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EXPERIMENTAL DESIGN TECHNIQUE APPLIED TO THE VALIDATION OF AN INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS PROCEDURE

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ABSTRACT

In this study optimization of procedures and standardization of Instrumental Neutron Activation Analysis (INAA) method were carried out for the determination of the elements bromine, chlorine, magnesium, manganese, potassium, sodium and vanadium in biological matrix materials using short irradiations at a pneumatic system. 2^k experimental designs were applied for evaluation of the individual contribution of selected variables of the analytical procedure in the final mass fraction result. The chosen experimental designs were the 2^3 and the 2^4, depending on the radionuclide half life. Different certified reference materials and multi-element comparators were analyzed considering the following variables: sample decay time, irradiation time, counting time and sample distance to detector. Comparator concentration, sample mass 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, interaction effects and better irradiation conditions.

1. INTRODUCTION

The essence of good experimental planning is to design an experiment so that it is able to provide exactly the kind of information we seek.

In a previous study, a 2^3 factorial planning was carried out with biological and geological samples, aiming the analysis of As, Co, Cr, Sb, Sc and Zn by Instrumental Neutron Activation Analysis (INAA), using long lived radionuclides [1].The purpose of this study is to explain the 2^3 factorial planning and other types of planning, such as 2^4, in the investigation of short irradiation elements such as Br, K, V, Mg and Mn.

The factorial design, also called experimental design, represents a set of tests established with scientific and statistical criteria, in order to determine the influence of several variables on the results of a given system or process [2].

Design of Experiments (DOE) is a multivariate methodology of factorial planning used in the optimization of methods and processes. Regardless of the model used, the use of DOE presents a number of advantages over the use of a univariate (conventional) methodology, such as the possibility of reduction in the number of rounds and experiments, indication of

the major variable of the investigated process and the form As the most diverse variables correlate with each other [3].

1.1. Instrumental Neutron Activation Analysis

INAA is used to determine the concentrations of elements present in several matrices. The technique has the advantage of being multielemental and determining a wide range of elements in small samples (around 50 to 200 mg) [4]. Recently, the character of INAA as a primary method of analysis has been proven, a fact that is of great relevance for its application in the certification of reference materials [5].

The INAA comparative method consists of subjecting elemental samples and standards simultaneously to the thermal neutron flux produced in a nuclear reactor and, after an appropriate decay period, to perform the element concentration determination by comparing the peak area of the samples with the Area of the peaks of the known standard, obtained by gamma spectrometry [4].

1.2. Factorial Planning

Experiment planning and analysis techniques are basically used to improve product quality characteristics of manufacturing processes, reduce the number of tests and optimize the use of company resources (material, time of employees, availability of equipment, etc.) [6].This general objective can be divided into other secondary objectives [7];

- identify the variables (control factors) of the process that most influence the response parameters of interest;
- assign values to influential variables of the process so that the variability of the response of interest is minimal or that the value of the result: (quality parameter) is close to the nominal value;
- assign values to the influential variables of the process so that the effect of the non-controllable variables is reduced.

Factorial planning is indicated for the initial phase of the experimental procedure when it is necessary to define the most important factors and to study the effects on the chosen response variable. It is a fixed effects model, that is, the analysis of the effects caused by the factors, cannot be transferred to levels other than that analyzed in the planning [8].

The most appropriate method to treat several factors is a *factorial experiment*. In this approach, the factors vary together, rather than one at a time. If we have k factors, each with two levels, the experiment will be 2^k factorial.

For experiments with four, five or more factors, it is not necessary to use all possible combinations. For cases like these, the technique to be used will be the fractional factorial experiment, which is a variation of basic factorial experiment.

The three basic principles of experiment planning are randomization, replication, and blocking. Through randomization the allocation of the experiment material and order in which the observations of the experiment are performed and determined at random is defined.

Replication is an independent repetition of each combination of factors. Replication has two important properties: it allows obtaining an estimate of experimental error; this estimate is used to evaluate the difference between the statistically significant data.

Blocking is a planning technique to improve the accuracy of how the comparison of factors of interest is done. Generally, blocking is used to decrease or eliminate the variability transmitted by factors that may influence the response, but are not of interest to us (noise factors). Blocking can be defined as a set of relatively homogeneous conditions of the experiment [9].

Factorial schedules are more efficient to study the effects of two or more factors in an experiment, because in each replication, all combinations of factor levels are investigated. The variation of the response produced by a factor variation is what we call the effect of a factor. By often referring to the primary factors of interest, we call it the principal effect. The high and low levels are denoted respectively by "+" and "-" [9].

