



Statistical mixture design development of digestion methods for Oyster tissue using inductively coupled plasma optical emission spectrometry for the determination of metallic ions

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ABSTRACT

The quantitative determination of chemical elements in organic or biological samples is an important analytical problem. Normally the elements to be determined in the organic matrix must be transformed into a simple inorganic form. A digestion method by heating on a block digester has been developed for the determination of Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mg, Mn, V and Zn in Oyster tissue by ICP OES. A simplex centroid statistical mixture design has been used to study the effects of changing HNO₃, HCl and H₂O₂ reagent proportions on the digestion of these samples. Response surface and principal component analyses show that the species Ca, Cd, Cu, Fe, Mg, Mn and Zn have very similar analytical tendencies under this experiment. By means of mixture modeling maximum recoveries for these ions were predicted using 19%, 18% and 63% of the HCl, HNO₃ and H₂O₂ pseudocomponent mixtures, respectively. This corresponds to 21.4%, 30.8% and 47.8% of the HCl, HNO₃ and H₂O₂ commercial solutions. Furthermore the As, Co and V ions present large recoveries for these mixtures as well. The Al and Ba ion recoveries are seen to be independent of the mixture proportions. The analysis of Oyster tissue reference material (SRM 1566b – NIST) under optimized conditions at the selected wavelengths resulted in ion recoveries between 90% and 100%.

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1. Introduction

Decomposition methods of biological samples are generally executed using two or more reagents because their different chemical properties can be exploited for the complete destruction of the organic material. Nitric and sulfuric acids, used individually or together with hydrogen peroxide are common digestion mediums. A reliable decomposition method that results in the complete solubilization of the biological sample while retaining the volatile components, reducing the contaminants, having low blank readings as well as an acceptable analytical frequency is one of the most critical steps of a spectrochemical analytical technique [1–4].

Biological materials rich in proteins and fats require high decomposition temperatures or the use of concentrated oxidizing mineral acid or a mixture of an oxidizing acid with hydrogen peroxide in open or closed systems with conventional or microwave heating [3].

Microwave (MW) radiation has become an analytical tool for sample decomposition. The use of high pressure flasks and oxidizing acids results in faster digestion permitting the processing

of a larger number of samples while eliminating losses of volatile species. This technique, however, is sensitive to several factors such as sample mass, acid type, reagent proportions, temperature, pressure and heating time. Different researchers have employed MW ovens or steel bombs for the digestion of biological materials although sample mass and acid volumes were as small as possible to avoid a very acidic final solution as well as for safety reasons.

In these solutions trace elements are preferably determined using mass spectrometry with inductively coupled plasma (ICP-MS) [5] or atomic absorption spectrometry with electrothermal atomization (ET AAS) [6–8] but atomic emission spectrometry [9,10] can also be used.

Some researchers describe the use of acidic or alkaline solubilization [11,12] in open systems [13–16], with larger sample masses and reagent volumes than those used in closed systems, for the determination of trace elements in addition to major elements, although these processes are slow and inadequate to determine volatile elements.

Based on the above considerations the objective of this study is to optimize mixtures of an oxidizing (HNO₃) and non-oxidizing (HCl) acids and an auxiliary oxidizing agent (H₂O₂) to determine macro- and micro-constituents in Oyster tissue using block digester heating under reflux (cold finger).

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Unfortunately the optimum mixture of these components cannot be determined from chemical principles or existing information. For this reason statistical mixture designs are employed in this work to determine the mixture proportions that result in maximum percentage recoveries of the 12 metals from the organic material by inductively coupled argon plasma optical emission spectrometry (ICP OES) [17–20]. These designs are especially appropriate for this study since they not only permit a determination of the linear blending properties of each solution but also the second and third order synergic and antagonistic interaction effects among the mixture components for each of the metallic elements investigated.

2. Experimental

2.1. Reagents and solutions

All the solutions were prepared with analytical reagent grade and ultra pure water, obtained by a Milli-Q system (Millipore, USA, with a conductivity of $0.054 \mu\text{S cm}^{-1}$). All the glassware and plastic flasks used were cleaned with a neutral detergent (5%, v/v), dilute nitric acid (10%, v/v for 24 h) and then with deionised water.

