

# OPTIMIZATION OF INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS METHOD BY MEANS OF 2<sup>k</sup> EXPERIMENTAL DESIGN TECHNIQUE AIMING THE VALIDATION OF ANALYTICAL PROCEDURES

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## ABSTRACT

In this study optimization of procedures and standardization of Instrumental Neutron Activation Analysis (INAA) methods were carried out for the determination of the elements arsenic, chromium, cobalt, iron, rubidium, scandium, selenium and zinc in biological materials. The aim is to validate the analytical methods for future accreditation at the National Institute of Metrology, Quality and Technology (INMETRO). The 2<sup>k</sup> experimental design was applied for evaluation of the individual contribution of selected variables of the analytical procedure in the final mass fraction result. Samples of Mussel Tissue Certified Reference Material and multi-element standards were analyzed considering the following variables: sample decay time, counting time and sample distance to detector. The standard multi-element concentration (comparator standard), mass of the sample and irradiation time were maintained constant in this procedure. By means of the statistical analysis and theoretical and experimental considerations it was determined the optimized experimental conditions for the analytical methods that will be adopted for the validation procedure of INAA methods in the Neutron Activation Analysis Laboratory (LAN) of the Research Reactor Center (CRPq) at the Nuclear and Energy Research Institute (IPEN - CNEN/SP). Optimized conditions were estimated based on the results of z-score tests, main effect and interaction effects. The results obtained with the different experimental configurations were evaluated for accuracy (precision and trueness) for each measurand.

## 1. INTRODUCTION

Currently, the implementation of a quality management system is absolutely essential to a measurement laboratory. By means of the processes of certification and/or accreditation, the laboratory can demonstrate that it has facilities, equipment and suitable analytical measurement methods to the quality assurance of generated results [1]. These processes can be formalized by adapting to a wide series of standards and protocols, depending on the activities performed in the laboratory. In Brazil, for measurements in chemistry, the ABNT NBR ISO/IEC 17025 [2] stands out as the most important regulation standard. In this context, validation of measurement procedures is a prerequisite for the implementation and suitability of the quality system in a laboratory.

A first strategy adopted in the validation process must be the optimization of analytical conditions. Thereby, it is possible to identify the importance of each factor to the

measurement process, as well as the uncertainty associated with it. Experimental design is a very useful tool for the optimization of analytical procedures [3-6]. By means of main effect and interaction contrasts, it is possible a correct understanding of the influence caused by each factor to the end result of analysis [7].

Depending on the purpose of the use, experimental conditions and number of variables to be investigated, different models of experimental designs can be used, among them,  $2^k$  (2 levels for k variables),  $3^k$  (3 levels for k variables) and generically  $x^k$  (x levels for k variables), from that  $x \geq 2$  and  $k \geq 2$ . In the context,  $2^k$  experimental design is very useful method for the investigation of preliminary studies with two different levels for each variable k. By means of this strategy, it is possible to evaluate and understand the influence that each variable “carries” to the final result. Nevertheless, the major advantage of the experimental design methods is the conduction of a multivariate optimization, explaining how the different variables are correlated and the importance of this correlation for the optimization of analytical method [8].

For a better understanding of the  $2^k$  experimental design carried in this work, it is necessary a knowledge of some terms and definitions, such as factor, level, main effect, interaction contrast and standard error [8]. These concepts are presented below.

**Factor:** each variable of study. In this work, sample decay time, sample distance to detector and sample counting time are the investigated factors;

**Level:** experimental condition assigned to each factor investigated. Usually, in a traditional experimental design, two different levels are assigned for each factor. These levels are denominated level - (assigned to less favorable condition) and level + (assigned to more favorable condition). Furthermore, a standard condition (current configuration procedure) is also required for a correct analysis of results. This condition is denominated level 0;

**Main Effect:** result obtained with the variation of different levels (- to +) assigned to a factor. It can be calculated according to Equation 1.

$$ME = 2 (\Sigma x^+ - \Sigma x^-) / n \quad (1)$$

where:

ME is the main effect result;

$x^+$  is the experimental result of level +;

$x^-$  is the experimental result of level -;

and n is the number of experiments.