The factorial experiment with *k* factors, each with two (levels), is called the 2^k experiment. The experimental process of this technique consists of performing tests with each of the combinations of the experimental matrix, to then determine and interpret the main effects and interactions of the investigated factors of the product or manufacturing process.

The major advantage of using a DOE is the realization of a multivariate optimization, where through the calculations of main effect and interaction effects it is possible to understand how the different factors are correlated and the importance that a factor carries in the final result. In addition, the method requires a smaller number of experiments when compared to the univariate optimization methodology. This implies lower expenses and less time required for the execution of the planning. [3].

The designs used in this study were the 2^3 and 2^4, complete. The respective designs were investigated in two different levels, in which one expects to obtain more favorable results (level +) and the other one is expected less favorable results (level -), for the accuracy of the results in INAA.

The factors evaluated in the 2^3complete delineation for the INAA method optimization study were: irradiation time (A), sample distance to detector (B) and counting time (C), as presented in Table 1.1.

Table 1.1: Typical experimental matrix for a complete 2^3 DOE.

Experiment	Contribution	Main Effect			Interaction Effect			
		A	B	C	AB	AC	BC	ABC
1	$A_0B_0C_0$	-	-	-	+	+	+	-
2	$A_1B_0C_0$	+	-	-	-	-	+	+
3	$A_0B_1C_0$	-	+	-	-	+	-	+
4	$A_1B_1C_0$	+	+	-	+	-	-	-
5	$A_0B_0C_1$	-	-	+	+	+	-	+
6	$A_1B_0C_1$	+	-	+	-	-	-	-
7	$A_0B_1C_1$	-	+	+	-	+	+	-
8	$A_1B_1C_1$	+	+	+	+	-	+	+

In the mathematical model, the 2^3 complete DOE is represented by Equation 1 [10].

$$y_{ijk} = \mu + \tau_i + \beta_j + \gamma_k + (\tau\beta_{ij}) + (\tau\gamma_{ik}) + (\beta\gamma_{jk}) + (\tau\beta\gamma_{ijk}) + \varepsilon_{ijk} \quad (1)$$

where:

μ is the average of results;
τ_i is the main effect of factor A;
β_j is the main effect of factor B;
y_k is the main effect of factor C;
$(\tau\beta_{ij})$ is the interaction effect between the factors A and B;
$(\tau\gamma_{ik})$ is the interaction effect between the factors A and C;
$(\beta\gamma_{jk})$ is the interaction effect between the factors B and C;
$(\tau\beta\gamma_{ijk})$ is the interaction effect between the factors A, B and C;
ε_{ijk} is the default effect error.

In the complete design, the factors that were evaluated for the optimization were: irradiation time (A), sample distance to detector (B), counting time (C) and decay time (D), shown in Table 1.2.

Table 1.2: Typical experimental matrix for a complete 2^4 DOE.

Exp.	Contribution	Main effect				Interaction Effect										
		A	B	C	D	AB	AC	AD	BC	BD	CD	ABC	ABD	ACD	BCD	ABCD
1	$A_0B_0C_0D_0$	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+
2	$A_1B_0C_0D_0$	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-
3	$A_0B_1C_0D_0$	-	+	-	-	-	+	+	-	-	+	+	+	+	-	-
4	$A_1B_1C_0D_0$	+	+	-	-	+	-	-	-	-	+	-	-	+	+	+
5	$A_0B_0C_1D_0$	-	-	+	-	+	-	+	-	+	-	+	-	+	+	+
6	$A_1B_0C_1D_0$	+	-	+	-	-	+	-	-	+	-	-	+	-	+	+
7	$A_0B_1C_1D_0$	-	+	+	-	-	-	+	+	-	-	-	+	+	-	+
8	$A_1B_1C_1D_0$	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-
9	$A_0B_0C_0D_1$	-	-	-	+	+	+	-	+	-	-	-	+	-	+	-
10	$A_1B_0C_0D_1$	+	-	-	+	-	-	+	+	-	-	+	-	+	+	+
11	$A_0B_1C_0D_1$	-	+	-	+	-	+	-	-	+	-	+	+	+	-	+
12	$A_1B_1C_0D_1$	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-
13	$A_0B_0C_1D_1$	-	-	+	+	+	-	-	-	-	+	+	+	-	-	+
14	$A_1B_0C_1D_1$	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-
15	$A_0B_1C_1D_1$	-	+	+	+	-	-	-	+	+	+	-	-	-	+	-
16	$A_1B_1C_1D_1$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

2. EXPERIMENTAL

The experimental part of this study was carried out in the following steps:
- definition of the factor parameters for the 2^3 and 2^4 experimental designs;
- assembly of the experiments for irradiation;
- assembly of samples after irradiation.

