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In-house method validation, estimating measurement uncertainty and the occurrence of fumonisin B_1 in samples of Brazilian commercial rice

Mateus Henrique Petrarca*, Elizeu Antonio Rossi, Célia Maria de Sylos

Department of Food and Nutrition, Faculty of Pharmaceutical Sciences, São Paulo State University (UNESP), P. O. Box 502, 14801-902 Araraquara, SP, Brazil

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ABSTRACT

Fumonisin B_1 was investigated in samples of rice intended for human consumption, including polished parboiled rice, whole grain rice and whole grain parboiled rice. Until the present, no studies on the occurrence of fumonisin B_1 have been performed on these types of rice that are commercially available in the south-eastern region of Brazil. A careful intralaboratory validation was carried out to demonstrate the fitness-of-purpose of the applied method for determining fumonisin B_1 in the three studied rice types. The performance criteria – selectivity, reliable limits of detection (50 μg kg^{-1}) and quantification (100 μg kg^{-1}), linearity (range 100–2500 μg kg^{-1}), precision (RSD values ≤ 17.0%) and recovery (71.7–112.0 %) were evaluated, and the expanded measurement uncertainty was estimated by using the data obtained from precision and recovery experiments. Matrix-matched calibration standards were employed to quantify the mycotoxin levels in the rice samples, in which the residual normality, homoscedasticity and independence were confirmed. In addition, the measurement uncertainty values are consistent with the maximum acceptable uncertainty established by European Union regulation for analytical methods for controlling mycotoxins in foodstuffs. Among the thirty-one commercial samples of rice analysed in the present study, five samples presented detectable levels of the mycotoxin, and these levels ranged from 64.8 to 163.0 μg kg^{-1}.

1. Introduction

Fumonisin B_1, a secondary metabolite of the *Fusarium* and *Alternaria* fungal genera, has been associated with the incidence of human oesophageal cancer in areas of the Transkei region of South Africa (Shephard et al., 2007) and with neural tube defects along the Texas–Mexico border (Missmer et al., 2006). This mycotoxin is classified under group 2B as a possible human carcinogen (IARC, 2011). In animals, fumonisin B_1 has been shown to cause porcine pulmonary oedema and equine leukoencephalomalacia, as well as species-specific targeted tissue damage, such as hepatotoxicity in rodents and nephrotoxicity in rabbits and sheep (Smith, 2007).

The highest levels and incidence of fumonisin B_1 have been reported in corn and corn-based foods (Soriano & Dragacci, 2004); however, this mycotoxin has also been associated with other cereals, such as rice (Tanaka, Sago, Zheng, Nakagawa, & Kushiro, 2007). Because of the toxicity of this mycotoxin and its processing stability (Bullerman & Bianchini, 2007), the occurrence of fumonisin B_1 in rice implies a potential risk to populations in regions of the world in which rice is a dietary staple. Specifically, in Brazil, the average daily consumption of rice is approximately 160 g per person (IBGE, 2011), and rice production was estimated to be 12,151.5 thousand tonnes in 2015 (CONAB, 2015).

Recently, we detected fumonisin B_1 in one sample of rice that was purchased at a local retail store in the south-eastern region of Brazil; however, that study was restricted to polished rice samples (Petrarca, Rodrigues, Rossi, & Sylos, 2014). Until the present, no studies on the occurrence of fumonisin B_1 have been performed on other types of rice intended for human consumption such as the polished parboiled rice, whole grain rice and whole grain parboiled rice that are commercially available in the south-eastern region of Brazil. Parboiled rice is the product that is obtained from the parboiling process, in which unpeeled rice is submerged in drinking water at a temperature above 58 °C, followed by partial or full gelatinization of its starch and then drying. Whole grain rice is the product that results when only the husk of the grain has been removed, and it can also be subjected to a parboiling process. The maximum moisture content permitted in these products is 14% (MAPA, 2009).

Several analytical methods have been explored to investigate fumonisin B_1 levels in rice and its products (Khayoon et al., 2010;

* Corresponding author.
E-mail address: petrarcamh@gmail.com (M.H. Petrarca).

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Kim, Scott, Lau, & Lewis, 2002; Lombaert et al., 2003; Park, Choi, Hwang, & Kim, 2005; Scott, Lawrence, & Lombaert, 1999; Seo et al., 2009). Many of these methods include laborious sample preparation steps as well as considerable amounts of sample and extraction solvent, and these methods require solid phase extraction (SPE) cartridges or immunoaffinity columns, which makes them costly. However, simple and low residue generation methods, which are based on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure, have been successfully applied to determine fumonisin B_1 in barley, corn, oats and wheat (Vaclavik, Zachariasova, Hrbek, & Hajslova, 2010; Yang & Wu, 2012; Zachariasova et al., 2010), rice (Becker-Algeri, Heidtmann-Bemvenuti, Hackbart, & Badiale-Furlong, 2013; Koesukwiwat, Sanguankaew, & Leepipatpiboon, 2014; Petrarca et al., 2014), cereal flours (Desmarchelier et al., 2010), and other food matrices and feed (Mol et al., 2008; Trebstein, Lauber, & Humpf, 2009). High performance liquid chromatography (HPLC), with fluorescence detection or coupled to mass spectrometry (MS), have been extensively employed for identifying and quantifying the mycotoxin in food matrices (Arranz, Baeyens, Van der Weken, De Saeger, & Van Peteghem, 2004; Köppen et al., 2010; Maragos & Busman, 2010).