**Interaction Contrasts:** is the comparison of results on one or more levels of a factor, iterating the results obtained with those found for other factors. Briefly, it can be defined as the change in the level of a factor affecting the result obtained for another factor [9].

**Standard Error:** measurement error in the result of an effect. It is the same value for the main effect and interaction contrast (Equation 2).

$$E = s / (2^{k-1})^{1/2} \quad (2)$$

where:

E is the standard error of main effect and interaction contrasts;

s is the standard deviation of the results;

and k is the number of factors.

In the study, a 2<sup>3</sup> experimental design (two levels for three variables) was used to identify the importance of selected factors to the accurate final result, aiming the optimization and validation of analytical procedure for the determination of arsenic, chromium, cobalt, iron, rubidium, scandium, selenium and zinc in biological matrix materials, performing Instrumental Neutron Activation Analysis (INAA).

Samples of a Mussel Tissue Reference Material (CRM) [10] were analyzed by INAA comparative method [11]. The determination of the main effect and interaction contrasts for each experiment, were developed considering the relative error of mean results obtained for three replicate analyses. The equations and results for quantification of measurands were calculated in Microsoft Office Excel spreadsheet [12] and results are presented as mean values and one standard deviation.

## 2. EXPERIMENTAL

### 2.1 Sample, Elemental and Multi-elemental Standard Preparation

Mussel Tissue CRM samples of approximately 150 mg were weighed in polyethylene bags using a Shimadzu AEM-5200 analytical balance. Elemental (for As) and multielemental standards (for Cr, Co, Fe, Rb, Sc, Se and Zn) were prepared by pipetting solutions with predetermined mass onto Whatman paper filters using Eppendorf pipettes with variable volume (20 µL to 100 µL). Elemental and multielemental stock solutions were prepared in volumetric flasks using different Spex standard element solution. After drying, paper filters were folded and stored in polyethylene bags with the same geometry of the samples. Polyethylene bags containing samples and standards were sealed and stored until the analysis was performed. Three replicate analyses for each sample were randomly performed, using the same irradiation time and thermal neutron flux, but on different days.

### 2.2 Irradiation and Element Determination

Samples of Mussel Tissue CRM, element and multi-element standards (comparator standard), were simultaneously irradiated during 8 h under a thermal neutron flux of 10<sup>13</sup> n cm<sup>-2</sup> s<sup>-1</sup> at IEA-R1 Nuclear Research Reactor. <sup>76</sup>As, <sup>51</sup>Cr, <sup>60</sup>Co, <sup>59</sup>Fe, <sup>86</sup>Rb, <sup>46</sup>Sc, <sup>75</sup>Se and <sup>65</sup>Zn radionuclides were quantified by gamma-ray spectrometry, using a Canberra GC2018 hyperpure Ge detector coupled a DSA 1000 multichannel analyzer. Genie 2000 – Gamma Acquisition & Analysis 3.1 software was used to perform the processing of the gamma-ray spectrum. The concentration of the measurands was obtained by comparing the photopeak area of the interest element in the spectrum of the sample with that of the multi-element standard by means of Equation 3 [13].

$$C = (A_s w_{st} C_{st}) e^{-\lambda(t_s - t_{st})} / (A_{st} w_s) \quad (3)$$

where:

$C$  is the sample element concentration (in  $\text{mg kg}^{-1}$ );

$C_{st}$  is the standard element concentration (in  $\text{mg kg}^{-1}$ );

$A_s$  is the activity of the element in the sample (in cps);

$A_{st}$  is the activity of the element in the standard (in cps);

$w_s$  and  $w_{st}$  are the weights of the sample and standard (in g), respectively;

$\lambda$  is the element decay constant;

and  $(t_s - t_{st})$  is the difference of the counting time between the sample and standard.

### 2.3 Experimental Design

Mussel tissue CRM samples were analyzed considering the following factors: sample decay time (A), distance of sample to detector (B) and counting time (C). Measurands were arranged in two groups according to half-life of the daughter radionuclide. Group 1 was composed by As and Group 2 by Co, Cr, Fe, Rb, Se, Sc and Zn. Table 1 presents the analytical peak and half-life of measurands [14].