2.1. Definition of the factor parameters for the 2^3 and 2^4 experimental designs.

When planning any experiment, the first thing that must be done is to decide which factors and the answers of interest. A typical DOE presents two levels to be investigated, denominated level +1 and level -1. At level +1, it is assigned to the level that will get better results and at level -1 it is attributed to the level that will obtain worse results. Some specific models may also require information on the factor configuration that is assigned to the default level, which is called the 0 level.

In the 2^3 planning, the parameters of configuration are described in Table 2.1.

Table 2.1: Factors and levels of the 2^3 DOE.

Factor		Level +	Level -	Level 0
A	Irradiation time	60 s	15 s	30 s
B	distance sample - detector	0 cm	6.2 cm	3.0 cm
C	counting time	300 s	100 s	600 s

In the 2^4 factorial planning, the parameters were similar to the factorial planning 2^3, but irradiation time was also considered as presented in Table 2.2.

Table 2.2: Factors and levels of the 2^4 DOE.

Factors		Level +	Level -	Level 0
A	Irradiation time	60 s	15 s	30 s
B	distance sample - detector	0 cm	6.2 cm	3.0 cm
C	counting time	2700 s	1800 s	2100 s
D	Decay time	1 h	30 min	45 min

2.2. Assembling the experiment for irradiation

The Certified Reference Material (CRM) chosen for the factorial planning was a mussel tissue reference material, produced at IPEN – CNEN/SP [11].

The standards and samples were inserted into a polyethylene vial and placed in a plastic container called "rabbit".

Multielemental standards for Mg and V (MV standards), along with subsamples of the mussel tissue CRM were assembled in eight experiments, that is, in eight rabbits, according to the complete 2^3design.

Multielemental standards for Na, K and Mn (NKM standards) and for Cl and Br (CB standards) were assembled in 16 rabbits, according to the complete 2^4 design. Both designs were subjected to irradiation and analysis of 3 test samples in each experiment.

Standards and samples were irradiated in the CRPq IEA-R1 research reactor at 4.5 MW, under a neutron flux of 0.5 to 1.0 x 10^{12} cm^{-2} s^{-1}.

2.3. Assembly of samples after irradiation

After irradiation, samples and standards were sealed in stainless steel holders prior to analysis. Samples and standards were transferred to the stainless steel support and after, were placed in lead containers called "castles", and finally were taken to the gamma spectrometer measuring room.

Gamma radiation measurements were performed using a Canberra GC2018 HP Ge high purity germanium detector coupled to a Canberra DSA-1000 digital spectrum analyzer. Gamma-ray spectra were collected and processed using the Genie 2000 program, version 3.1 (Canberra). The calculation of the concentration of the elements was done using a spreadsheet (Microsoft Excel).

3. RESULTS AND DISCUSSION

The results obtained in the planning of the 2^3 DOE are presented in Table 3.1.

Table 3.1: Results for the 2^3 DOE for Mg and V (mean ± standard deviation, n=3).

Experiment	Factor and level			Concentration	
	A	B	C	Mg, %	V, mg kg^{-1}
1	-1	-1	-1	0.29 ± 0.00	2.80 ± 0.21
2	+1	-1	-1	0.31 ± 0.04	2.89 ± 0.11
3	-1	+1	-1	0.32 ± 0.05	2.91 ± 0.06
4	-1	-1	+1	0.32 ± 0.13	2.11 ± 0.03
5	+1	+1	-1	0.32 ± 0.06	2.49 ± 0.03
6	+1	-1	+1	0.35 ± 0.21	2.74 ± 0.04
7	-1	+1	+1	0.31 ± 0.08	2.43 ± 0.03
8	+1	+1	+1	0.38 ± 0.52	2.76 ± 0.10

Using the multivariate DOE, calculations for the effects and interactions were performed as presented in Table 3.2. The Sum of Squares (SS) is defined as a measure of the total variability of the data.

Table 3.2: Results of the effects and interactions of multivariate 2^3 DOE for Mg and V.