Multielement standards of Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mg, Mn, V and Zn were prepared by dilution of $1000 \mu\text{g L}^{-1}$ standard solutions (Merck) of the elements. Analytical curves were made using the following concentrations of the analytes: (i) 0.5; 1.0; 2.0; 4.0; $6.0 \mu\text{g mL}^{-1}$ for Al, Cu and Fe; (ii) 0.02; 0.04; 0.10; 0.20; $0.40 \mu\text{g mL}^{-1}$ for As, Ba, Cd, Co, Mn, and V; (iii) 5.0; 10; 15; 20; $40 \mu\text{g mL}^{-1}$ for Ca, Mg and Zn.

2.2. Sample digestion

The solutions for each point of the mixture design were prepared weighing approximately $0.5000 \pm 0.0001 \text{ g}$ of reference material (SRM 1566b – NIST) in a digestion tube and adding volumes of 37% (v/v) HCl and 65% (v/v) HNO_3 , as listed in Table 1. The tubes were put in a TECNAL model TE 040/25 block digester. Cold finger condensers with water refrigeration were attached to the tubes. The system remained in operation overnight, at a temperature of 50°C . After this step 30% (w/v) H_2O_2 was added and the temperature gradually raised until about 140°C , with digestion occurring for 4 h at this temperature. After digestion the tubes were taken off the block digester and cooled to room temperature. The solutions were then transferred to a 25.0 mL volumetric flask with the volume being completed with ultra pure water [21]. The Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mn, Mg, V and Zn concentrations were determined at the selected wavelengths shown in Table 2.

2.3. Statistical mixture design

Mixture properties are governed by reagent proportions and not by the total quantity of each reagent. The mixture proportions are

Table 2

Instrument operating conditions for the determination of metallic elements in Oyster tissue (SRM 1566b-NIST).

Sample flow rate (mL min^{-1})	1.0
Radio frequency power (kW)	1.4
Principal argon flow rate (L min^{-1})	15
Auxiliary argon flow rate (L min^{-1})	0.5
Nebulization flow rate (L min^{-1})	0.6
Read delay (s)	30
Auto integration (s, min.–max.)	1–5
Radial observation height (mm)	15
Replicates	3
Wavelengths (nm)	Al I: 396.152; As I: 188.977; Ba II: 455.403; Ca II: 315.887; Cd II: 226.502; Co II: 228.616; Cu I: 324.754; Fe II: 238.204; Mg II: 279.077; Mn II: 257.610; V II: 292.402; Zn I: 213.856;
Background correction	2 points

not independent of one another since

$$f_{\text{HCl}} + f_{\text{HNO}_3} + f_{\text{H}_2\text{O}_2} = 1$$

where the f values are the volume fractions of the commercial grade acids. Since digestion is not recommended using pure acid or peroxide solutions only ternary mixtures of these solvents were investigated. To facilitate the statistical interpretation of the mixture models the component proportions were converted to pseudocomponents using the usual equation:

$$x_i = \frac{f_i - L_i}{1 - \sum L_i}$$

where L_i are the lower limits of the proportions of the components used in the mixture design, and f_i are the volume proportions. The subscript i represents HCl, HNO_3 and H_2O_2 . The volumes of HCl, HNO_3 and H_2O_2 solutions used to make the mixtures and the corresponding pseudocomponent values are included in Table 1. Fig. 1 shows the compositions of the 10 mixtures investigated here. The vertices correspond to the pseudocomponent mixtures of the commercial solutions: (1) 10% HCl, 20% HNO_3 and 70% H_2O_2 , (2) 70% HCl, 10% H_2O_2 and 20% HNO_3 and (3) 10% HCl, 10% H_2O_2 and 80% HNO_3 . Since the pseudocomponents are linearly related to the volume proportions, model interpretation in terms of pseudocomponents is easily converted into laboratory values.