Considering that the same sample preparation method is employed to investigate fumonisin B_1 in the rice samples that were selected for the present study, the co-extractives and their amounts may vary between the analysed rice types, and consequently may affect the method performance characteristics and interfere with the generation of quantitatively accurate results. Thus, estimating the measurement uncertainty for each matrix studied is important to ensure the quality of the analytical results and to demonstrate the suitability of the analytical method (Boleda, Galceran, & Ventura, 2013). Different procedures have been applied to calculate the measurement uncertainty associated with analyses of antibiotics (Borecka et al., 2013), chlorides and fatty acids (Quintela, Báguena, Gotor, Blanco, & Broto, 2012), ochratoxin A (Fernandes, Barros, & Câmara, 2013), pharmaceuticals (Boleda et al., 2013), pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Aslan-Sungur, Gaga, & Yenisoy-Karakas, 2014; Kmellár et al., 2008; Planas, Puig, Rivera, & Caixach, 2006) in environmental and food matrices. However, some of these models require certified reference material, which is not always available. Therefore, the procedure proposed by Boleda et al. (2013) based on single-laboratory validation was selected to calculate the expanded measurement uncertainty in this study. Thus, the uncertainty was estimated by using the data obtained from precision and recovery experiments, i.e., within-laboratory repeatability and reproducibility standard deviations.

In the present study, fumonisin B_1 was analysed in samples of commercial rice that were available in the south-eastern region of Brazil; these types of rice have not been studied until the present, and they include polished parboiled rice, whole grain rice and whole grain parboiled rice. We evaluated the performance criteria, namely, the selectivity, limits of detection and quantification, linearity, matrix effects, extraction efficiency and precision of a simple and cost-effective method of sample preparation based on the QuEChERS procedure for each matrix. In-house validation data were used to estimate the expanded measurement uncertainty for the mass fraction of the mycotoxin detected in the rice samples.

2. Material and methods

2.1. Standard and chemicals

A fumonisin B_1 standard (98% purity) was obtained commercially from Sigma–Aldrich, Inc. (St. Louis, MO, USA). A fumonisin B_1 stock solution was made in acetonitrile: water (1:1, v/v) at 1000 μg ml^{-1}, and standard working solutions were prepared at 20 μg ml^{-1}. All solutions were kept in amber flasks at −18 °C.

Ortho-phthaldialdehyde (OPA) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA), sodium tetraborate was purchased from LabSynth (Diadema, SP, Brazil) and 2-mercaptoethanol was purchased from Vetec (Rio de Janeiro, RJ, Brazil). To prepare the derivatisation reagent, OPA (40 mg) was dissolved in 1 ml of methanol, and then 5 ml of 0.1 M sodium tetraborate solution (pH 9.0 ± 0.1) and 50 μl of 2-mercaptoethanol were added (Trucksess, 2005). This solution was prepared weekly and stored at room temperature in an amber flask.

HPLC-grade acetonitrile, glacial acetic acid and methanol were obtained from J.T. Baker, Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). The water used in the chromatographic analyses was prepared by using a Milli-Q system (Millipore, Milford, MA, USA). Anhydrous sodium sulphate was purchased from Qhemis (Indaiatuba, SP, Brazil), diatomaceous earth (Celite) was purchased from Almeria S.A. (Guadalajara, Mexico), phosphoric acid was purchased from LabSynth (Diadema, SP, Brazil), and monosodium phosphate 1-hydrate and sodium chloride were bought from Merck S.A. (Rio de Janeiro, RJ, Brazil).

2.2. Sampling

Samples of rice intended for human consumption were purchased in the commercially available size of 1 kg from 5 supermarkets, 1 grain store and 1 natural foods store in the city of Araraquara, SP, in the south-eastern region of Brazil, between September and October of 2011. A total of 31 different brands of three rice types were randomly collected, including 10 brands of polished parboiled rice, 6 brands of whole grain rice and 15 brands of whole grain parboiled rice. These cereals were ground in a food processor (Arno, SP, Brazil) to obtain homogenous samples, sieved through a 0.84 mm mesh and stored in polypropylene flasks until the time of analysis. The analyses were performed on the same day the cereal was ground.