**Table 1: Radionuclides, half-life and analytical peak**

Radionuclide	Half-life (days)	Analytical peak (keV)
<sup>76</sup> As	1.078	559.10
<sup>51</sup> Cr	27.70	320.08
<sup>60</sup> Co	1925	1173.23
<sup>59</sup> Fe	44.50	1099.25
<sup>86</sup> Rb	18.63	1077.00
<sup>46</sup> Sc	83.79	889.28
<sup>75</sup> Se	119.8	264.66
<sup>65</sup> Zn	244.3	1115.54

Different levels for each factor are shown in Table 2 (for As) and Table 3 (for Cr, Co, Fe, Rb, Sc, Se and Zn). The variables standard multi-element concentration, mass of the sample and irradiation time were maintained constant for this procedure.

**Table 2: Arsenic experimental design: levels and conditions.**

Factor	Level -	Level 0	Level +
Sample decay time (A)	5 days	7 days	9 days
Distance of sample to detector (B)	Shelf 3	Shelf 1	Shelf 0
Counting time (C)	1 hour	2 hours	3 hours

**Table 3: Chromium, cobalt, iron, rubidium, scandium, selenium and zinc experimental design: levels and conditions.**

Factor	Level -	Level 0	Level +
Sample decay time (A)	10 days	15 days	20 days
Distance of sample to detector (B)	Shelf 3	Shelf 1	Shelf 0
Counting time (C)	6 hours	8 hours	10 hours

Variables arising from the preparation of standards and samples, detection system and irradiation process, were assumed to be constant terms in this study. The matrix of experimental design and configuration of each different experiment is shown in Table 4.

**Table 4: Matrix of 2<sup>k</sup> experimental design**

Experiment	*Contributions	A	B	C	AB	AC	BC	ABC
y <sub>1</sub>	a <sub>0</sub> b <sub>0</sub> c <sub>0</sub>	-	-	-	+	+	+	-
y <sub>2</sub>	a <sub>1</sub> b <sub>0</sub> c <sub>0</sub>	+	-	-	-	-	+	+
y <sub>3</sub>	a <sub>0</sub> b <sub>1</sub> c <sub>0</sub>	-	+	-	-	+	-	+
y <sub>4</sub>	a <sub>1</sub> b <sub>1</sub> c <sub>0</sub>	+	+	-	+	-	-	-
y <sub>5</sub>	a <sub>0</sub> b <sub>0</sub> c <sub>1</sub>	-	-	+	+	-	-	+
y <sub>6</sub>	a <sub>1</sub> b <sub>0</sub> c <sub>1</sub>	+	-	+	-	+	-	-
y <sub>7</sub>	a <sub>0</sub> b <sub>1</sub> c <sub>1</sub>	-	+	+	-	-	+	-
y <sub>8</sub>	a <sub>1</sub> b <sub>1</sub> c <sub>1</sub>	+	+	+	+	+	+	+

\* where 0 and 1 match to different levels (- and + respectively)

The results obtained with different experimental configurations were evaluated for accuracy (precision and trueness) for the different measurands. Optimized conditions were estimated based on the results of z-score tests (Equation 4), relative error (Equation 5) [15], main effect and interaction contrasts.

$$Z = (C_s - C_{CRM}) / u_{CRM} \quad (4)$$

where:

Z is a z-score value for experiment;

C<sub>s</sub> the mean result for three replicates;

C<sub>CRM</sub> the measurand certified concentration in the CRM;

and u<sub>CRM</sub> the combined uncertainty in the CRM.

$$RE = 100 \times (C_s - C_{CRM}) / C_{CRM} \quad (5)$$

where:

RE is a relative error to mean result of measurement;

$C_s$  the mean result for three replicates;

and  $C_{CRM}$  the measurand certified concentration in the CRM.

For each experiment, three replicates analysis were conducted, totalizing twenty four experiments at this  $2^3$  experimental design for INAA.

### 3. RESULTS AND DISCUSSION

#### 3.1 Levels and Factors

Initially, aiming to check whether the values assigned to the different levels had been properly delineated, the samples were analyzed in -, 0 e + conditions (see Table 4). Figure 1 shows the z-score values of the results obtained in different conditions (-, 0, +) for each measurand. Results of experiments are presented in Table 5 as mean  $\pm$  standard deviation, z-score values and relative standard deviation (RSD).

