Accuracy evaluation in thiamin quantification in food matrices

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Abstract: Performance evaluation of the method showed its suitability. Intermediate precision relative standard deviation was under 8% for all food matrices tested. For all reference materials, the obtained Z-scores were < 1.5. The estimated expanded measurement uncertainty was 13%.

INTRODUCTION

The evaluation of a method performance is essential to assess the accuracy and associated measurement uncertainty of the obtained results. Accuracy evaluation, the estimation of the closeness of agreement between a quantity value obtained by measurement and the true value of the measurand, involves precision and trueness studies and these studies are essential to uncertainty estimation.

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

METHODS

Pre Analytical Procedure

Food samples were grinded, homogeneized, aliquoted and frozen at -18ºC.

Quantification

Samples – 1 to 10 g
Extraction – 0.1 M HCl, 121°C, 30 min
Derivatization – Pre-column oxidation with potassium hexocyanoferrate III
Enzymatic treatment –Takadistase/β-amylase
Purification – SPE C18 55-105 µm, 50/pk
Separation – Phenomenex Luna 5µm C18 1000A column (250x4,6mm)
Mobile phase – 0.05 M acetate buffer + methanol (70:30), 1 ml/min.
Detection – Fluorescence: excitation λ-366 nm, emission λ-435 nm
Quantification – External standard method
HPLC System – Waters 2695 HPLC, and 2475 fluorescent detector
Software - Empower

Accuracy Evaluation

Powder milk, green peas, corn flour and chicken were the matrices tested four times, in triplicate, for the **Precision Study**. Repeatability and intermediate precision standard deviations were calculated trough ANOVA (p<0.05). **Trueness** was evaluated through the Z-scores obtained from FAPAS 2183 (liquid vitamin supplement) and FAPAS 2186 (breakfast cereal).

Measurement uncertainty was estimated from bottom-up approach using the data obtained in the **precision** and **trueness** studies. The standard uncertainty associated to the method precision (μ_P) was calculated from pool relative standard deviation (RSDpool) and Trueness associated uncertainty (μ_T) was estimated by testing FAPAS 2186 five times over time.

Combined standard uncertainty (μ_C) was calculated:

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

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

$$BIAS = \left|\frac{\overline{X_{obs}} - X_{RM}}{X_{RM}}\right|$$

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

where, X_{RM} and U_{RM} are, respectively, the stated value and the expanded uncertainty of the RM (k=2), n is the number of tests and S_{obs} the average and standard deviation, respectively, of the obtained results.

To obtain expanded uncertainty (U), a coverage factor (K) of 2 was applied to $\mu_{C.}$

Additionally, combined standard uncertainty for each food matrix ***i*** was calculated, replacing μ_P by RSD for matrix i.

RESULTS

Table 1. Precision data (mg/100g). Sr – Repeatability standard deviation, r – repeatability limit, SPi – Intermediate precision standard deviation, Pi - Intermediate precision limit.

	S_r	r	S_{Pi}	Pi	$\overline{x}$
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

Table 2. Measurement uncertainty data (µP - Precision uncertainty; µT - Trueness uncertainty; µc - Combined uncertainty; U - Expanded uncertainty; k=2).

	Powder milk	Green peas	Corn flour	Chicken brest	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

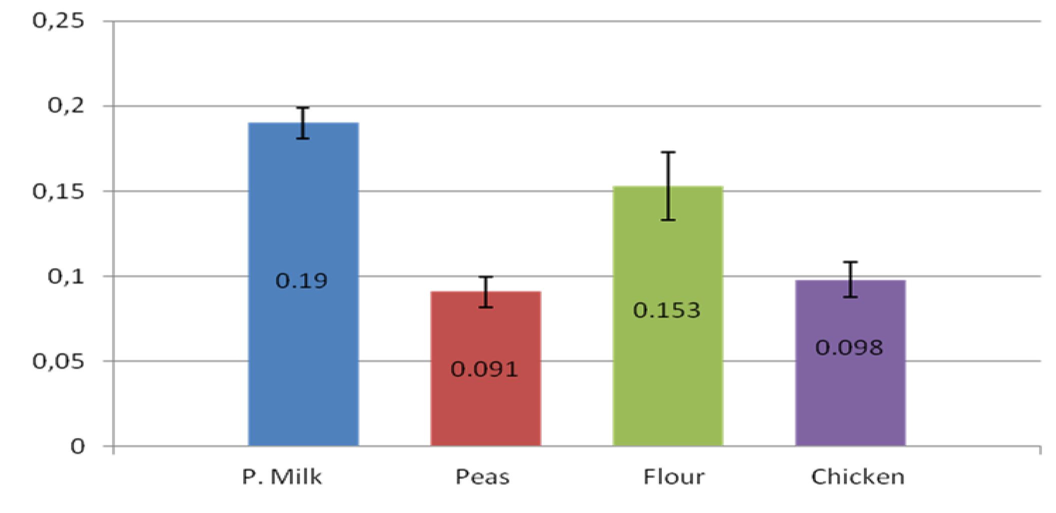

Figure 1. Mean values of thiamin concentration (mg/100g). Error bars represent +/-1 standard deviation.

