UNCERTAINTY ESTIMATION OF AN ANALYTICAL METHOD FOR ANALYSIS OF FUMONISIN B_1 IN FOUR TYPES OF RICE INTENDED FOR HUMAN CONSUMPTION

Mateus Henrique Petrarca*; Eliseu Antonio Rossi; Célia Maria de Sylos

Department of Food and Nutrition, Faculty of Pharmaceutical Sciences, São Paulo State University, Araraquara, SP, Brazil. *E-mail: petrarcamh@gmail.com*

INTRODUCTION

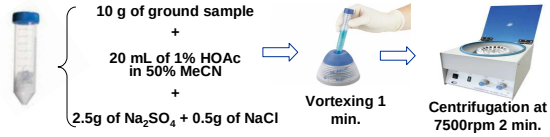
Fumonisin B_1 has been associated with neural tube defects and an increased incidence of oesophageal cancer in humans. Due to the toxicity of the mycotoxin, different analytical methods have been reported for quantitative analysis of fumonisin B_1 in cereals. Considering that the matrices of cereal are complex and their composition varies from cereal to cereal, and the undetected co-eluting sample components may affect the method performance characteristics and consequently interfere with quantitatively accurate results, we evaluated the fitness-for-purpose as well as the expanded measurement uncertainty of a sample preparation method for analysis of fumonisin B_1 in four types of rice intended for human consumption.

MATERIAL AND METHODS

➢ **Sampling:** 51 commercial samples of rice, including polished rice, polished parboiled rice, whole grain rice and whole parboiled grain rice were randomly collected from local markets in the city of Araraquara, SP, Brazil.

➢ **Determination of fumonisin B_1:**

▪ **Extraction:**



▪ **Clean-up by Dispersive SPE (d-SPE):**

▪ **Precolumn derivatisation with *ortho*-phthaldialdehyde (OPA) according to AOAC (2005);**

▪ **Injection of 10 uL into HPLC with fluorescence detection:** ODS-Hypersil column (250 x 4.6 mm; 5 µm); 1.0 mL/min.; isocartic elution; mobile phase: MeCN:0.1M sodium phosphate buffer adjusted to pH 3.3 with phosphoric acid (44:56). Fluorescence detector set at excitation and emission wavelengths of 335 and 440 nm, respectively.

➢ **In-house validation:** the method performance criteria – selectivity, limits of detection (LOD) and quantification (LOQ), linearity, matrix effects, trueness and precision, were evaluetd according to Eurachem (1998).

➢ **Measurement uncertainty:** the expanded uncertainty was estimated according to Boleda, Galceran and Ventura (2013) using the Eq. (1):

$$U = k \times \sqrt{u_s^2 + u_{RV}^2 + u_{SD}^2 + u_{corr}^2}$$

where U is the expanded uncertainty of the mass fraction of the analyte (µg/kg), k is the coverage factor (2), u_S is the uncertainty of (im)precision of the measurements in terms of repeatability, u_{RV} is the uncertainty estimate for the reference value used (spiking level), u_{SD} is the uncertainty of (im)precision of the measurements in terms of reproducibility, and u_{corr} is the uncertainty of the corrected analyte mass fraction.

RESULTS AND DISCUSSION

The selectivity was evaluated on the ability of the method to determine accurately the fumonisin B_1-OPA derivative (FB_1-OPA) in presence of other components from matrix (Figure 1).

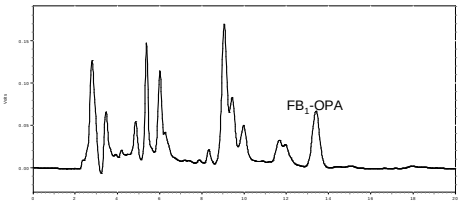


Figure 1. HPLC-FLD chromatogram of extract of rice spiked with fumonisin B_1 standard.

The LOD (50 µg/kg) and LOQ (100 µg/kg) were set using the signal-to-noise ratio of 3:1 and 10:1, respectively. The high significance ($p < 0.001$) of the regression and no significance of the lack of fit ($p > 0.05$) indicated linearity in the range from 100 to 2500 µg/kg for the solvent and matrix-matched calibration curves. The accuracy of the method was also evaluated (Table 1).

Table 1. Trueness and precision of the method for the determination of fumonisin B_1 in four types of rice.

	Level (µg/kg)	Polished Rice	Polished Parboiled Rice	Whole Grain Rice	Whole Parboiled Grain Rice
R (%)*	100	108.0	112.0	109.7	104.7
	1000	99.4	106.5	81.4	88.8
	2500	94.0	96.3	71.7	81.8
RSDr (%)	100	2.8	5.5	2.9	0.9
	1000	7.6	2.9	4.3	5.4
	2500	5.0	8.3	3.2	2.4
RSD_R (%)	100	5.6	4.9	4.8	16.7
	1000	12.9	2.0	17.0	12.6
	2500	13.7	2.7	6.7	8.8

*n = 3; RSDr: relative standard deviation under repeatability conditions (n = 3); RSD_R: relative standard deviation within-reproducibility conditions (n = 9).

Matrix effect was verified, thus matrix-matched calibration curves were used to quantify fumonisin B_1 in the samples. The mycotoxin was detected in 12% of total of analysed samples and their levels ranged from 70.66 to 258.7 µg/kg.

Data on precision experiments i.e. the within-laboratory repeatability standard deviation and the within-laboratory reproducibility standard deviation were employed to estimate the expanded measurement uncertainty, as well as the data on trueness experiments (Table 2).

Table 2. Estimation of the measurement uncertainty for fumonisin B_1 in four types of rice.

	Level (µg/kg)	Polished Rice	Polished Parboiled Rice	Whole Grain Rice	Whole Parboiled Grain Rice
u_S	100	1.7	3.6	1.8	0.5
	1000	4.4	1.8	2.0	2.8
	2500	2.8	4.6	1.3	1.1
u_{SD}	100	2.0	1.8	1.7	5.8
	1000	4.2	0.7	4.6	3.7
	2500	4.3	0.9	1.6	2.4
U	100	5.4	8.1	5.2	11.7
	1000	12.2	4.0	10.1	9.3
	2500	10.3	9.5	4.3	5.4

The measurement uncertainty values were lower than the maximum standard uncertainty established by the European Community (2006) for methods of analysis for control of mycotoxin in foodstuffs, indicating the suitability of the analytical method for the determination of fumonisin B_1 in rice.

ACKNOWLEDGMENT: CNPq