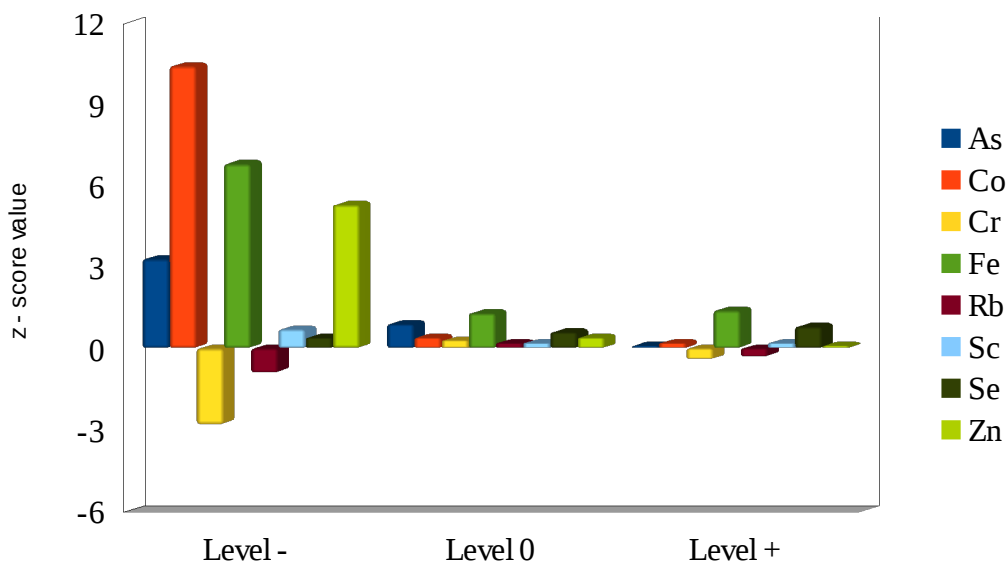


Figure 1: Z-Score values obtained for the different levels

**Table 5: Mean results, z-score values and relative standard deviations (RSD) obtained at conditions -, 0 and + for each measurand.**

Measurand	Level	* Mean Result (mg kg <sup>-1</sup> )	Certified Concentration (mg kg <sup>-1</sup> )	z-score value	RSD
Arsenic	-	16.4 ± 2.0	13.6 ± 1.7	3.3	12 %
	0	12.8 ± 0.59		0.9	4.7 %
	+	13.6 ± 0.88		0.0	6.6 %
Cobalt	-	1.229 ± 0.091	0.829 ± 0.077	10	7.4 %
	0	0.842 ± 0.077		0.4	9.1 %
	+	0.836 ± 0.045		0.2	5.4 %
Chromium	-	0.85 ± 0.16	1.24 ± 0.28	-2.8	19 %
	0	1.28 ± 0.24		0.3	19 %
	+	1.19 ± 0.26		-0.4	22 %
Iron	-	774 ± 110	593 ± 53	6.8	14 %
	0	628 ± 71		1.3	11 %
	+	631 ± 36		1.4	5.7 %
Rubidium	-	4.52 ± 0.14	4.93 ± 0.92	-0.9	3.1 %
	0	5.03 ± 0.21		0.2	4.2 %
	+	4.81 ± 0.35		-0.3	7.3 %
Scandium	-	0.208 ± 0.005	0.199 ± 0.023	0.7	2.4 %
	0	0.201 ± 0.003		0.2	1.5 %
	+	0.201 ± 0.002		0.2	1.0 %
Selenium	-	4.52 ± 0.38	4.42 ± 0.45	0.4	8.4 %
	0	4.55 ± 0.39		0.6	8.6 %
	+	4.59 ± 0.35		0.8	7.6 %
Zinc	-	143.6 ± 12.6	118.5 ± 9.5	5.3	8.8 %
	0	120.4 ± 5.6		0.4	4.7 %
	+	118.1 ± 6.9		0.1	5.8 %

\* mean results ± standard deviation (n=3)

Analyzing the results by overlapping the experimental result (mean and standard deviation) with the CRM certified value, it was observed that, with exception of the results for Co and Zn in the condition - ( $1.229 \pm 0.0091$  mg kg<sup>-1</sup> and  $143.6 \pm 12.6$  mg kg<sup>-1</sup> respectively), all other results are inside these rough acceptance criteria. By means of the z-score analysis, it can be seen that the results for condition + presented a greater degree of trueness than of condition -, with exception of Se, where the condition - was most favorable condition.