Mixture models are determined for each of the metallic ions being analyzed. The recovery value for each ion, y , is expressed as a function of the mixture pseudocomponents by linear, quadratic and special cubic models incorporated in the equation:

$$y = b_{\text{HCl}}x_{\text{HCl}} + b_{\text{HNO}_3}x_{\text{HNO}_3} + b_{\text{H}_2\text{O}_2}x_{\text{H}_2\text{O}_2} + b_{\text{HCl-HNO}_3}x_{\text{HCl}}x_{\text{HNO}_3} + b_{\text{HCl-H}_2\text{O}_2}x_{\text{HCl}}x_{\text{H}_2\text{O}_2} + b_{\text{HNO}_3-\text{H}_2\text{O}_2}x_{\text{HNO}_3}x_{\text{H}_2\text{O}_2} + b_{\text{HCl-HNO}_3-\text{H}_2\text{O}_2}x_{\text{HCl}}x_{\text{HNO}_3}x_{\text{H}_2\text{O}_2} + e$$

Table 1

Volume proportions and pseudocomponent values for the mixtures of the statistical design shown in Fig. 1.

Mixture	V_{HCl}	V_{HNO_3}	$V_{\text{H}_2\text{O}_2}$	x_{HCl}	x_{HNO_3}	$x_{\text{H}_2\text{O}_2}$
1,11	3.0	1.0	1.0	0.8333	0	0.1667
2,12	0.5	4.0	0.5	0	1	0
3,13	1.0	1.0	3.0	0.1667	0	0.8333
4,14	0.5	2.3	2.2	0	0.4167	0.5833
5,15	2.2	2.3	0.5	0.5833	0.4167	0
6,16	2.0	1.0	2.0	0.5	0	0.5
7,17	1.6	1.7	1.7	0.3888	0.2222	0.3888
8,18	2.8	1.1	1.1	0.7777	0.0283	0.1950
9,19	1.1	2.8	1.1	0.1950	0.6117	0.1950
10,20	1.1	1.1	2.8	0.1950	0.0283	0.7777

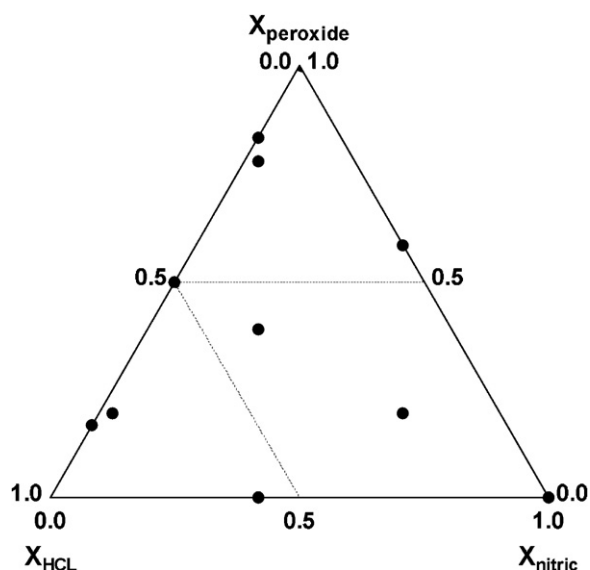


Fig. 1. Mixture design points, 1–10, of Table 1. The pseudocomponents correspond to the vertices of the inner triangle.

For additive mixture behavior a linear model including only the first three terms is adequate to describe percentage recovery. The b_{HCl} , b_{HNO_3} and $b_{\text{H}_2\text{O}_2}$ are linear blending terms reflecting the importance of each pure pseudocomponent on the percentage recovery. Synergic and antagonistic interactions between pairs of mixture pseudocomponents are given by the next three terms in the equation. Quadratic models contain all the terms in the above equation except the ternary mixture term that describes the interaction involving all three mixture components. The special cubic model includes all the terms in the above equation.

Linear, quadratic and special cubic models were determined for each of the metallic ions analyzed here, Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mg, Mn, V and Zn. Attempts to obtain the best recovery percentage for all these ions involved determining individual linear, quadratic and special cubic models for each ion. These models were compared using ANOVA (ANALYSIS OF VARIANCE) and by calculating standard errors for each model coefficient. Models can be validated if they do not have statistically significant lack of fit at the 95% confidence level but have statistically significant regression results at this level. For those ions for which more than one model meets these criteria, the preferred model can be chosen based on the statistical significance of individual model coefficients.

The mixture that is optimum for the recovery of one element may not be optimum for the other ones. For this reason the validated models for all 12 ions must be examined to determine the most appropriate mixture for recovery of all the elements studied. In this case principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to treat all the experimental data simultaneously rather than individually. These methods allow the

user to extract the maximum amount of useful information from multivariate data sets.

