Determination of Al in cake mix: Method validation and estimation of measurement uncertainty.

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Abstract: An analytical method for the determination of Al in cake mix was developed. Acceptable values were obtained for the following parameters: linearity, detection limit – LOD (5.00 mg·kg^{-1}) quantification limit – LOQ (12.5 mg·kg^{-1}), the recovery assays values (between 91 and 103%), the relative standard deviation under repeatability and within-reproducibility conditions (<20.0%) and measurement uncertainty tests (<10.0%) The results of the validation process showed that the proposed method is fitness for purpose.

Keywords: In house validation; measurement uncertainty; aluminum; cake mix.

1. INTRODUCTION

Aluminum (Al) is the third most abundant element in the earth's crust and is also present in water and food. For over a century it has been recognized as a neurotoxic element [1].

One of the main routes of exposure to aluminum in the general population is food consumption, especially of those with food additives containing Al [2]. Additives and Contaminants) evaluated the toxicity of Al and established the Provisional Tolerable Weekly Intake (PTWI) 2 mg Al / kg body weight per week, from all sources, including additives. As reported in sixty-seventh meeting, some population groups could exceed Al, especially children, due to the consumption of food additives containing aluminum added in its composition [3]. The aluminum content in food has been studied in several countries. However, in Brazil, there are few data addressing the Al concentration.

Considering the relevance of the Al presence in the diet of the population, the aim of this work is to present a detailed procedure for in-house method validation and evaluate the uncertainty sources for determining the levels of Al in cake mix by ICP OES. The analytical performance of the entire procedure, such as linearity, detection, limit, quantification limit, precision and accuracy were assessed statistically to evaluate the developed procedures.

2. MATERIAL AND METHODS

2.1. Instrumentation

The acid digestion of cake mix samples was performed using a closed-vessel microwave system (Model D Start, Milestone, Italy). The device can work up to the power of 1000 W

during the time set by the user, providing that a vessel does not exceed 100 bars pressure or 300°C.

The table 1 summarizes the operating conditions used in ICP OES with radially viewed configuration (Optima 3000, Perkin Elmer, USA)

Table 1 - Instrumental conditions of the ICP OES.

Parameter	Value
RF incident power	1500
Nebulizer	Cross-flow
Nebulizer argon flow rate	1.0 L·min^{-1}
Sample flow rate	1.5 mL·min^{-1}
Auxiliary argon flow rate	0.5 L·min^{-1}
Plasma argon flow rate	15 L·min^{-1}
Flow pumping	1.5 mL·min^{-1}
Diameter of the injector tube	2.0 mm
Height of view radial	10 mm
Wavelength of the Al (atomic line)	396.153 nm
Ionization Energy	9.13 eV[1]

2.2. Reagents and solutions

High purity deionized water (resistivity 18MΩ cm) was obtained from a Milli-Q Plus water purification system (Millipore Corp, EUA). Analytical reagent grade HNO_3 65% (w·w^{-1}) (Merck, Germany) previously was purified by sub-boiling (Berghof, Germany). For the preparation of standards was used certified multielement solution (SPEX, Certipur, Lot 27200JB), containing 500 mg·L^{-1} of Al.

2.3. Contamination care

In order to prevent contaminations all operations were performed in a clean room ISO Class 7 equipped with islands of laminar flow ISO class

5. The materials used in the test consists of Teflon or polyethylene and have been thoroughly decontaminated solution with HNO_3 10% (w·w^{-1}) for 24 hours, then washed with ultrapure water, dried and kept in laminar flow.

2.4. Digestion procedure

The digestion procedure for all samples was performed as [4], [5] and [6]. Aliquots (0.2-0.3 g) of homogenized sample were digested with 3 mL of 65% nitric acid (w·w^{-1}) and 3 ml of high purity deionized water in a closed microwave. The heating program for digestion of the samples was previously optimized [6]. The samples were transferred to 50 mL volumetric flasks.

2.5. Calibration and sample analysis

Two sets of standards were used for calibration: one for low concentrations of Al (20 µg·L^{-1} to 200 µg·L^{-1}). Analytical blanks were also prepared in order to evaluate the noise and zero adjustment instrumental, but were not included in the calculations. The calibration curves were assessed through ordinary least squares estimation, outlier detection and lack of fit tests [7]

2.6. Validation

The performance characteristics of the method were established by assays employing standards solutions, sample blanks and spiked samples. Linearity, matrix effects, lack of trueness (recovery), precision under repeatability and within-reproducibility conditions, detection and quantification limits were the parameters evaluated [8].

All the tests for linearity assumption were also done for these curves [7].The slopes and intercepts were compared by t-test for a 0.05 after evaluation the assumption of homogeneity of variances. The standard addition method was used to check the matrix effects. Due the absence certified reference material for matrix studied, the

parameters lack of trueness and precision were estimated through recovery.

The recovery and precision were established by spiked samples in four different days, with three independent replicates at the same levels of linearity test. Blank samples were also prepared, in triplicate, for each curve. The values obtained were analyzed. The repeatability and within-reproducibility were estimated by ANOVA [7].