By means of relative standard deviation, it was carried out an estimation of precision to the mean results in different conditions (- and +). It was verified that for measurands As, Co, Fe, Sc, Se and Zn, more precise results were obtained in the condition +, with values between 1.0% (Sc) and 7.6% (Se). Cr and Rb presented the least relative standard deviation in the condition - (18.6 % and 3.1 % respectively). Table 6 presents the most favorable condition for accuracy (trueness and precision) of the results for each measurand.

**Table 6: Trueness and precision (most favorable condition)**

Measurand	Precision		Trueness	
	Level -	Level +	Level -	Level +
Arsenic		√		√
Cobalt		√		√
Chromium	√			√
Iron		√		√
Rubidium	√			√
Scandium		√		√
Selenium		√	√	
Zinc		√		√

Taking into account the discussed results, the level + showed best results for trueness and precision for As, Co, Fe, Se and Zn measurands. Cr and Rb presented the more precise results to level -, but not more exact. Selenium presented more exact results to level -, but not more precise. However, the mean of results presented to rubidium, scandium and selenium measurands are statistically equivalent on the different levels. Hence, it can be inferred that INAA is a very robust method for the quantification of rubidium, scandium and selenium in biological matrix materials.

### 3.2 Experimental Design

Eight experiments were simultaneously performed aiming to evaluate the influence caused by changing of the different levels into each factor (Table 4). Z-score values are presented in Table 7 and relative error to the results are shown in Table 8. Table 9 presents mean results of experiments for different measurands.

Analysis for main effect and interaction contrasts for each measurand were performed considering the relative error of mean results obtained for three replicate analyses. For assessing the statistical significance of the result, a t-test (t-Student) was carried to the 95% confidence level.

In this work,  $H_0$  (null hypothesis) was accepted when there is no statistically significant difference for the means with 95% confidence, in other words, when the means are statistically equivalent.  $H_1$  (alternative hypothesis) was accepted when there is a statistically



significant difference for the means with 95% confidence, to the means that are not statistically equivalent.

For  $p \geq 0.05$  (to 5% significance level)  $H_0$  was accepted. Otherwise,  $H_0$  was rejected and  $H_1$  accepted. Results were performed in experimental design spreadsheet [16]. Main effects and interaction contrasts results are presented in the Table 10.

**Table 7: Experimental Design z-score values**

Design	As	Co	Cr	Fe	Rb	Sc	Se	Zn
y <sub>1</sub>	3.3	10	-2.9	6.8	-0.9	0.7	0.4	5.3
y <sub>2</sub>	-4.2	0.7	-2.7	1.5	-1.9	0.7	-0.6	0.7
y <sub>3</sub>	2.3	4.8	-0.7	2.9	-0.9	0.6	1.6	0.8
y <sub>4</sub>	-1.5	0.3	-0.3	1.6	-0.4	0.1	1.5	-0.1
y <sub>5</sub>	2.8	9.6	-0.5	3.4	-0.8	0.6	0.7	4.9
y <sub>6</sub>	-3.5	0.7	-2.3	1.3	-0.7	0.5	0.1	1.0
y <sub>7</sub>	1.3	4.5	0.4	2.8	-0.7	0.7	0.8	0.2
y <sub>8</sub>	0.0	0.2	-0.4	1.4	-0.3	0.2	0.7	0.1

**Table 8: Experimental Design Relative Error of Mean Results**

Design	As	Co	Cr	Fe	Rb	Sc	Se	Zn
y <sub>1</sub>	21 %	48 %	-33 %	30 %	-8.3 %	4.5 %	2.3 %	21 %
y <sub>2</sub>	-26 %	3.0 %	-30 %	6.7 %	-18 %	4.0 %	-2.9 %	2.9 %
y <sub>3</sub>	14 %	22 %	-7.3 %	13 %	-8.1 %	3.5 %	7.7 %	3.1 %
y <sub>4</sub>	-9.6 %	1.2 %	-2.4 %	6.6 %	-4.1 %	0.5 %	7.7 %	-0.3 %
y <sub>5</sub>	17 %	45 %	-5.6 %	15 %	-7.5 %	3.0 %	3.2 %	20 %
y <sub>6</sub>	-22 %	2.9 %	-27 %	5.7 %	-6.5 %	3.0 %	0.7 %	4.1 %
y <sub>7</sub>	8.1 %	21 %	4.0 %	12 %	-6.9 %	4.0 %	3.8 %	0.3%
y <sub>8</sub>	0.0 %	0.8 %	-4.0 %	6.4 %	-2.4 %	1.0 %	3.4 %	-0.3%