Factors	Mg			V		
	Effect	SQ	Contribution, %	Effect	SQ	Contribution, %
A	0.06	0.01	0.81 %	-0.01	0.01	0.17
B	-0.71	2.02	9.22 %	-0.08	0.03	5.18
C	0.13	0.07	3.53 %	0.19	0.15	2.48
AB	0.02	0.03	0.15 %	-0.10	0.04	6.74
AC	0.10	0.04	1.87 %	-0.20	0.16	27
BC	-0.06	0.01	0,07 %	-0,22	0,19	3.2
ABC	0.067	0.018	0.8 %	0.06	0.015	2.4

As observed in Table 3.2, factors B (distance sample-detector) and C (counting time) presented the higher contributions for the main effect model, despite only the AC interaction was more representative. Similar information was observed in the SS column.

In order to better verify the variability of the results, Analysis of Variance (ANOVA) was performed. The objective of this technique is to analyze the mean variation of test results and to demonstrate which are the factors that actually produce significant (main and interaction) effects on the responses of a system. ANOVA is used to statistically accept or reject the investigated hypotheses. In this case, the null hypothesis is that there are no significant differences among the results of the various experiments for each design of experiments (DOE).

In Table 3.3, the ANOVA output is presented, which consists of the variability of the data in components in the same bottle.

Table 3.3: ANOVA test for Mg and V variability, for the results of INAA.

Elements	*F*	*p* value	*Fc*
Mg	0.84	0.44	3.46
V	0.30	0.74	3.46

Table 3.3 shows that for both elements, Mg and V, $F < F_c$ and the p value > 0.05 then the null hypothesis H_0 is accepted, since there is no difference between the groups. It means that in this specific study the mean results are equivalent, independent from the irradiation conditions.

In a similar fashion, in the 2^4 DOE, the results were analyzed by means of the mean and standard deviation and the ANOVA test.

The results obtained in the planning of the 2^4 DOE are presented in Table 3.4. Using the multivariate DOE, calculations for the effects and interactions were performed as presented in Tables 3.5 and 3.6.

Table 3.4: Results for the 2^4 DOE for Na, K, Mn, Cl and Br (mean ± standard deviation, n = 3).

Exp.	Factor and Level				Concentration				
	A	B	C	D	Na, %	K, %	Mn, mg kg^{-1}	Cl, mg kg^{-1}	Br, mg kg^{-1}
1	-1	-1	-1	-1	3.48 ± 0.69	0.73 ± 0.06	26.46 ± 0.53	3.87 ± 0.46	268± 0.4
2	+1	-1	-1	-1	3.54 ± 0.31	0.86 ± 0.06	27.26 ± 1.32	3.45 ± 0.78	265 ± 0.8
3	-1	+1	-1	-1	3.61 ± 0.34	0.76 ± 0.55	27.46 ± 0.54	3.46 ± 0.73	255 ± 1.6
4	-1	+1	-1	-1	3.75 ± 0.29	0.76 ± 0.83	25.73 ± 0.40	3.52 ± 0.99	258 ± 1.2
5	-1	-1	+1	-1	3.69 ± 0.08	0.81 ± 0.81	27.73 ± 0.89	3.65 ± 0.86	261 ± 0.8
6	+1	-1	+1	-1	3.59 ± 0.08	0.77 ± 0.31	22.9 ± 0.65	3.61 ± 0.93	266 ± 0.8
7	-1	+1	+1	-1	3.64 ± 0.24	0.66 ± 0.07	23.63 ± 0.77	3.54 ± 0.99	277 ± 1.3
8	+1	+1	+1	-1	3.62 ± 0.24	0.70 ± 0.12	23.51 ± 0.81	3.57 ± 0.77	274 ± 0.8
9	-1	-1	-1	+1	3.63 ± 0.33	0.86 ± 0.14	23.26 ± 0.90	3.73± 0.48	274 ± 1.7
10	+1	-1	-1	+1	3.53 ± 0.79	0.87 ± 0.85	24.27 ± 0.69	3.44 ± 0.63	276 ± 0.8
11	-1	+1	-1	+1	3.59 ±0.60	0.69 ± 0.57	24,04 ± 0.16	3.44 ± 0.68	265 ± 2.6
12	+1	+1	-1	+1	3.43 ± 0.92	0.99 ± 0.35	23.28 ± 2.53	3.33 ± 0.56	266 ± 0.8
13	-1	-1	+1	+1	3.62 ± 0.56	0.82 ± 0.33	24.62 ± 0.86	3.65 ± 1.08	276 ± 0.9
14	+1	-1	+1	+1	3.63 ± 0.29	0.88 ± 0.25	24.16± 0.38	3.22 ± 0.62	263 ± 1.2
15	-1	+1	+1	+1	3.64 ± 0.43	0.86 ± 0.95	22.50 ± 0.74	3.20 ± 0.34	259 ± 2.1
16	+1	+1	+1	+1	3.62 ±0.27	0.70 ± 0.73	26.16 ± 0.75	3.48 ± 1.20	257 ± 0.8

Table 3.5: Results of the effects and interactions of multivariate 2^4 DOE for Na, K and Mn.

