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ACCURACY EVALUATION IN THIAMIN QUANTIFICATION IN FOOD MATRICES

Cristina Flores , *M. Graça Dias*, *Mariana Santos*

National Health Institute Doutor Ricardo Jorge, IP, Food and Nutrition Department
Av. Padre Cruz, 1649-016 Lisbon, Portugal E-mail: cristina.flores@insa.min-saude.pt

Abstract

The performance evaluation of an RP-HPLC method to determine thiamine using different food matrices and reference materials, showed the method suitability to its purpose and to be submitted to accreditation. Intermediate precision evaluate through the relative standard deviation (RSD%) was under 8% for all food matrices tested an all the z-scores obtained for the reference materials were < 1.5.The estimated expanded measurement uncertainty was 13%.

Keywords: Thiamin, accuracy, foods, HPLC.

1. INTRODUCTION

Reliable data on the nutrient composition of foods can only be obtained by the careful performance of appropriate, accurate analytical methods in the hands of trained analysts. The choice of the appropriate methods carried out under quality assurance schemes is the second crucial element in ensuring the quality of the values in a food composition database., The laboratory has, since 2009, submitted to accreditation several analytical methods for vitamin quantification. This process aims the obtainment of accurate and traceable data, for food vitamin content, to nutrition studies, food labeling or to the regular updating of the Portuguese Food Composition Table.

The evaluation of a method performance is essential to assess the accuracy and associated uncertainty of the obtained results. Accuracy evaluation involves precision and trueness studies as well as uncertainty estimation [1,2].

In this work we validated a standard method for thiamin quantification in food matrices. Precision was evaluated in matrices of four different food groups: legumes, grains, dairy products and meat.

2. MATERIAL and METHODS

2.1. Food Samples

Powder milk, green peas, corn flour and chicken were the matrices selected to the precision study. Trueness was evaluated through quantification of two reference materials (RM) proficiency testing samples; FAPAS 2183 (liquid vitamin supplement) and FAPAS 2186 (breakfast cereal) with stated standard uncertainties of 0.308 mg/mL and 0.031 mg/100 g, respectively.

2.2. Reagents and standards solutions

Methanol of HPLC grade and ultrapure type I water were used. All the other reagents were of analytical grade. Standard stock solutions were prepared every four weeks from thiamin standards of purity ≥98% and their exact concentration determined spectrophotometrically.

Derivatization solution - 15% NaOH, 0,04 % K3 [Fe(CN)6] - and standard working solutions (0,1-1 µg/ml) were prepared daily.

Takadistase (86247) and β-amylase (A-7130), from Sigma, were used in the enzymatic treatment.

2.3. Analytical methodology

Thiamin was quantified by RP-HPLC with pre-column derivatization and fluorometric detection [3,4,5].

Samples, 2 to 10 g, were submitted to acid hydrolysis with 0.1 M HCl for 30’ in an autoclave at 121ºC. After cooling pH was adjusted to 4.5 with 2.5 M sodium acetate and samples were submitted to an overnight enzymatic treatment at 37ºC, using takadistase (0.25 g/sample) and β-amylase (0.05 g/sample, with agitation. Extracts were diluted to 100 mL with water and filtred trough a qualitative paper filter. 2 mL of derivatization solution was added to 1 mL of each standard, sample or blank and, following thiamine oxidation to thiochrome. Solutions were purified through a solid phase extraction column,
(WATERS 020515) and diluted to 10 ml.

The final solutions, after filtration through 0.45 µm PVDF filters were injected in a HPLC system and separated at 37 ºC in a Phenomenex Luna 5 µm C18 1000A column (250 x 4,6 mm).

The mobile phase consisted of 0.05 M acetate buffer + methanol (70:30) with a flow rate of 1 ml/min. Excitation and emission wavelengths were 366 nm and 435 nm, respectively.

The HPLC system was a Waters 2695 Separations Module equipped with a sample cooler, a column heater/cooler and a 2475 multi-wavelength fluorescence detector.

Data were collected and analysed using the “Empower” software. Peak identification was performed comparing the retention times with those from pure standards. Quantification was made using a six point external standard calibration (0.01 µg/mL – 0.1 µg/mL).

2.4. Accuracy evaluation

Each of the matrices evaluated for precision was tested in triplicate in four different days. Repeatability and intermediate precision standard deviations were calculated, for each matrix, trough ANOVA ($p<0.05$) [6]. Repeatability (r) and intermediate precision (Pi) limits, were calculated using the following equations [7] :

$$r = 2.8 * S_r \qquad (1)$$

$$Pi = 2.8 * S_{Pi} \qquad (2)$$

Trueness was evaluated from the z-scores obtained; FAPAS 2183 was tested once and FAPAS 2186 was tested 5 times, in intermediate precision conditions, over a three months period. FAPAS samples were tested in duplicate.

Mesurement uncertainty was estimated from bottom-up approach using the validation data obtained in the mentioned precision and trueness studies.