2.3. Determination of fumonisin B_1

The sample preparation method applied in this study was optimized to determine fumonisin B_1 in polished rice, and it is described elsewhere (Petrarca et al., 2014). In this method, 10 g of ground sample, 20 mL of 50% acetonitrile aqueous solution and 0.2 mL of glacial acetic acid were added to a 50 mL polypropylene centrifuge tube (Nalgene, Thermo Scientific, Rochester, NY, USA) and vortexed for 1 min. Then, 2.5 g of anhydrous sodium sulphate and 0.5 g of sodium chloride were added to mixture and vortexed again for 1 min and centrifuged at 7500 rpm for 2 min. After the centrifugation step, 5 mL of supernatant, 0.3 g of anhydrous sodium sulphate and 0.1 g of diatomaceous earth were added to a 50 mL polypropylene centrifuge tube and this mixture was vortexed for 30 s, and then centrifuged at 7500 rpm for 2 min.

The final extract was filtered through a 0.22 μm syringe filter, and then the precolumn derivatisation reaction was performed. A 225 μl aliquot of the derivatisation reagent was mixed with 25 μl of filtered extract for 30 s at room temperature and protected from light, and then 10 μl of this mixture was injected into the HPLC system within 2 min of adding the derivatisation reagent to the extract (Trucksess, 2005).

2.4. Chromatographic conditions

An HPLC system (Shimadzu, Kyoto, Japan) equipped with a LC-10AT VP quaternary pump, a SIL-10A automatic injector and an RF-10A XL fluorescence detector (FLD) that was set at excitation

and emission wavelengths of 335 nm and 440 nm, respectively, was employed to determine the fumonisin B_1 mass fractions. Separation was achieved at 20 °C on a reversed phase analytical column (250 × 4.6 mm i.d., 5-μm particle size; ODS-Hypersil, Thermo, UK) with a flow rate of 1.0 ml min^{-1} and isocratic elution. The mobile phase was acetonitrile: 0.1 M sodium phosphate buffer solution adjusted with phosphoric acid to pH 3.3 (44:56), and the total run time was 20 min. The fumonisin B_1 identification was performed according to Trucksess (2005) by comparing the retention time of the fumonisin B_1-OPA derivative in the extracts with the retention time observed for the fumonisin B_1 standard.

2.5. In-house method validation

The selectivity of the method was assessed by using HPLC-FLD chromatograms of extracts from blank rice samples, rice samples spiked with fumonisin B_1 standard, and rice samples that were naturally contaminated with the mycotoxin. Samples of the three types of rice in which fumonisin B_1 was not detected, were used as blank samples, and then extracts of these samples were used as a representative matrix to establish the method's limits and to obtain matrix-matched calibration curves. The limits of detection (LOD) and limits of quantification (LOQ) were set by using signal-to-noise ratios of 3:1 and 10:1, respectively. The linearity was assessed for solvent and matrix-matched calibration curves according to Souza and Junqueira (2005). To obtain matrix-matched calibration curves, appropriate volumes of standard working solution were added to blank extracts to provide 100, 500, 1000, 1500, 2000 and 2500 μg kg^{-1} of standard equivalent in the samples, and for solvent calibration curves, the same concentrations were prepared in 1 ml of acetonitrile: water (1:1, v/v). By using regression analysis, the calibration curves were checked for outliers by considering the range $\pm\, t_{(1-\alpha/2;\ n-2)}s_{res}$, where s_{res} is the square root of the residual variance. The normality, homoscedasticity, and independence of the regression residuals were evaluated by using the Ryan–Joiner test, the Brown-Forsythe or the modified Levene test, and the Durbin–Watson test, respectively (Souza & Junqueira, 2005). An *F* test was employed to evaluate the linear regression and lack of fit for solvent and matrix-matched calibration curves. The slopes obtained for solvent and matrix-matched calibration curves were used to calculate the matrix effect (Economou, Botitsi, Antoniou, & Tsipi, 2009) by employing Eq. (1) as follows:

$$\text{Matrix effect}\,\% = (1 - \text{matrix slope}/\text{solvent slope}) \times 100 \qquad (1)$$

To evaluate the extraction efficiency and precision, blank rice samples were spiked with the fumonisin B_1 standard at levels of 100, 1000, and 2500 μg kg^{-1}. The mean recovery (%) was calculated from the nine independent replicates of spiked samples, which were obtained from three independent replicates at each level and analysed on three different days, and the precision was expressed in terms of relative standard deviations (RSD) (Thompson, Ellison, & Wood, 2002). Under the repeatability conditions, the precision was obtained from the three independent replicates of spiked samples at each level that were analysed on the same day by the same analyst under the same chromatographic conditions. Under within-reproducibility conditions, the precision was calculated from the three independent replicates of spiked samples at each analysed level on three different days by the same analyst under the same chromatographic conditions, totalling nine independent replicates for each level. The mass fraction of fumonisin B_1 in the spiked samples was determined by employing matrix-matched calibration curves.