Factors	Na			K			Mn		
	Effect	SQ	Contr.%	Effect	SQ	Contr.%	Effect	SQ	Contr.%
A	-0.25	0.25	0.04	0.43	0.76	1.66	-0.34	0.47	0.10
B	0.22	0.20	0.03	-0.58	1.38	2.99	-0.61	1.50	0.34
C	0.60	1.47	0.22	-2.46	24.3	52.68	-0.76	2.32	0.53
D	-0.29	0.34	0.05	0.81	2.64	5.73	-1.48	8.85	2.02
AB	0.08	0.02	0.04	0.06	0.01	0.03	-5.13	105.4	24.04
AC	-0.09	0.03	0.05	-0.71	2.03	4.41	-0.07	0.02	0.04
AD	-0.44	0.79	0.12	-1.61	10.4	22.5	1.25	6.26	1.49
BD	-0.27	0.28	0.04	-0.28	0.33	0.71	-0.28	0.32	0.073
BC	-0.57	1.30	0.19	0.087	0.03	0.06	0.91	3.33	0.76
CD	0.20	0.17	0.02	0.062	0.01	0.03	1.047	4.38	1.00
ABC	-9.02	325	49.9	-0.33	0.45	0.98	1.33	7.15	1.63
ABD	-0.32	0.41	0.06	0.08	0.03	0.06	6.33	160.2	36.55
ACD	8.93	319	48.9	-0.28	0.33	0.72	1.15	5.37	1.22
BCD	0.63	1.60	0.24	0,08	0.03	0.06	5.758	132.6	30.25
ABCD	0.03	0.04	0.06	-0.91	3.33	7.23	0.141	0.07	0.018

In planning 2^4 of the Na, K and Mn elements, the significant effects were C (counting time) and D (decay time). The interactions that stood out were AD, BC, AC and AB and those of three Factors were ABC, ACD and ABD. Although A shows significance in interactions, in both the two and three factors, the most prevalent effect is C and D.

Table 3.6: Results of the effects and interactions of multivariate 2^4 DOE for Cl and Br.

Factors	Cl			Br		
	Effect	SQ	Contr.%	Effect	SQ	Contr.%
A	-1.32	6.96	13.49	-1.46	8.55	0.02
B	-1.22	6.01	11.64	-4.74	89.7	0.22
C	-0.24	0.24	0.47	-1.31	6.89	0.01
D	-1.32	6.99	13.54	70.1	1.96	48.1
AB	1.94	15.17	29.37	0.99	3.90	0.09
AC	0.92	3.40	6.59	-1.93	15.0	0.03
AD	-0.05	0.01	0.02	-1.89	14.3	0.03
BD	0.35	0.49	0.96	4.98	99.5	0.24
BC	-0.27	0.29	0.57	-5.71	130.5	0.32
CD	-0.74	2.23	4.33	-7.13	203.7	0.49
ABC	-0.02	0.03	0.00	-0.38	0.60	0.01
ABD	0.24	0.24	0.47	1.31	6.89	0.01
ACD	-0.32	0.42	0.83	-2.5	25.0	0.06
BCD	0.20	0.16	0.31	28.9	3.34	8.19
ABCD	1.49	8.97	17.36	-65.66	17.2	42.2

In the 2^4 planning of Br, the effects that mostly stood out were B (distance sample-detector) and D (decay time). The interactions in the two factors were AB, AC and BD and in the three factors were BCD. On the other hand, in the Cl analysis, the effects that were evidenced were A, B and D, those of two factors AB, AC, BD and CD and the three factors were ACD and BCD. Factor B and D were the most prominent effects between Cl and Br, although A was strongly related to other interactions.

The number of interactions involved in the factorial design using the NKM and CB standards, are still under investigation, in order to get a better, more detailed resolution of the effects.

The result of ANOVA calculation for NKM and CB standards is presented in Table 3.7.

Table 3.7: ANOVA test for Na, K, Mn, Cl and Br variability, for the results of INAA.