**Table 9: Experimental Design Results (in mg kg<sup>-1</sup>) (mean results ± standard deviation, n=3)**

Experiment	As	Co	Cr	Fe	Rb	Sc	Se	Zn
		<i>13.6 ± 1.7*</i>	<i>0.829 ± 0.077*</i>	<i>1.24 ± 0.28*</i>	<i>593 ± 53*</i>	<i>4.93 ± 0.92*</i>	<i>0.199 ± 0.023*</i>	<i>4.42 ± 0.45*</i>
y <sub>1</sub>	16.4 ± 2.0	1.229 ± 0.091	0.83 ± 0.26	774 ± 110	4.52 ± 0.14	0.208 ± 0.005	4.52 ± 0.38	143.6 ± 12.6
y <sub>2</sub>	10.0 ± 2.6	0.854 ± 0.071	0.87 ± 0.24	633 ± 54	4.04 ± 1.11	0.207 ± 0.004	4.29 ± 0.42	121.9 ± 8.0
y <sub>3</sub>	15.5 ± 1.0	1.014 ± 0.084	1.15 ± 0.43	670 ± 84	4.53 ± 0.15	0.206 ± 0.006	4.76 ± 0.59	122.2 ± 9.3
y <sub>4</sub>	12.3 ± 0.8	0.839 ± 0.071	1.21 ± 0.27	632 ± 33	4.73 ± 0.32	0.200 ± 0.003	4.73 ± 0.27	118.2 ± 6.7
y <sub>5</sub>	15.9 ± 1.4	1.200 ± 0.087	1.17 ± 0.23	682 ± 102	4.56 ± 0.14	0.205 ± 0.004	4.56 ± 0.38	141.7 ± 11.4
y <sub>6</sub>	10.6 ± 2.0	0.853 ± 0.067	0.91 ± 0.46	627 ± 44	4.61 ± 0.79	0.205 ± 0.002	4.45 ± 0.30	123.3 ± 6.5
y <sub>7</sub>	14.7 ± 0.9	1.001 ± 0.080	1.29 ± 0.33	667 ± 93	4.59 ± 0.14	0.207 ± 0.004	4.59 ± 0.47	118.8 ± 8.9
y <sub>8</sub>	13.6 ± 0.9	0.836 ± 0.045	1.19 ± 0.26	631 ± 36	4.81 ± 0.35	0.201 ± 0.002	4.57 ± 0.35	118.1 ± 6.9

\* CRM certified values, uncertainties are expanded uncertainties, k = 2.