2.6. Estimation of measurement uncertainty

The expanded measurement uncertainty was estimated by using the data obtained from in-house method validation according to Boleda et al. (2013). Data on precision experiments, i.e., the within-laboratory repeatability standard deviation and the within-laboratory reproducibility standard deviation, were employed to estimate the uncertainty, as well as the data on recovery experiments, for each rice matrix studied at the 100 μg kg^{-1} level by applying the following Eq. (2):

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

where *U* is the expanded measurement uncertainty of the analyte mass fraction (μg kg^{-1}), *k* is the coverage factor, u_s is the uncertainty of (im)precision of the measurement in terms of repeatability, u_{RV} is the uncertainty estimate for the reference value used, u_{SD} is the uncertainty of (im)precision of the measurement in terms of reproducibility, and u_{corr} is the uncertainty of the corrected analyte mass fraction.

2.7. Moisture content

The moisture contents of the rice samples were determined gravimetrically by employing an infrared balance, model ID 200 (Marte, SP, Brazil).

2.8. Decontamination of the materials

All the materials were treated with 5% sodium hypochlorite solution for at least 12 h, then a 5% acetone solution, and finally washed with water.

3. Results and discussion

3.1. In-house method validation

Accurate and sensitive methods have been developed for multi-mycotoxin analysis in foodstuffs including fumonisin B_1; however, many these analytical methods demand costly equipment, such as HPLC-tandem MS and high-resolution MS analysers (Köppen et al., 2010). Therefore, the fitness-of-purpose of a simple and cost-effective method, which included precolumn derivatisation with OPA and HPLC-FLD analysis, was evaluated for fumonisin B_1 control in three different rice matrices, that is, polished parboiled rice, whole grain rice and whole grain parboiled rice.

The selectivity was evaluated based on the ability to determine the fumonisin B_1-OPA derivative (FB_1-OPA) accurately in the presence of other components from the matrix (Eurachem, 1998). The retention time for the FB_1-OPA derivative was 13.5 min, and based on the profile for the HPLC-FLD chromatograms of extracts from blank rice sample, rice sample spiked with fumonisin B_1 standard at a 1000 μg kg^{-1} level and rice sample naturally contaminated with fumonisin B_1, we verified the separation of the FB_1-OPA derivative from matrix interferences, demonstrating the selectivity of the applied method (Fig. 1).

The method LOD was set at the lowest contamination level of fumonisin B_1 that was reliably detectable in the spiked rice extracts but not necessarily quantifiable, and could be distinguished from noise (Eurachem, 1998). The resulting LOD was 50 μg kg^{-1} for the three types of rice studied here. This same limit was reported by Arranz et al. (2004) as the LOD that is commonly obtained when precolumn derivatisation with *ortho*-phthaldialdehyde is employed for fumonisin B_1 analysis. However, LOD values of 25 μg kg^{-1} (Seo