Element	*F*	*P value*	*Fc*
Na	5.44	2.9E-05	1.99
K	4.65	1.2E-04	1.99
Mn	6.42	5.4E-06	1.99
Cl	8.15	3.9E-07	1.99
Br	6.17	2.7E-19	1.99

It was observed in Table 3.7 that $F > F_c$ and the value of $p < 0.05$ for all elements. Then it was concluded that the null hypothesis H_0 is rejected and the alternative hypothesis H_1 is accepted, considering that there are differences and interactions between the means of the results and obtained results may vary according to the measurement protocol.

The *z*-score, according to Andriotti, is the number of standard deviations by which any value is distant from the mean, where x_i represents each observation, $\bar{X}$ represents the mean and SD is the standard deviation [12].

The values of *z*-score in the Mg, V, Na, K, Mn, Cl and Br experiments were calculated in Table 3.8 and 3.9, in order to verify the best conditions to be used as a INAA protocol and to compare with the Mussel Tissue reference material certified values. A mean *z*-score |*z*| was also calculated as the mean of the absolute *z*-scores for each experiment, in order to provide an overall index of the quality of the experiments.

Table 3.8: Results of the *z*-score in the Mg and V experiments.

Experiment	*z*-score		|*z*|
	Mg	V	
1	-1.63	-0.11	0.87
2	-1.15	0.00	0.58
3	-1.03	0.03	0.53
4	-1.05	-0.95	1.00
5	-1.00	-0.49	0.75
6	-0.30	-0.18	0.24
7	-1.25	-0.56	0.91
8	0.53	-0.16	0.34
Certified value [11]	0.36 ± 0.04, %	2.89 ± 0.82, mg kg^{-1}	

Table 3.9: Results of the *z*-score in the Na, K, Mn, Cl and Br experiments.

Experiments	*z*-score					|*z*|
	Na	K	Mn	Cl	Br	
1	4.04	-0.73	0.32	0.60	0.44	1.23
2	4.26	0.45	0.40	-0.70	0.36	1.24
3	4.48	-0.45	0.42	-0.36	0.12	1.17
4	4.94	-0.36	0.19	-0.23	0.20	1.18
5	4.73	0.09	0.45	0.08	0.26	1.12
6	4.40	-0.55	-0.05	0.00	0.38	1.08
7	4.57	-1.36	0.02	-0.18	0.65	1.36
8	4.50	-1.00	0.01	-0.11	0.57	1.24
9	4.55	0.45	-0.01	0.27	0.58	1.17
10	4.20	0.64	0.09	-0.40	0.62	1.19
11	4.41	-1.09	0.06	-0.42	0.37	1.27
12	3.87	1.64	-0.01	-0.67	0.38	1.31
13	4.51	0.09	0.12	0.08	0.63	1.09
14	4.54	0.73	0.08	-0.92	0.32	1.32
15	4.57	0.45	-0.09	-0.97	0.23	1.26
16	4.48	-0.91	0.29	-0.32	0.17	1.23
Certified value [11]	2.27±0.30,%	0.81±0.11,%	23.4±3.1,mg kg^{-1}	3.62±0.43,mg kg^{-1}	250±42,mg kg^{-1}	

Except for Na, *z*-score results were considered adequate for the INAA methods used as $-2 < z < 2$. The unfavorable Na results are still under investigation.

In Table 3.8, the smallest value of $|z|$ was observed for the Experiment number 6. Then, the established condition to be worked is the irradiation time of 60 s, the distance of the sample in the detector of 6.2 cm and the counting time 300 s.

In Table 3.9, it is also observed that smallest value of $|z|$ was observed for the Experiment number 6. The same established condition of irradiation time of 60 s, distance of the sample in the detector of 6.2 cm, but counting time of 2700 s and decay time of 30 min.

4. CONCLUSIONS

In the study, the concentrations of Na, Cl, Br, K, Mg, Mn and V were determined by INAA as part of a factorial planning design, with the intention to choose the best conditions for the irradiation and to investigate the most comprehensive effects that may occur. In the 2^3 planning, the results obtained in ANOVA showed that there are no differences between groups, regardless of the variations in the irradiations, while in the 2^4 planning ANOVA results showed significant differences between groups. Using a *z*-score analysis, it was observed that the best irradiation conditions for the optimization of the INAA method were similar. It was concluded that with the two designs (2^3 and 2^4) the same analysis protocol can be chosen to optimize the analytical results. In the near future, the design of experiments will be evaluated using the gross activities values for the irradiations, in order to better investigate the factorial design output and optimize the method for element determination in biological samples via INAA.

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