2.4. Instrumentation

The analytical measurements were made with a Perkin-Elmer Inductively Coupled Optical Emission Spectrometer, model Optima 3000DV (Norwalk, CT, USA), equipped with a peristaltic pump, a cross-flow nebulizer coupled to a Rytan double pass spray chamber (Scott type) and a ceramic central torch tube injector with an internal diameter of 2.0 mm. This instrument has a solid-state segmented array charge coupled device (SCD) detector and operates in radial and axial torch configurations. For the axial viewing mode a shear gas interface was used to strip off the cool plasma recombination area. The entire system is controlled with PE Winlab software. The spectrometer conditions [22] of operation are presented in Table 2.

3. Results and discussion

3.1. Statistical mixture design

Table 3 contains the recoveries percentage for Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mg, Mn, V and Zn ions determined by digestion of the NIST 1556b reference material using the 10 mixtures of the statistical design. All determinations were performed in duplicate in order to validate the mixture models. Decompositions performed with the 2.8 mL HCl, 1.1 mL HNO₃ and 1.1 mL H₂O₂ solutions were not successful because the Oyster tissue was carbonized.

Linear, quadratic and special cubic models were determined for all the metallic ions. The coefficients and their standard errors for the preferred models for each ion are given in Table 4 for all ions except Al and Ba. The linear and quadratic models for Al suffered from statistical lack of fit at 95% confidence level whereas the special cubic model was not significant at that level. For the Ba ion all models showed insignificant regression results. As such the recoveries of the Al and Ba ions are predicted to be independent of the component proportions of the digestion mixture. Significant mixture models with no statistical lack of fit were found for all the other ions. Quadratic models were most adequate for describing the As, Ca, Cd, Cu, Fe, Mg, Mn and Zn ion recoveries. For all these models at least one binary interaction model coefficient was significant at the 95% confidence level. Furthermore none of the special cubic models for these ions had significant ternary interaction model coefficients. Linear models were validated for the Co and V ions. Their quadratic models did not contain any significant binary interaction model coefficients.

For each of these models the *F* distribution statistical for lack of fit was calculated using ANOVA. All the values given in Table 4 are much smaller than the 95% confidence critical *F* value demonstrating that the models accurately predict the recovery values within experimental error. Furthermore the *F* statistical values calculated for regression significance, also given in Table 4, are con-

Table 3
Percentage recoveries of the ions for the mixtures of statistical design.

Mixture	Al	As	Ba	Ca	Cd	Co	Cu	Fe	Mg	Mn	V	Zn												
1	53	46	85	78	86	63	96	88	96	87	54	54	97	88	82	94	86	93	84	78	78	92	84	
2	54	39	78	76	112	68	75	74	62	68	14	40	70	69	70	71	76	74	74	73	26	69	73	75
3	55	36	94	89	94	55	101	94	105	95	121	54	94	89	93	86	98	94	98	92	87	69	97	91
4	75	61	106	97	139	117	97	94	97	92	81	52	88	86	87	87	93	92	90	90	60	67	92	89
5	15	38	74	72	**	68	89	79	83	77	40	40	81	73	80	69	86	79	84	77	34	59	86	78
6	40	18	86	83	31	**	96	97	96	94	80	54	90	90	91	89	94	94	93	92	77	86	92	92
7	57	88	90	93	93	**	98	95	97	94	81	67	89	85	92	89	96	92	94	90	69	78	94	90
9	39	41	89	90	54	37	94	93	90	91	54	54	85	85	87	88	91	91	89	89	52	78	89	88
10	38	47	88	95	42	67	87	101	86	98	120	67	80	91	80	92	84	97	85	95	78	78	81	96

Table 4
Model coefficients and their standard errors for the ion recoveries and calculated and reference *F*-distribution values for model validation.