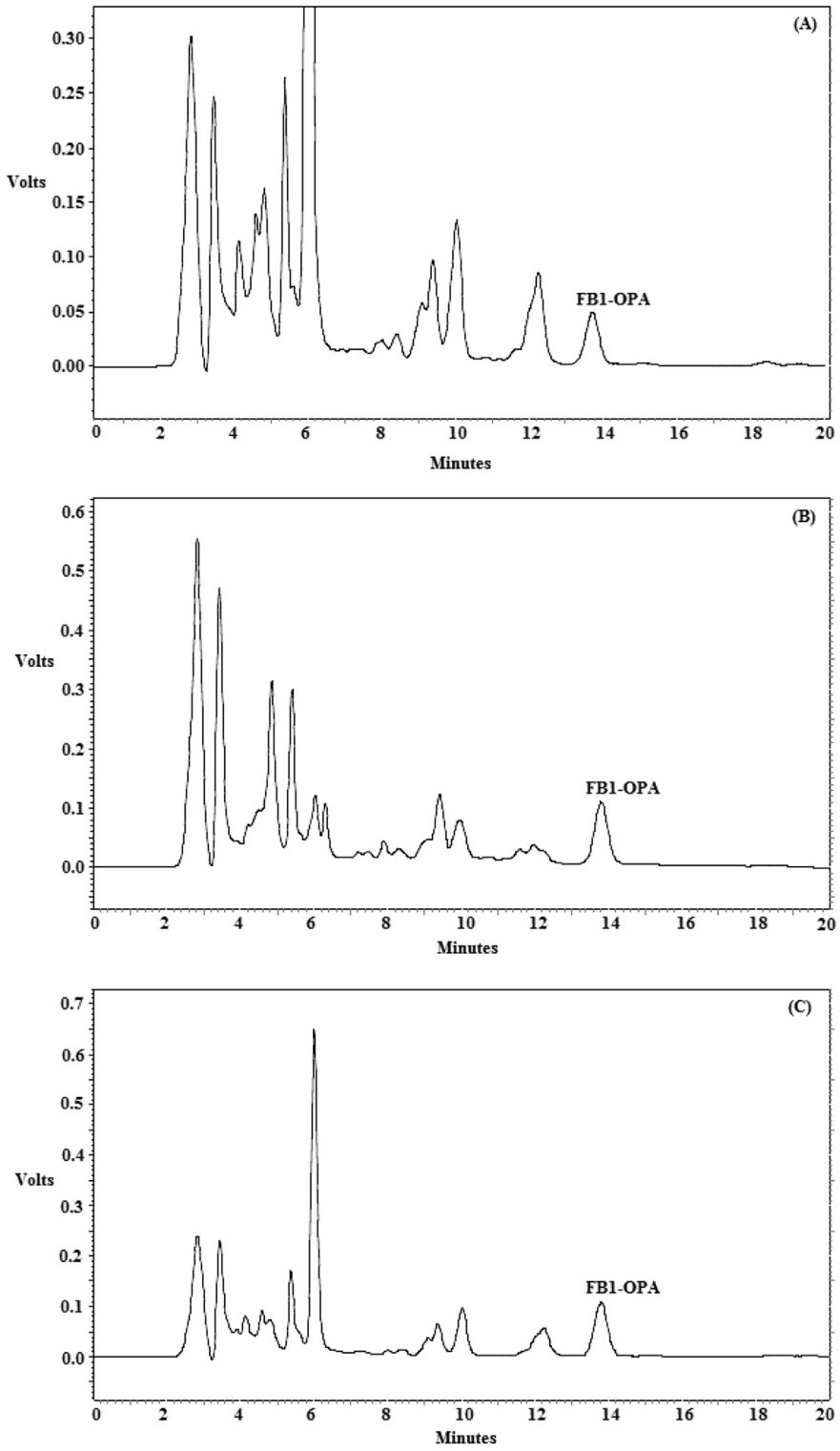

Fig. 1. HPLC-FLD chromatograms of extracts from polished parboiled rice (A), whole grain rice (B), and whole grain parboiled rice (C), which were spiked with fumonisin B_1 standard at a 1000 μg kg^{-1} level. Chromatographic conditions: ODS-Hypersil column (250 × 4.6 mm, 5 μm); mobile phase: acetonitrile: 0.1 M sodium phosphate buffer solution adjusted with phosphoric acid to pH 3.3 (44:56); flow rate: 1 ml min^{-1}; isocratic elution; and fluorescence detector set at excitation and emission wavelengths of 335 and 440 nm, respectively. *FB_1-OPA*: fumonisin B_1-*ortho*-phthaldialdehyde derivative.

et al., 2009), 30 μg kg^{-1} (Becker-Algeri et al., 2013), and 35 μg kg^{-1} (Park et al., 2005; Scott et al., 1999) have been reported for fumonisin B_1 in rice samples.

The LOQ was set as the smallest contamination level of fumonisin B_1 that could be detected and quantified in the rice samples with acceptable values of trueness and precision (Eurachem, 1998). Fumonisin B_1 was detected in samples of polished parboiled rice, whole grain rice and whole grain parboiled rice that were spiked at 100 μg kg^{-1}, the recovery values of which were within a range from 60 to 120 % with RSD values ≤30% and ≤60%, under repeatability

and within-reproducibility conditions, respectively; thus, this contamination level was defined as the method LOQ for the three studied matrices.

The linear range including the LOQ was evaluated from 100 to 2500 μg kg^{-1} for solvent and matrix-matched calibration curves. Two outliers at 1000 and 2500 μg kg^{-1} levels were identified by regression analysis in the solvent calibration curve, one outlier at 2500 μg kg^{-1} level in the matrix-matched calibration curve obtained from polished parboiled rice extract, four outliers at 1000, 1500 and 2000 μg kg^{-1} levels in the matrix-matched calibration curve obtained from whole grain rice extract, and four outliers at 1500 and 2000 μg kg^{-1} levels in the matrix-matched calibration curve obtained from whole grain parboiled rice extract. These outliers were then removed by considering the 22.2% limit on the original amount of data for each calibration curve according to Souza and Junqueira (2005).

The normality, homoscedasticity and independence of the regression residuals from solvent and matrix-matched-calibration curves were evaluated (Table 1). The coefficient correlation indicated no significant deviation ($p > 0.05$) from normal distribution, according to the Ryan–Joiner test. The homoscedasticity was verified by Levene t statistics, which were not significant ($p > 0.05$), indicating that the residual variance across all levels was constant. The regression residuals were independent, and we verified that the autocorrelation was not significant ($p > 0.05$) by Durbin–Watson test. Once the regression residuals are normally distributed, homoscedastic and independent, linearity in the range from 100 to 2500 μg kg^{-1} was verified for solvent and matrix-matched calibration curves because the regression was significant ($p < 0.05$) and the lack of fit was not significant ($p > 0.05$) (Table 1).