**Table 10: Main Effects (A, B, C) and Interaction Contrasts (AB, AC, BC, ABC)**

Arsenic					Cobalt				
Effect	Result	Error	t-value	p-value	Effect	Result	Error	t-value	p-value
Mean	0,24	± 1,98	0,12	0,90567	Mean	18.0	± 1.87	9.64	<0.00001
A	-29,3	± 3,96	7,41	<0.00001	A	-32.0	± 3.73	8.58	<0.00001
B	5,99	± 3,96	1,51	0,14939	B	-13.5	± 3.73	3.61	0.00233
C	1,21	± 3,96	0,31	0,76334	C	-1.42	± 3.73	0.38	0.70897
AB	13,7	± 3,96	3,47	0,00318	AB	11.6	± 3.73	3.10	0.00693
AC	5,99	± 3,96	1,51	0,14939	AC	1.18	± 3.73	0.32	0.75662
BC	0,84	± 3,96	0,21	0,83376	BC	0.39	± 3.73	0.11	0.91761
ABC	1,95	± 3,96	0,49	0,62928	ABC	-0.51	± 3.73	0.14	0.89240
Chromium					Iron				
Effect	Result	Error	t-value	p-value	Effect	Result	Error	t-value	p-value
Mean	-13.0	± 4.58	2.85	0.01169	Mean	12.1	± 2.59	4.65	0.00027
A	-5.66	± 9.16	0.62	0.54486	A	-11.4	± 5.19	2.19	0.04337
B	21.2	± 9.16	2.32	0.03402	B	-4.89	± 5.19	0.94	0.35996
C	9.98	± 9.16	1.09	0.29190	C	-4.30	± 5.19	0.83	0.41945
AB	4.06	± 9.16	0.44	0.66336	AB	5.14	± 5.19	0.99	0.33633
AC	-8.91	± 9.16	0.97	0.34502	AC	3.71	± 5.19	0.71	0.48492
BC	-5.15	± 9.16	0.56	0.58165	BC	3.96	± 5.19	0.76	0.45614
ABC	2.45	± 9.16	0.27	0.79269	ABC	-3.54	± 5.19	0.68	0.50470
Rubidium					Scandium				
Effect	Result	Error	t-value	p-value	Effect	Result	Error	t-value	p-value
Mean	-7.73	± 2.15	3.59	0.00244	Mean	2.95	± 0.41	7.26	<0.00001
A	-0.05	± 4.30	0.01	0.99075	A	-1.63	± 0.81	2.00	0.06220
B	4.72	± 4.30	1.10	0.28955	B	-1.36	± 0.81	1.70	0.10921
C	3.80	± 4.30	0.88	0.39010	C	-0.38	± 0.81	0.46	0.65066
AB	4.31	± 4.30	1.00	0.33164	AB	-1.38	± 0.81	1.70	0.10921
AC	2.79	± 4.30	0.65	0.52629	AC	0.13	± 0.81	0.16	0.87808
BC	-2.38	± 4.30	0.55	0.58751	BC	0.88	± 0.81	1.08	0.29533
ABC	-2.59	± 4.30	0.60	0.55645	ABC	-0.12	± 0.81	0.15	0.88046
Selenium					Zinc				
Effect	Result	Error	t-value	p-value	Effect	Result	Error	t-value	p-value
Mean	3.28	± 1.66	1.97	0.06633	Mean	6.31	± 1.57	4.01	0.00100
A	-1.92	± 3.33	0.58	0.57159	A	-9.44	± 3.15	3.00	0.00847
B	4.98	± 3.33	1.49	0.15440	B	-11.2	± 3.15	3.56	0.00259
C	-0.79	± 3.33	0.24	0.81504	C	-0.83	± 3.15	0.26	0.79452
AB	1.92	± 3.33	0.58	0.57159	AB	7.48	± 3.15	2.38	0.03028
AC	0.68	± 3.33	0.20	0.84104	AC	1.40	± 3.15	0.45	0.66167
BC	-3.05	± 3.33	0.92	0.37260	BC	-0.62	± 3.15	0.20	0.84570
ABC	-0.68	± 3.33	0.20	0.84104	ABC	0.01	± 3.15	0.00	0.99737

### 3.2.1 Experimental design considerations for arsenic

Results for arsenic were considered satisfactory to all experiments by interpolation of the experimental results with the certified value (see Table 6). Nevertheless, it was observed a great difference for variance of results in the different experiments. Z-score values were between -4.2 ( $y_2$ ) and 2.8 ( $y_5$ ) and relative error between -26 % and 17 %. Best results for trueness were observed to experiments  $y_8$  and  $y_7$  respectively. Experiments  $y_4$ ,  $y_7$  and  $y_8$  presented the most precise results.

Main effect and interaction contrast analysis presented the factor A as the most important factor for optimization of this analytical procedure and that best results are closely related to levels attributed to B (Main effect:  $p < 0.00001$ ; Contrast AB:  $p = 0.00318$ ).

In this case, a multivariate optimization procedure proved to be of value. If this method was not implemented,  $y_8$  experiment would be considered the best analytical condition. However, it is noticed that factor C has no significant influence on the results. In other words, measuring for 1 or 3 hours does not cause significant differences to the result.

Thus, according to the results and based on the AB contrasts, it was decided to keep the standard condition for the A and B factors (level 0) as the best condition, once the measurement for 9 days (level + of factor A) generated results with a increased uncertainty associated to analytical measurement (in countings per second). As factor C was not directly related to results, level - was considered as the optimal level.