Coefficient	As	Ca	Cd	Co	Cu	Fe	Mg	Mn	V	Zn
b_{HCl}	75.91 (± 4.72)	84.41 (± 6.32)	84.10 (± 7.31)	47.48 (± 12.54)	90.55 (± 6.14)	74.56 (± 6.22)	83.23 (± 5.80)	81.57 (± 5.54)	71.33 (± 8.56)	82.14 (± 6.23)
b_{HNO_3}	77.21 (± 2.47)	75.27 (± 3.31)	66.00 (± 3.83)	31.01 (± 10.57)	70.52 (± 3.21)	71.32 (± 3.26)	75.68 (± 3.04)	74.22 (± 2.90)	45.32 (± 7.21)	74.51 (± 3.26)
$b_{\text{H}_2\text{O}_2}$	91.03 (± 4.21)	90.16 (± 5.63)	91.72 (± 6.51)	99.91 (± 10.64)	85.37 (± 5.47)	78.72 (± 5.54)	88.46 (± 5.17)	88.12 (± 4.93)	82.83 (± 7.26)	87.31 (± 5.55)
$b_{\text{HCl-HNO}_3}$	-8.32 (± 13.91)	19.82 (± 18.61)	22.65 (± 21.51)	-	-14.29 (± 18.08)	13.02 (± 18.31)	15.52 (± 17.08)	14.22 (± 16.30)	-	16.77 (± 18.34)
$b_{\text{HCl-H}_2\text{O}_2}$	14.21 (± 19.98)	40.70 (± 26.74)	34.29 (± 30.91)	-	11.83 (± 25.98)	60.60 (± 26.31)	36.14 (± 24.54)	34.93 (± 23.41)	-	31.40 (± 26.35)
$b_{\text{HNO}_3-\text{H}_2\text{O}_2}$	72.66 (± 13.23)	52.76 (± 17.71)	62.86 (± 20.47)	-	38.44 (± 17.20)	53.88 (± 17.43)	43.24 (± 16.26)	36.86 (± 15.51)	-	38.45 (± 17.45)
$b_{\text{HCl-HNO}_3-\text{H}_2\text{O}_2}$	-	-	-	-	-	-	-	-	-	-
F_{adj}	0.96	0.81	2.02	0.17	1.95	1.35	1.33	1.42	0.54	0.80
F_{crit}	3.86	3.86	3.86	3.37	3.86	3.86	3.86	3.86	3.37	3.86
F_{reg}	18.61	7.54	10.92	9.45	7.18	6.61	6.66	7.50	6.52	5.04
F_{crit}	3.11	3.11	3.11	3.68	3.11	3.11	3.11	3.11	3.11	3.11

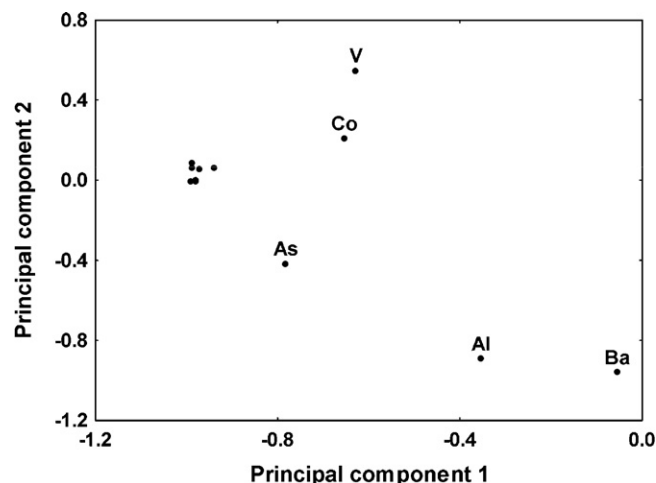


Fig. 2. Principal component loading plot of the ion recoveries for the experiments of the mixture design. The cluster of points represents the Ca, Cd, Cu, Fe, Mg, Mn and Zn ions.

siderably higher than their corresponding critical values showing that recovery values indeed depend on the HCl, HNO₃ and H₂O₂ proportions.

The quadratic models in Table 4 contain some striking similarities. First, for all the ions except for As and Cu, $b_{\text{H}_2\text{O}_2} > b_{\text{HCl}} > b_{\text{HNO}_3}$. For the As model $b_{\text{H}_2\text{O}_2} > b_{\text{HCl}} = b_{\text{HNO}_3}$ and for Cu $b_{\text{HCl}} = b_{\text{H}_2\text{O}_2} > b_{\text{HNO}_3}$ within experimental error. Second, the HNO₃-H₂O₂ interaction coefficients are two to four times larger than the corresponding standard errors in each model with all these coefficients being significant at the 95% confidence level. The linear models for Co and V have some similarities to the quadratic models. Both models have $b_{\text{H}_2\text{O}_2} > b_{\text{HCl}} > b_{\text{HNO}_3}$ although no binary interaction terms were found to be significant.