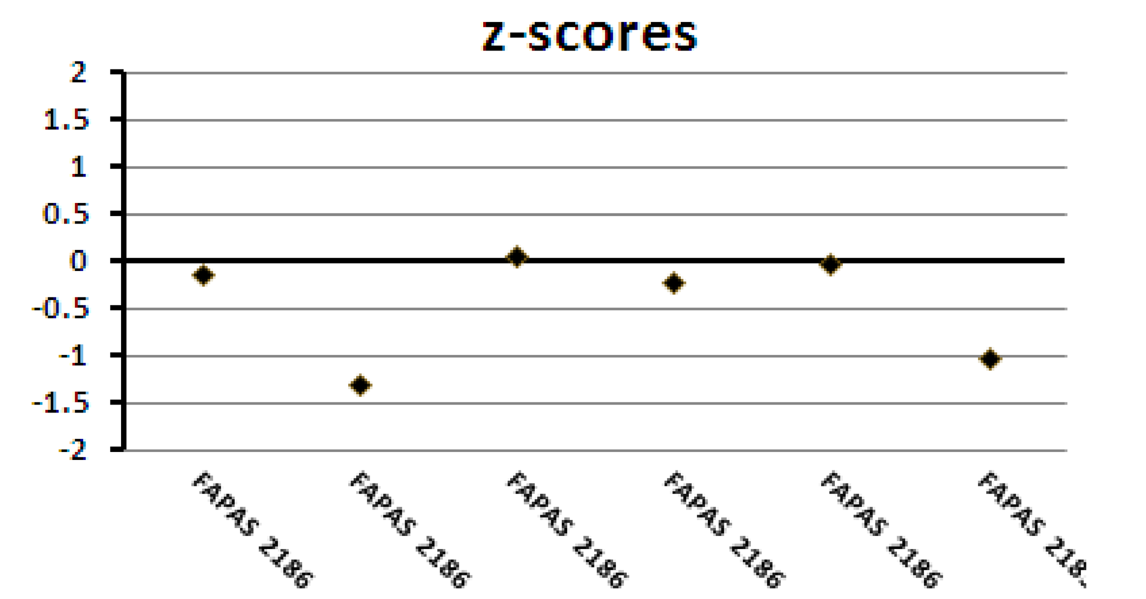

Figure 2. Trueness data.

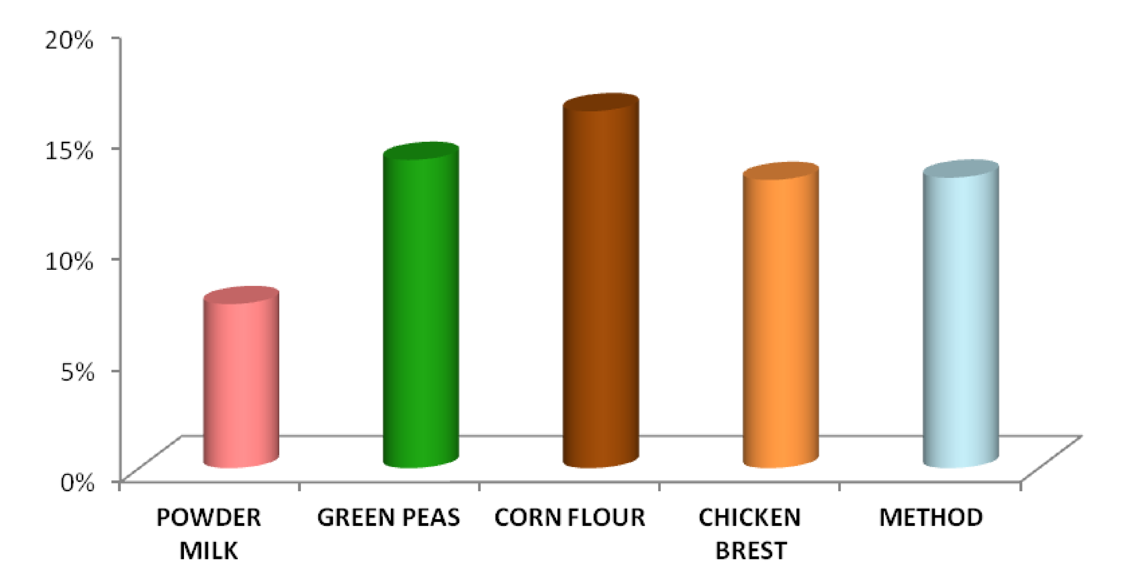

Figure 3. Relative expanded uncertainty (U), k=2.

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 .

Trueness 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 from precision. Thiamin determination in powder milk matrix showed the lowest measurement uncertainty (7.4%).According to the obtained values for measurement 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.

REFERENCES

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