Homocedasticity of the results obtained for the different matrices tested for precision was evaluated through an F-test to validate the calculated pool relative standard deviation - RSD_{pool} - (3) as the standard uncertainty associated to the method precision (μ_P) [8] were n_i is the number of different days in which the matrix i was tested and RSD_i is the relative standard deviation of the 4 daily mean results obtained, for each matrix.

$$\mu_P = RSD_{pool} = \sqrt{\frac{\sum(n_i-1)*RSD_i^2}{\sum(n_i-1)}} \qquad (3)$$

Trueness associated uncertainty (μ_T) was estimated from the data obtained from FAPAS 2186, according to [9] (4,5):

$$BIAS = \left|\frac{\overline{X}_{obs}-X_{RM}}{X_{RM}}\right| \qquad (4)$$

$$\mu_T = \sqrt{(BIAS)^2 + \left(\frac{S_{obs}*100}{\sqrt{n}*\overline{X}_{obs}}\right)^2 + \left(\frac{\frac{U_{MR}}{k}}{X_{MR}}\right)^2} \qquad (5)$$

were, X_{RM} and U_{RM} are, respectively, the stated value and the expanded uncertainty of the RM (k=2), n the number of tests and $\overline{X}_{obs}$ and S_{obs}, the average and standard deviation, respectively, of the obtained results.

Combined standard uncertainty (μ_C) was calculated using equation (6) and, to obtain expanded uncertainty (U), a coverage factor (K) of 2 was applied to μ_C.

$$\mu_c = \sqrt{(\mu_P)^2 + (\mu_T)^2} \qquad (6)$$

Additionally, combined standard uncertainty for each food matrice *i*, was calculated using (6), replacing μ_P by RSD_i.

3. RESULTS

Precision data are presented, below, in table 1 and Fig. 1. All the │Z-score│ values obtained in the RM tests were < 1.5 and within the acceptance criterion of │Z-score│< 2. (Fig. 2).

Table 1. Precision data (mg/100 g). S_r – Repeatability standard deviation, r – repeatability limit, S_{Pi} – Intermediate precision standard deviation, Pi - Intermediate precision limit, $\bar{x}$ – Average

	S_r	r	S_{Pi}	Pi	$\bar{x}$
Powder milk	0.009	0.024	0.009	0.025	0.19
Green peas	0.003	0.008	0.01	0.028	0.091
Corn flour	0.005	0.014	0.02	0.057	0.153
Chicken chest	0.004	0.012	0.01	0.029	0.098

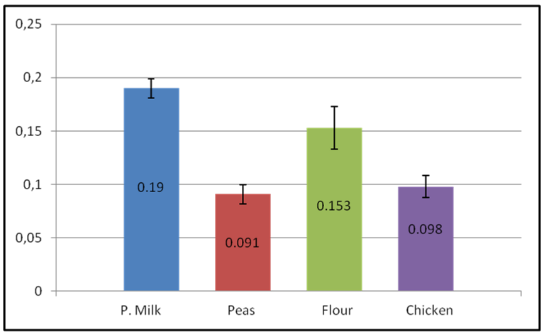

Fig.1. Mean values of thiamin concentration (mg/100 g). Error bars represent +/- 1 standard deviation.

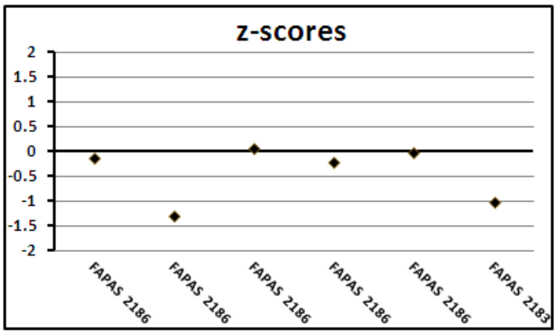

Fig.2. Trueness data

RSD_{pool}, was validated as the standard uncertainty associated to the method precision (μ_P); the calculated F value, 5.29, is < than critical F value; F(3, 3, 0.01) = 29.45.

The calculated values for the uncertainties associated to the results obtained with the method; μ_p, μ_T,. μ_c. and U were, respectively, 6.0%, 2.6%, 6.6% and 13%.

Uncertainty values calculated for individual matrices are presented in table 2 and expanded uncertainties are compared to the method uncertainty, in Fig.3.

Table 2.Ucertainty data (μ_P - Precision uncertainty μ_T - Trueness uncertainty, μc - Combined uncertainty, U - Expanded uncertainty, k=2).

	Powder milk	Green peas	Corn flour	Chicken chest	FAPAS 2186	Method
μ_p%	2.7	6.5	7.4	6.2	—	6.0
μ_T%	2.6	2.6	2.6	2.6	2.6	2.6
μ_c%	3.7	7	8.1	6.7	—	6.6
U %	7.4	14	16	13	—	13

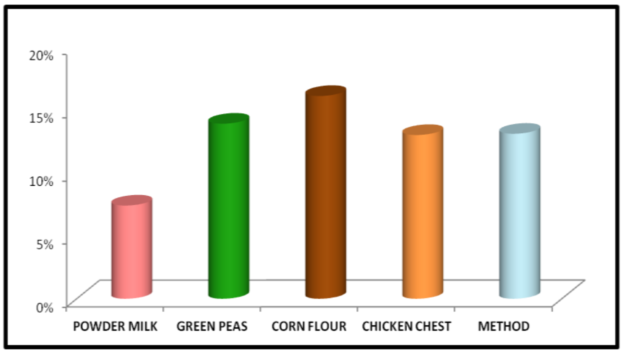

Fig.3. Relative expanded uncertainty (U), k=2.

4. DISCUSSION

The relative standard deviation of repeatability values for thiamin, obtained, in this study were, for all food matrices, in accordance with the ones presented in the method standard [5].

Truness evaluation using liquid vitamin supplement and breakfast cereals, showed a good method/laboratory performance.

The major contribution to the measurement uncertainty by the present method was the precision. According to the obtained value for measurment uncertainty, results should be reported with two significant figures.

Method accuracy evaluation and uncertainty estimation, showed the method suitability for its purpose and to be submitted to accreditation.

5. REFERENCES

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