The above similarities show that the percentage recoveries of these ions have similar dependencies on the HCl, HNO₃ and H₂O₂ proportions. The linear terms indicate that recoveries for these ions are highest for the H₂O₂ pseudocomponent (10% HCl, 20% HNO₃ and 70% H₂O₂ solution) followed by the HCl pseudocomponent (70% HCl, 10% H₂O₂ and 20% HNO₃) with the HNO₃ pseudocomponent (10% HCl, 10% H₂O₂ and 80% HNO₃) promoting the smallest recovery. The synergistic HNO₃-H₂O₂ interaction term provides significant contributions to the recoveries of As, Ca, Cd, Cu, Fe, Mg, Mn and Zn ions. The HCl-H₂O₂ binary interaction terms are positive for all these ion recoveries and usually are larger than their corresponding standard errors. However they are not significant at the 95% confidence level for any of these ions.

Principal component (PC) graphs are useful for illustrating the correlations between the analytical results of the different ions. Fig. 2 shows the loading graph for the first two principal components that represent 80% of the total variance of the data set in Table 4. The ion recoveries more adequately described by the quadratic models have almost identical loadings and their points form a tight cluster in the PC plot except for As point that is closer to this cluster than any of the other ion points. This indicates that ion recoveries for the experiments of the mixture design are highly correlated. Indeed correlation coefficients for all the ions in the tight cluster are 0.95 or more and between 0.72 and 0.78 for all these ions with the As ion. The V and Co ion points are close to each other and farther from the cluster. Their ion recoveries follow a linear model with $b_{\text{H}_2\text{O}_2} > b_{\text{HCl}} > b_{\text{HNO}_3}$ the same numerical ordering in the linear terms as have most of the quadratic models, but without any significant binary interaction. The Al and Ba ion recoveries with models indicating no dependence on the type of mixture used for

Table 5

Equivalent background concentration (BEC), limits of detection (LOD) and limits of quantification (LOQ) for the elements studied.

Element	Wavelength (nm)	Instrumental BEC ($\mu\text{g mL}^{-1}$)	BEC ($\mu\text{g mL}^{-1}$)	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
Al	396.153	0.95	0.076	0.0025	0.0082
As	188.979	4.55	0.0028	0.012	0.04
Ba	455.403	0.04	0.018	0.00074	0.0025
Ca	315.887	0.73	0.00053	0.000022	0.000074
Cd	226.502	0.11	0.00015	0.000043	0.00014
Co	228.616	0.23	0.00014	0.000056	0.00019
Cu	324.754	0.18	0.00031	0.000022	0.000074
Fe	238.204	0.15	0.060	0.005	0.016
Mg	279.077	1.00	0.012	0.00046	0.0015
Mn	257.610	0.05	0.00039	0.00001	0.000034
V	292.402	0.25	0.00022	0.000084	0.00028
Zn	213.856	0.06	0.0083	0.00001	0.000033

digestion have loading points at the bottom right far removed from the other points.

The contour lines for the response surface for Cd recovery are shown in Fig. 3. It is representative of all the elements that are described by quadratic models for ions with points in the tight cluster. The vertex for the H_2O_2 pseudocomponent is predicted to have higher recoveries than those for HCl pseudocomponent. The HNO_3 pseudocomponent is predicted to have the smallest recovery value. However does exist a high plateau region in the upper portion of the triangle with ion recoveries expected to be 95% or more. The plateau represents mixtures for which the synergic binary interactions have important contributions that increase ion recovery. A maximum recovery is predicted for 19%, 18% and 63% of the HCl, HNO_3 and H_2O_2 pseudocomponents, respectively. This corresponds to 21.4%, 30.8% and 47.8% of the HCl, HNO_3 and H_2O_2 commercial solutions used here.

3.2. Figures of merits

BEC, SBR and LOD were calculated according to the literature [23], relating to the analyte concentration and SBR (analytical signal and signal background ratio). Limit of quantification was calculated as 3.3 LOD [24].