The matrix effect was assessed by employing the slopes obtained from calibration curves of the same concentration levels in solvent and blank matrix extracts, and we verified the low matrix effects of +8.0%, − 3.0%, and +4.0% for polished parboiled rice, whole grain rice, and whole grain parboiled rice, respectively. In addition, matrix effects can induce errors in the quantitative analysis, and matrix-matched calibration standards thus become an efficient method for matrix effects compensation when a blank matrix is available (Economou et al., 2009; Lehotay et al., 2010; Wiest et al., 2011). Therefore, the matrix-matched calibration curves were employed to calculate the contamination level of fumonisin B_1 in the rice samples. The regression equations and determination coefficients (R^2) were $y = 923.76x - 18662$ ($R^2 = 0.9997$), $y = 1032.4x - 8055.2$ ($R^2 = 0.9997$) and $y = 964.62x + 47003$ ($R^2 = 0.9959$) for the matrix-matched calibration curves obtained from polished parboiled rice, whole grain rice and whole grain parboiled rice extracts, respectively.

In the absence of certified reference material available for fumonisin B_1 in rice, spiking/recovery experiments were employed in this study (Thompson et al., 2002). Blank rice samples were spiked with a standard solution of the mycotoxin, and, before the extraction procedure, this mixture was allowed to stand for 12 h for better interaction between the analyte and matrix. The average recovery values ranged from 96.3 to 112.0 % for polished parboiled rice, from 71.7 to 109.7 % for whole grain rice, and from 81.8 to 104.7 % for whole grain parboiled rice (Table 2). Different recovery values were observed for the analysed rice matrices, showing that unknown matrix components can potentially affect the extraction efficiency. Additionally, all resulting recovery values were consistent with the performance criteria established by the Commission Regulation (EC) No. 401/2006, with acceptable recoveries between 60 and 120% for fumonisin B_1 contamination levels less than or equal to 500 μg kg^{-1}, and from 70 to 110 % for levels greater than 500 μg kg^{-1}.

The precision was evaluated under repeatability conditions, with RSD values ranging between 2.9 and 8.3%, 2.9 and 4.3%, and 0.9 and 5.4% for polished parboiled rice, whole grain rice and whole grain parboiled rice, respectively (Table 2). Under within-reproducibility conditions, the RSD values varied from 2.1 to 4.9 % for polished parboiled rice, from 4.8 to 17.0 % for whole grain rice and from 8.8 to 16.7 % for whole grain parboiled rice (Table 2). For the control of mycotoxin levels in foodstuffs, RSD values ≤30% and ≤60% (under repeatability and within-reproducibility conditions, respectively) are acceptable for fumonisin B_1 levels ≤ 500 μg kg^{-1}. For fumonisin B_1 levels greater than 500 μg kg^{-1}, the RSD values must be ≤ 20% under repeatability conditions and ≤30% within-reproducibility conditions (Commission Regulation, 2006).

3.2. Estimation of measurement uncertainty

The expanded measurement uncertainty (U) of the fumonisin B_1 in polished parboiled rice, whole grain rice and whole grain parboiled rice, at a 100 μg kg^{-1} level (LOQ), was estimated according to

Table 1
Evaluation of residual normality, homoscedasticity and independence in addition to ANOVA statistics for regression, including the lack-of-fit test for the matrix-matched calibration curves.

Statistic	Matrix-matched calibration curve		
	Polished parboiled rice	Whole grain rice	Whole grain parboiled rice
n	17	14	14
Normality			
R	0.987	0.945	0.979
p	>0.05	>0.05	>0.05
Homoscedasticity			
t_L	0.942	0.398	1.040
p	>0.05	>0.05	>0.05
Independence			
d	1.962	2.769	2.027
p	>0.05	>0.05	>0.05
Regression			
F	5.295×10^4	3.989×10^4	2.897×10^3
p	<0.05	<0.05	<0.05
Lack of fit			
F	2.875	0.420	0.687
p	>0.05	>0.05	>0.05

n: number of observations after the treatment of outliers; *R*: Ryan–Joiner correlation coefficient; t_L: Levene t statistic, *d*: Durbin–Watson statistic; *F*: variance ratio; and *p*: significance.

Table 2
Recovery, repeatability and reproducibility data for the fumonisin B_1 in the three rice matrices at different mass fraction levels.

Level (μg kg^{-1})	Polished parboiled rice			Whole grain rice			Whole grain parboiled rice		
	R (%)	RSD_r (%)	RSD_R (%)	R (%)	RSD_r (%)	RSD_R (%)	R (%)	RSD_r (%)	RSD_R (%)
100	112.0	5.5	4.9	109.7	2.9	4.8	104.7	0.9	16.7
1000	106.5	2.9	2.1	81.4	4.3	17.0	88.8	5.4	12.6
2500	96.3	8.3	2.7	71.7	3.2	6.7	81.8	2.4	8.8

R: mean recovery (n = 9); RSD_r: relative standard deviation under repeatability conditions (n = 3); and RSD_R: relative standard deviation under within-reproducibility conditions (n = 9).

the procedure presented by Boleda et al. (2013), and the data are presented in Table 3.

