL}$, Zn^{MP}, Sr^{MT}, and Sb^{TL}. Contrarily, concentrations were overestimated by the three procedures only for Mn^{MT}. Thus, Tables 5(a), 5(b), and 5(c) can provide so guidance for selecting the most appropriate procedure for any particular analyte depending on the matrix, but a general recommendation cannot be established for all three digestion procedures. Independent of the selected digestion procedure, the use of a sequence of digested masses should provide a more robust tool for quality control and absolute quantification with acceptable precision.

The digestion with HNO_3 alone quantitatively extracted almost all the elements in the three SRMs. For the majority of elements analyzed in the samples, there was no clear improvement in the recovery when using HNO_3 + HCl or HNO_3 + H_2O_2 in the digestions compared to HNO_3 alone. Incomplete recoveries were found for Fe, Sr, Al, V, Cr, Fe, Cu, and Sb, which could indicate a poor digestion of refractory Si likely affecting the recovery of silicon-bound elements. On the other hand, adding HCl to the HNO_3 could carry out disadvantages because Cl^- ion is retained in the final sample matrix and could create interferences in the determination of some elements, such as Va, Cr, and As [41], although this has not been noticed in this study.

4. Conclusions

Microwave-assisted digestion procedures with simplified reagents (HNO_3 alone and mixtures of HNO_3/HCl and HNO_3/H_2O_2) were compared for quantitative determination of 25 elements (Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Pb, Th, and U) in biological reference materials provided by NIST (mussel tissue, tomato leaves and milk powder) by ICP-MS.

The corresponding method detection limits were determined from reagent blanks, being comparable to those published in the scientific literature when the dilution factor is taken into account. For most of the elements, the choice of the digestion procedure had little influence on the MDL.

From scaling masses in procedure A, a linear relationship among instrumental signal (background corrected through reagent blank) and digested sample mass could be constructed at 99% CL for most of the target analytes. No linear relationship was found for those present at very low concentrations (below their respective MDL) or interfered by internal standards. The slope of this linear fit provided, along with the applied dilution factor, the estimation of concentration in the sample, while the ordinate in origin allowed for the identification of matrix interferences which were absent in the reagent blank and which were not properly resolved by the implemented interference correction equations. When available, the so quantified concentrations were in good agreement with certified/reference values. This methodology provides a robust mean for evaluating both, the analyte recovery resulting from the digestion method and the reliability of the treatment of interferes. The best compromise between accuracy and precision was found when a sample amount of 600 mg was used in the digestion, which corresponds to a sample mass to acid volume ratio of 60 mg mL^{-1}.

The digestion with HNO_3 alone (procedure A) quantitatively extracted almost all the elements in the three reference materials, although incomplete recoveries in the digestions were found for Fe and Sr in MT and for Al, V, Cr, Fe, Cu, and Sb in TL, probably because these elements commonly occur as silicate compounds which are not solubilized efficiently using only HNO_3.

There were not statistically significant differences among different digestion procedures for the following set of elements (matrices are indicated as superindexes): Mn^{MP}, Co^{MT}, Cu^{MP}, $Zn^{MT,TL}$, As^{MT}, $Sr^{MP,TL}$, Mo^{MP}, $Ag^{MT,TL}$, $Cd^{MT,TL}$, $Ba^{MT,TL}$, Th^{MT}, and Pb^{MT}. Nevertheless, most of these

Table 5: (a) Measured concentrations (mg kg^{-1}) in SRM 2976 mussel tissue for target analytes using different digestion procedures. (b) Measured concentrations (mg kg^{-1}) in SRM 1549 nonfat milk powder for target analytes using different digestion procedures. (c) Measured concentrations (mg kg^{-1}) in SRM 1573a tomato leaves for target analytes using different digestion procedures.

(a)