Table 5 presents the values of BEC, LOD and LOQ. Comparing the values of BEC from the manufacturer with those obtained experimentally it is possible to conclude that the instrumental conditions of this work were robust ones as can be seen by the MgII/MgI ratio of 9.2 and by the low background signal.

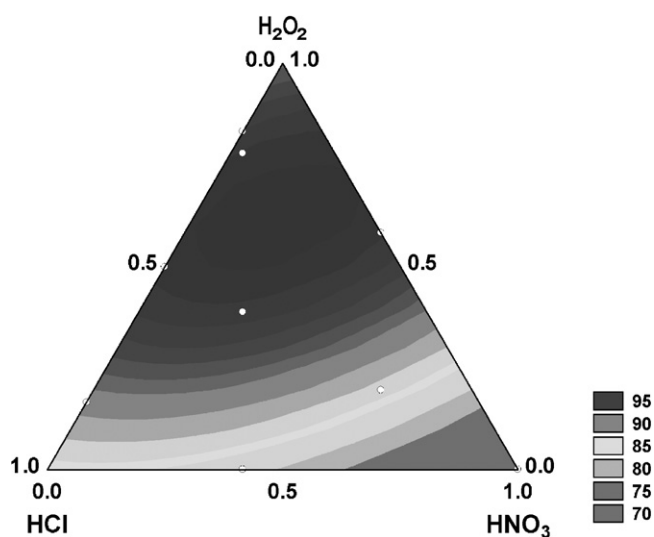


Fig. 3. Contour curves for the response surface of the Cd ion determined from the results of the mixture design.

Table 6

Concentration and recovery values for the analytes in the certified reference material (CRM 1566b).

Element	Concentration of CRM		Recovery (%)
	Declared value ($\mu\text{g g}^{-1}$)	Obtained value ($\mu\text{g g}^{-1}$)	
Al	197.2 ± 6.0	94 ± 8	47.9 ± 4.2
As	7.65 ± 0.65	7.52 ± 0.17	98.4 ± 2.2
Ba	8.6 ± 0.3	7.11 ± 0.39	92.9 ± 5.1
Ca	838 ± 20	877 ± 12	104.7 ± 1.4
Cd	2.48 ± 0.08	2.677 ± 0.56	108.0 ± 2.2
Co	0.371 ± 0.009	0.311 ± 0.017	83.8 ± 4.5
Cu	71.6 ± 1.6	72.8 ± 1.4	101.7 ± 1.9
Fe	205.8 ± 6.8	205.3 ± 2.7	99.8 ± 1.3
Mg	1085 ± 23	1106 ± 15	101.9 ± 1.4
Mn	18.5 ± 0.2	20.22 ± 0.47	109.3 ± 2.5
V	0.577 ± 0.023	0.393 ± 0.037	68.0 ± 6.4
Zn	1424 ± 46	1415 ± 61	99.3 ± 4.3

3.3. Validation of method

An evaluation of the accuracy of the digestion method using a digestion block was carried out under the optimized instrumental conditions. Table 6 shows good efficiency of digestion procedure with recoveries between 84% and 110%. Concentrations for Co and V in the certified reference material are lower than their LOQ values resulting in low recoveries. Aluminum also showed problems and this can be understood considering the need to add fluoridric acid in the sample for the recovery of aluminum since fluoride ion reacts with refractory oxides and forms a complex in order to increase the solubility [1].

Statistical tests using one-way ANOVA as well as the multiple Student and Newman–Keuls comparison methods were carried out to verify the existence of significant differences between results from the decomposition method with those of the reference material for the analyzed ions [25]. The null hypothesis indicating no significant difference at the 95% confidence level between the results of the proposed digestion method and the certified values was obtained for the As, Ba, Ca, Cd, Cu, Fe, Mg, Mn and Zn ions.

4. Conclusion

An optimum 21.4%, 30.8% and 47.8% mixture of HCl, HNO_3 and H_2O_2 commercial solutions was determined using a statistical simplex centroid design for the digestion of a biological sample and the subsequent determination of the Al, As, Ba, Ca, Cd, Co, Cu, Fe, Mn, Mg, V and Zn concentrations by ICP OES.

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