The uncertainty associated with the (im)precision of the measurements in terms of repeatability (u_s) was calculated from the standard deviation of the mean mass fraction (μg kg^{-1}) obtained from repeated measurements that were performed on the same day, as indicated in the following Eq. (3):

$$u_s = \frac{SDr}{\sqrt{n}} \tag{3}$$

where SDr is the standard deviation of the within-laboratory repeatability, and n is the number of measurements. In this study, three independent replicates of spiked samples were analysed on the same day by the same analyst under the same chromatographic conditions, and the u_s values ranged from 0.5 μg kg^{-1} for whole grain parboiled rice to 3.5 μg kg^{-1} for polished parboiled rice (Table 3).

The uncertainty obtained for the reference value used, or u_{RV}, was obtained from Eq. (4) as follows:

$$u_{RV} = \frac{u_{assoc}}{k} \tag{4}$$

where u_{assoc} is the uncertainty associated with the mycotoxin level in the spiked sample, and k is the coverage factor.

The coverage factor is usually 2 for normally distributed data, and it yields an expanded uncertainty that can be used to construct a 95% coverage interval (Eurolab, 2007). To obtain the u_{assoc}, the uncertainties associated with the weight of the standard (u_m), the dilution volume of the standard solution (u_{Vdil}) and the purity of the standard (u_P) were combined (Díaz, Vàzquez, Ventura, & Galceran, 2004) as indicated in Eq. (5) as follows:

$$u_{assoc} = \sqrt{u_m^2\left(\frac{P}{V_{dil}}\right)^2 + u_{V_{dil}}^2\left(m\frac{P}{V_{dil}^2}\right)^2 + u_P^2\left(\frac{m}{V_{dil}}\right)^2} \tag{5}$$

Table 3
Estimation of expanded measurement uncertainty (U, μg kg^{-1}) for fumonisin B_1 in three types of rice at a 100 μg kg^{-1} level.

Terms	Polished parboiled rice	Whole grain rice	Whole grain parboiled rice
u_s	3.5	1.8	0.5
u_{RV}	0.5	0.5	0.5
u_{SD}	1.8	1.7	5.8
u_{corr}	12.0	9.7	4.7
U	25.3	20.0	15.0

u_s: uncertainty obtained from the (im)precision of the measurements in terms of repeatability; u_{RV}: uncertainty estimate for the reference value used; u_{SD}: uncertainty obtained from the (im)precision of the measurements in terms of reproducibility; u_{corr}: uncertainty of the corrected analyte mass fraction; and U: expanded measurement uncertainty.

where P is the purity of the standard; V_{dil} is the dilution volume (ml) and m is the weight of the standard (mg).

The uncertainty associated with the (im)precision of the measurements in terms of reproducibility (u_{SD}) was calculated as indicated in Eq. (6) as follows:

$$u_{SD} = \frac{SD_R}{\sqrt{N}} \tag{6}$$

where SD_R is the within-laboratory reproducibility standard deviation of the mean mass fraction (μg kg^{-1}) obtained from replicate measurements, which were performed on different days, and N is the number of replicates. In our study, three independent replicates of spiked samples were analysed on three different days by the same analyst under the same chromatographic conditions, totalling 9 independent replicates for each matrix studied here. The u_{SD} values varied between 1.7 μg kg^{-1} for whole grain rice and 5.8 μg kg^{-1} for whole grain parboiled rice (Table 3).

The uncertainty of the corrected analyte mass fraction (u_{corr}), which is associated with the recovery, was calculated by using Eq. (7) as follows:

$$u_{corr} = |C_{RV} - C_{SD}| \tag{7}$$

where C_{RV} is the spiked concentration and C_{SD} is the average concentration obtained from reproducibility experiments, which were performed on three different days. In this study, the u_{corr} values varied from 4.7 μg kg^{-1} to 12.0 μg kg^{-1} for the rice matrices (Table 3), which made the largest contribution to the final expanded measurement uncertainty for the mass fraction of mycotoxin detected in the polished parboiled rice and whole grain rice samples.

Finally, the uncertainty obtained from the (im)precision of the measurements in terms of repeatability (u_s), the uncertainty estimate for the reference value used (u_{RV}), the uncertainty obtained from the (im)precision of the measurements in terms of reproducibility (u_{SD}), and the uncertainty of the corrected analyte mass fraction (u_{corr}) were combined as presented in Eq. (2), and then the expanded measurement uncertainty (U) was estimated, the values of which ranged from 15.0 μg kg^{-1} for fumonisin B_1 in whole grain parboiled rice to 25.3 μg kg^{-1} for fumonisin B_1 in polished parboiled rice (Table 3).