Element	*m/z*	SRM 2976 mussel tissue			
		A	B	C	LSD at 95% CL
Be	9	0.014 ± 0.003	<MDL	<MDL	
B	11	20.5 ± 0.6	23.7 ± 0.3	22.7 ± 2.0	ab-a-b-ab-N
Al	27	184 ± 9	204.0 ± 2.0	133 ± 14	a-b-b-a-a
Ti	49	16.5 ± 0.3	28 ± 3	13.3 ± 2.4	a-a-b-a-N
V	51	2.24 ± 0.17	N.Q.	6.9 ± 0.4	a-b-N-c-N
Cr	52	0.597 ± 0.023	1.74 ± 0.05	<MDL	a-b-c-N-b
Mn	55	38.7 ± 0.3	38.1 ± 1.0	38 ± 3	b-b-b-b-a
Fe	56	110 ± 4	112 ± 9	110 ± 10	a-a-a-a-b
Co	59	0.663 ± 0.009	0.637 ± 0.015	0.63 ± 0.06	a-a-a-a-a
Ni	60	0.91 ± 0.04	1.02 ± 0.27	0.68 ± 0.23	ab-ab-b-a-ab
Cu	65	2.745 ± 0.014	2.920 ± 0.028	3.2 ± 0.03	c-a-ab-bc-d
Zn	66	138.7 ± 2.8	137 ± 3	133 ± 11	a-a-a-a-a
As	75	14.77 ± 0.17	12.8 ± 0.3	12.6 ± 1.1	a-a-a-a-a
Se	82	2.90 ± 0.06	1.6 ± 0.5	2.1 ± 0.3	b-b-a-a-a
Sr	88	69.3 ± 0.5	68.7 ± 0.9	68 ± 6	a-a-a-a-b
Mo	98	0.575 ± 0.022	0.486 ± 0.021	0.48 ± 0.04	a-c-b-b-N
Ag	107	0.020 ± 0.004	0.017 ± 0.004	0.014 ± 0.012	N-a-a-a-a
Cd	111	0.91 ± 0.03	0.90 ± 0.07	0.86 ± 0.10	a-a-a-a-a
Sb	123	0.0183 ± 0.0021	0.021 ± 0.004	0.0227 ± 0.0020	b-a-a-a-c
Cs	133	0.1687 ± 0.0015	0.33 ± 0.05	0.17 ± 0.06	N-b-c-b-a
Ba	137	0.636 ± 0.007	0.59 ± 0.04	0.68 ± 0.08	a-a-a-a-N
Tl	205				
Pb	208	1.214 ± 0.022	1.11 ± 0.0454	1.15 ± 0.10	a-a-a-a-a
Th	232	0.0167 ± 0.0015	0.0140 ± 0.0026	0.015 ± 0.004	a-a-a-a-a
U	238	0.2430 ± 0.0017	0.243 ± 0.0184	0.237 ± 0.018	a-a-a-a-N

LSD test for the five independent determinations, ordered as follows: (1) from slope and scaling masses; (2) digestion method A (HNO_3); (3) digestion method B (HNO_3 + HCl); (4) digestion method C (HNO_3 + H_2O_2); and (5) certified/reference values; N means nonincluded; low case letters a, b, c, and d are classes ordered from lower to upper values; N.Q.: nonquantified; <MDL: below method detection limit.
Certified/reference values and obtained from slope and scaling masses are shown in Table 4(a).

(b)

Element	*m/z*	SRM 1549 milk powder			
		A	B	C	LSD at 95% CL
Be	9				
B	11	3.2 ± 0.6	4.4 ± 0.3	4.0 ± 1.0	a-ab-b-ab-N
Al	27				
Ti	49				
V	51				
Cr	52				
Mn	55	0.203 ± 0.013	0.29 ± 0.08	0.23 ± 0.14	a-a-a-a-a
Fe	56				
Co	59				
Ni	60				
Cu	65	0.51 ± 0.07	0.41 ± 0.13	0.58 ± 0.05	a-a-a-a-a
Zn	66	42.7 ± 2.7	42.5 ± 2.2	42.0 ± 2.4	c-ab-a-bc-d
As	75				
Se	82				

(b) Continued.

Element	m/z	SRM 1549 milk powder			
		A	B	C	LSD at 95% CL
Sr	88	3.51 ± 0.17	3.53 ± 0.20	3.49 ± 0.25	a-a-a-a-N
Mo	98	0.330 ± 0.010	0.310 ± 0.027	0.33 ± 0.03	a-a-a-a-a
Ag	107				
Cd	111				
Sb	123				
Cs	133				
Ba	137	0.81 ± 0.05	0.73 ± 0.04	0.80 ± 0.06	b-ab-a-ab-N
Tl	205				
Pb	208				
Th	232				
U	238				

LSD test for the five independent determinations, ordered as follows: (1) from slope and scaling masses; (2) digestion method A (HNO_3); (3) digestion method B (HNO_3 + HCl); (4) digestion method C (HNO_3 + H_2O_2); and (5) certified/reference values; N means nonincluded; low case letters a, b, c, and d are classes ordered from lower to upper values; N.Q.: nonquantified; <MDL: below method detection limit.
Certified/reference values and obtained from slope and scaling masses are shown in Table 4(b).