Optimized condition for arsenic measurand in biological matrix samples by INAA adopted in this study was: 7 days to sample decay time, shelf 1 to sample distance to detector and 1 hour to counting time of sample.

### 3.2.2 Experimental design considerations for cobalt, chromium, iron, rubidium, scandium, selenium and zinc

According to the results, it was inferred that experiments  $y_8$  and  $y_4$  presented the best results for all measurands. Experiments  $y_1$  and  $y_5$  presented the worst results. Analyzing the configuration of the experiments  $y_8$  and  $y_4$ , it is a possible to verify that the factor C (counting time) was changed from the level + to -. Nevertheless, there was no significant change of the results because A and B were maintained in level +. In the context, counting time showed the lowest influence to the detection process for Co, Cr, Fe, Rb, Sc, Se and Zn measurands by INAA comparative method. This fact is even more evidenced in the analyses of results obtained to main effect and interaction contrasts.

By means of p-values for main effects and assuming a normal distribution of the results, it was verified that for factor C,  $H_0$  was accepted for all measurands. Therefore, the level change did not affect the final result. In other words, there is no evidence that change of the counting time for sample from 6 to 10 hours gives rise to a positive or negative effect on the final result.

Nonetheless, in this investigative step, it was obtained two distinct behaviors for measurands. The first was observed for Rb, Sc and Se, where main effect and interaction contrast results did not present statistically significant differences. For this group of measurands, different

levels settings for the factors did not influence the final result of analysis. This fact can be easily seen in the results shown in Tables 7 (mean results) and 8 (z-score value).

Co, Cr, Fe and Zn presented a distinct result. By means of p-values results of main effect and interaction contrast it is possible to observe an important influence assigned to the factor A for Fe ( $p = 0.04337$ ), Co ( $p < 0.00001$ ) and Zn ( $p = 0.00847$ ), factor B for Cr ( $p = 0.03402$ ), Co ( $p = 0.00233$ ) and Zn ( $p = 0.00259$ ) and AB interaction for Co ( $p = 0.00643$ ) and Zn ( $p = 0.03028$ ). For Co, the small p-value obtained for factor A ( $p < 0.00001$ ) can be attributed to the presence of spectral interferences for analytical photopeak utilized (factor A, level -).

In this case, the optimization of analytical procedure should be directed to the interactions between such variables. Thus, interaction contrast analysis arises as a lighthouse to guide the path to be followed.

The  $2^k$  experimental design showed to be an important tool for optimization of the analytical procedure for this group of measurands. If a univariate optimization procedure was used, conditions of the experiment y8 (level + to A, B and C factors) would be chosen as the optimized condition. However, a multivariate procedure was able to show that factor C does not present significant influence to the results. Thus, it was observed that A and B are the most important factors to the detection step in INAA. Furthermore, it was verified that the relationship between these variables is of great importance for best results, particularly to zinc and cobalt measurands.

AC, BC and ABC interactions did not present important interactions for the optimization of analytical procedures for the levels attributed to the different factors in this study.

Thus, optimal condition for the measurement of cobalt, chromium, iron, rubidium, selenium, scandium and zinc in biological matrix samples by INAA was: 20 days to sample decay time, shelf 0 to sample distance to detector and 6 hours to counting time of sample. Subsequent tests will indicate on the precision and trueness of analytical results for this configuration.

#### 4. CONCLUSION

In this study,  $2^k$  experimental design was an important tool for the optimization of element determination by INAA. By means of the statistical analysis and theoretical and experimental considerations it was determined the optimized experimental conditions for the analytical methods that will be adopted for the future steps of the validation procedure of INAA methods in the Neutron Activation Analysis Laboratory (LAN) of the Research Reactor Center (CRPq) at the Nuclear and Energy Research Institute (IPEN – CNEN/SP). For As, optimal analytical condition was: 7 days to sample decay time, shelf 1 to sample distance to detector and 1 hour to counting time of sample. For others measurands (Co, Cr, Fe, Rb, Sc, Se and Zn), the analytical optimized condition was: 20 days to sample decay time, shelf 0 to sample distance to detector and 6 hours to counting time of sample.

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