To assess the suitability of the analysis method, we calculated the maximum standard uncertainty (Uf) according to the methods of analysis criteria used to control mycotoxins in foodstuffs that were established by the Commission Regulation (EC) No. 401/2006, by employing the following Eq. (8):

$$Uf = \sqrt{\left(\frac{LOD}{2}\right)^2 + (\alpha \times C)^2} \tag{8}$$

where Uf is the maximum standard uncertainty (μg kg^{-1}), LOD is the limit of detection for the method (μg kg^{-1}), α is a constant

depending on the value of C, and C is the mass fraction of interest ($\mu g\ kg^{-1}$).

The numeric value used for α as the constant in Eq. (8) was 0.18 for the mass fraction at 100 $\mu g\ kg^{-1}$ (Commission Regulation, 2006); therefore, the maximum standard uncertainty (Uf) was 30.8 $\mu g\ kg^{-1}$. In brief, the analytical method provided agreement between the expanded measurement uncertainty value (U) and the maximum standard uncertainty (Uf) for the three rice types studied here, indicating the suitability of the analytical method for the determination of fumonisin B_1 in polished parboiled rice, whole grain rice and whole grain parboiled rice.

3.3. Occurrence of fumonisin B_1

Commercial samples of polished parboiled rice, whole grain rice and whole grain parboiled rice were investigated for the presence of fumonisin B_1. According to the packing information for the analysed products, the samples collected at local retail stores in the city of Araraquara, SP were produced in the Brazilian States of Rio Grande do Sul, Santa Catarina, São Paulo and Pernambuco. Among the 31 samples of rice analysed here, 5 samples presented detectable levels of the mycotoxin, and these levels were reported as the mean ($n = 3$) ± expanded measurement uncertainty (U) that was obtained for each rice matrix, namely, 25.3% for polished parboiled rice, 20.0% for whole grain rice and 15.0% for whole grain parboiled rice.

A total of 10 different commercial brands of polished parboiled rice was analysed, and two samples showed detectable levels of fumonisin B_1 at mass fractions of 109.4 ± 27.7 $\mu g\ kg^{-1}$ and 163.0 ± 41.2 $\mu g\ kg^{-1}$. During the sampling period, six different brands of whole grain rice were commercially available at local retail stores, among which one sample showed a detectable level of fumonisin B_1 at a mass fraction of 111.2 ± 22.2 $\mu g\ kg^{-1}$. Fumonisin B_1 was also investigated in fifteen different commercial brands of whole grain parboiled rice, and the mycotoxin was detected in two samples. One sample showed fumonisin B_1 at a mass fraction of 64.8 $\mu g\ kg^{-1}$, a contamination level that was lower than the LOQ obtained for the method; as a result, the quantification was performed without precision and trueness. For the other sample, the contamination level of fumonisin B_1 was 110.6 ± 16.6 $\mu g\ kg^{-1}$. In addition, no maximum *Fusarium* toxins levels have been proposed for rice and rice products (Commission Regulation, 2005). The moisture contents of all analysed samples ranged from 5.8 to 8.7 %, which are consistent with the established maximum limit of 14%.

Data regarding the occurrence of *Fusarium* toxins in foods from nine countries in the European Community indicated the presence of fumonisin B_1 in 2% of the 197 rice samples analysed, with a maximum contamination level of fumonisin B_1 at 77 $\mu g\ kg^{-1}$ (Brera & Miraglia, 2003). With respect to the of Brazilian rice samples, fumonisin B_1 was reported by Becker-Algeri et al. (2013) in rice samples from the southern region of Brazil, which showed varying contamination levels between 30 and 170 $\mu g\ kg^{-1}$. In a recent study, we detected the mycotoxin in one commercial sample of polished rice from the south-eastern region of Brazil at a mass fraction of 258.7 $\mu g\ kg^{-1}$ (Petrarca et al., 2014). In general, the incidence and contamination levels of fumonisin B_1 found in rice samples intended for human consumption from around the world are regarded as low (Park et al., 2005; Patel, Hazel, Winterton, & Gleadle, 1997; Patel, Hazel, Winterton, & Mortby, 1996; Scott et al., 1999; Seo et al., 2009; Tanaka et al., 2007).

4. Conclusions

A simple and cost-effective method of sample preparation was successfully applied to investigate the presence of fumonisin B_1 in three types of rice that were intended for human consumption. The fitness-of-purpose of the applied method was verified by its selectivity, reliable limits of detection and quantification, linearity, extraction efficiency and precision. The influence of the matrix type on the method performance characteristics was verified, and matrix-matched calibration standards were used to quantify the mycotoxin in the rice samples, in which the residual normality, homoscedasticity and independence were confirmed. By using data obtained through precision and recovery experiments, it was possible to estimate the expanded measurement uncertainty for the mass fraction of the mycotoxin detected in the rice samples. The values for these samples were consistent with the maximum acceptable uncertainty that was established for analytical methods for controlling mycotoxins in foodstuffs. A low incidence and a low level of fumonisin B_1 were verified in the polished parboiled rice, whole grain rice and whole grain parboiled rice that was commercially available in the south-eastern region of Brazil.

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