(c)

Element	m/z	SRM 1573a tomato leaves			
		A	B	C	LSD at 95% CL
Be	9	0.0127 ± 0.0022	<MDL	<MDL	
B	11	30.2 ± 0.4	34.1 ± 1.6	33.8 ± 1.4	a-a-b-b-N
Al	27	567 ± 23	550 ± 50	557 ± 14	a-ab-a-ab-b
Ti	49	24.0 ± 1.7	31 ± 3	25 ± 7	a-ab-b-ab-N
V	51	0.79 ± 0.04	N.Q.	N.Q	a-b-N-N-b
Cr	52	1.65 ± 0.10	2.2 ± 0.4	0.87 ± 0.06	b-bc-d-a-cd
Mn	55	219 ± 6	220 ± 8	226 ± 7	bc-a-a-ab-c
Fe	56	264 ± 10	281 ± 13	290 ± 7	c-a-ab-bc-d
Co	59	0.696 ± 0.019	0.708 ± 0.028	0.737 ± 0.017	c-b-b-bc-a
Ni	60	1.64 ± 0.06	1.78 ± 0.25	1.76 ± 0.08	b-a-ab-ab-a
Cu	65	3.29 ± 0.14	3.24 ± 0.16	3.59 ± 0.08	c-ab-a-bc-d
Zn	66	25.9 ± 0.9	26.4 ± 1.4	27.4 ± 1.7	a-a-a-a-a
As	75	0.149 ± 0.022	0.78 ± 0.14	0.66 ± 0.08	bc-a-ab-ab-c
Se	82				
Sr	88	76.9 ± 1.8	75 ± 3	78.0 ± 2.7	a-a-a-a-a
Mo	98	0.362 ± 0.015	0.336 ± 0.017	0.377 ± 0.011	c-b-a-bc-N
Ag	107	0.029 ± 0.018	0.027 ± 0.007	0.015 ± 0.004	a-a-a-a-a
Cd	111	1.47 ± 0.07	1.51 ± 0.07	1.50 ± 0.010	a-a-a-a-a
Sb	123				
Cs	133	0.017 ± 0.0020	0.030 ± 0.017	0.020 ± 0.013	ab-a-a-a-b
Ba	137	55.3 ± 2.0	54.0 ± 2.5	56.7 ± 1.4	a-a-a-a-a
Tl	205	0.041 ± 0.026	0.040 ± 0.004	0.042 ± 0.001	a-a-a-a-N
Pb	208	0.548 ± 0.0255	0.49 ± 0.0424	0.539 ± 0.003	c-bc-a-ab-N

(c) Continued.

Element	m/z	SRM 1573a tomato leaves			
		A	B	C	LSD at 95% CL
Th	232	0.095 ± 0.004	0.088 ± 0.0071	0.100 ± 0.007	ab-a-a-ab-b
U	238	0.0293 ± 0.0021	0.0243 ± 0.0031	0.033 ± 0.004	ab-ab-a-b-b

LSD test for the five independent determinations, ordered as follows: (1) from slope and scaling masses; (2) digestion method A (HNO_3); (3) digestion method B (HNO_3 + HCl); (4) digestion method C (HNO_3 + H_2O_2) and (5) certified/reference values; N means nonincluded; low case letters a, b, c and d are classes ordered from lower to upper values; N.Q.: nonquantified; <MDL: below method detection limit.
Certified/reference values and obtained from slope and scaling masses are shown in Table 4(c).

elements exhibited statistically significant differences in other matrices, and some of them were affected by spurious background contributions Concentrations were underestimated by the three digestion procedures for the following set of elements: $Fe^{MT,TL}$, $Cu^{MT,TL}$, Zn^{MP}, Sr^{MT}, and Sb^{TL}.

This illustrates the limitations of the assessment of different digestion methods throughout a direct quantification by the dilution factor, since it is mediated by a series of facts, as the nonuniform uncertainties, and by spurious background contributions by matrix effects which are not properly detected and treated by the quantification method.

Conflict of Interests

Some commercial identities are mentioned in this paper: (i) Merck, Darmstadt, Germany, (ii) Millipore, Bedford, USA, (iii) Cromlab, (iv) Thermo Fisher, Cambridge, UK, (v) CETAC, Omaha, NE, USA, and (vi) Anton Paar, Graz, Austria. None of the authors have any financial relation with any of those companies that could lead to a conflict of interests. The names of the companies have been written in the paper only as information for possible readers to know which kind of equipment has been used to get the results that we show.

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