The theoretical detection limit (LD) and quantification limit (LQ) were estimated using the background equivalent concentration, and after were confirmed by testing the recovery and precision.

2.8. Measurement uncertainty

Uncertainties were estimated according to the Guide to the Expression of Uncertainty in Measurement[9].Relevant sources for the uncertainty assessment have been divided into four groups: a) the uncertainty associated with sample preparation, b) preparation of standard solutions, c) the analytical curve, d) method precision. To calculate the expanded uncertainty (U), a coverage factor (k) of 2 was used which corresponds to a 95% interval for a normal probability distribution.

3. RESULTS AND DISCUSSION

3.1. Validation of method

Outliers were removed before statistical tests. The results obtained for the linearity assessment are shown in table 2. The assumptions that the errors are normally distributed, homoscedastic and independent were confirmed as proposed by [7].

All the regression assumptions were confirmed for the solvent and matrix-matched calibration curves. No significant matrix effects were detected when the slopes of the solvent and matrix-matched curves were compared.

Table 2 - Evaluation of Linearity for curves in nitric acid and the matrix in the concentration range studied.

Statistic	Curve in nitric acid	Curve in matrix
n	18	18
Outilers	0	2
R	0.9937	0.9894
Rcrític	0.9567	0.9529
p	>0.10	>0.10
d	1.516	2.116
p	>0.10	>0.10
tL	1.101	0.732
p	0.2870	0.4764
F^1	$2.545 \cdot 10^3$	$1.710 \cdot 10^3$
p	$4.57 \cdot 10^{-19}$	$4.89 \cdot 10^{-16}$
F	1.224	2.083
p	0.352	0.158

n = Number of observations; R = Ryan‑Joiner correlation coefficient. R crític = Ryan‑Joiner critics correlation coefficients; d = Durbin‑Watson statistic. t = Levene t-statistic.; F1= ratio of variances of the regression; F2= Variance ratio; p = significance.

The Table 3 presents the estimated recovery, relative standard deviations under repeatability (RSDr) and within-reproducibility (RSDR).

Table 3 - Mean recovery, relative standard deviation under repeatability and within-reproducibility conditions.

Concentration ($\mu g \cdot L^{-1}$)	MR (%)	RSDr (%)	RSDR (%)
20	93.16	16.88	19.39
50	101.98	4.29	5.59
80	101.98	3.10	4.13
120	97.71	3.24	6.46
160	93.72	7.33	7.33
200	91.70	4.81	6.20

MR = mean recovery, RSDr = repeatability relative standard deviation, RSDR = within reproducibility relative standard deviations.

These recovery results were considered acceptable and demonstrated no lack of trueness of the validated method. For the first level were obtained RSDr and RSDR above than the respective critical values (14.7%) [8],these results indicate accuracy and precision of the method in the range above of 12.5 mg ·kg^{-1}.

The theoretical detection and quantification limits were estimated in 5.0 mg kg^{-1} and 12.5 mg kg^{-1}, respectively. They were confirmed by recovery experiments.

The individual components of total uncertainty were estimated by the error propagation approach. The figure 1 shows diagram of cause and effect.
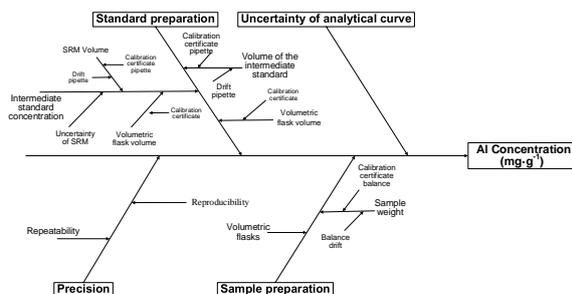

Figure 1 - Cause and effect diagram for the determination of Al.

The values of the standard uncertainty contribution from each source are shows in Figure 2. The uncertainties associated with the precision are the main contribution in the method.
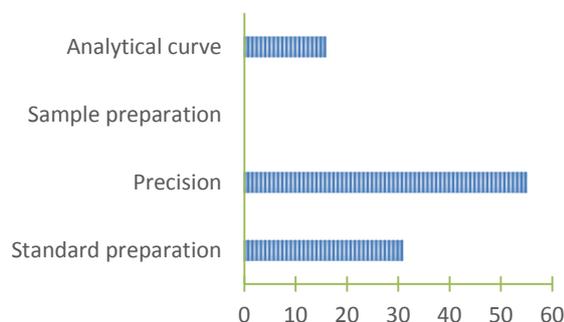

Figure 2 - Standard uncertainty contribution from each source.

For the analyzed sample, whose Al concentration determined, was 34.6 mg·kg^{-1}, the value of expanded uncertainty (U) was 2.0mg·kg^{1}, which corresponds less than 10% of the concentration value.

4. CONCLUSIONS

The method presented good results of selectivity, linearity, precision (repeatability and reproducibility), recovery and the expanded uncertainties. These results showed that the proposed method is fitness for purpose.

5. REFERENCES

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