

Occurrence of Aflatoxins and Ochratoxin A in Baby Foods in Portugal

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Abstract Infants have a more restricted diet and they generally consume more food on a body weight basis than adults. Therefore, the significance and potential health risk of any contaminant in foods consumed by infants is increased and diligent attention must be paid to this particular area. The present study aims to determine the occurrence of aflatoxin M₁ (AFM₁), aflatoxin B₁ (AFB₁) and ochratoxin A (OTA) in processed cereal-based foods (flours) and infant formulae (milk powder) available in the Portuguese market, both sold as conventional and organic origin. Mycotoxin determination was carried out using a method previously applied to duplicate diet samples. This method employed chloroform extraction, liquid–liquid extraction, immunoaffinity column (IAC) cleanup and HPLC analysis with fluorescence detection after post-column derivatisation. Quantification limits were 0.014, 0.004 and 0.028 µg kg⁻¹ for AFM₁, AFB₁ and OTA, respectively. These toxins could only be quantified in 12

of 27 analysed samples (15 positive results): two samples with AFM₁, two samples with AFM₁ and OTA, one sample with AFB₁ and OTA and seven samples with OTA. Positive results concerned four for AFM₁ (26%), one for AFB₁ (7%) and ten for OTA (67%). For these samples, contents ranged between 0.017–0.041 µg AFM₁ kg⁻¹, 0.034–0.212 µg OTA kg⁻¹, and one sample had a value of 0.009 µg AFB₁ kg⁻¹. Considering the presented results, we could provisionally conclude that the presence of these mycotoxins in baby foods does not constitute a public health problem. These are the first results concerning the occurrence of mycotoxins in marketed baby foods in Portugal and this is the first study using the HPLC method, proposed for duplicate diets, in baby food sample analysis.

Keywords Aflatoxins · Ochratoxin A · Baby Foods · HPLC · Portugal · Human Health

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Introduction

Infants are a vulnerable part of the population due to, in part, their physiology, a fairly restricted diet and a higher consumption relative to their body. Therefore, the significance and potential health risk of any contaminant in foods consumed by infants is increased and diligent attention must be paid to this particular area. Milk is a major nutrient for children, and cereals are an important source of nutrition in their diet and are among the first solid foods eaten. The presence of chemical contaminants in the human diet, and especially in the diet of vulnerable populations such as infants, is of great concern. Natural toxins such as those produced by fungi (mycotoxins) are among the most important chemical contaminants found in

foodstuffs and a primary concern when considering chronic health risks (Shephard 2006). Mycotoxins, particularly aflatoxins (AFTs) and ochratoxin A (OTA), pose significant threat to human health. Aflatoxins are potent carcinogens and, in association with hepatitis B virus, are responsible for many thousands of human deaths per annum, mostly in non-industrialised tropical countries (Shephard 2006). Ochratoxin A is a probable human carcinogen, and it was reported to cause urinary tract cancer and kidney damage in people from Eastern Europe. Exposure to OTA seems to be the biggest hazard correlated to microscopic fungi for the European consumers of cereals (Council of Europe 2006). EC Regulation 1881/2006 sets a limit of $0.25 \mu\text{g kg}^{-1}$ (dry product) for aflatoxin M_1 (AFM₁) for infant formulae and follow-on formulae, including infant milk and follow-on milk, and a limit of $0.10 \mu\text{g kg}^{-1}$ for aflatoxin B_1 (AFB₁) and $0.50 \mu\text{g kg}^{-1}$ for OTA for processed cereal-based foods and baby foods for infants and young children (European Commission 2006b).

Mycotoxin analysis is usually carried out by High-Performance Liquid Chromatography (HPLC) after immunoaffinity column cleanup or by enzyme-linked immunosorbent assay tests. These methods involve determination of single compounds (Spanjer et al. 2008). Aflatoxins are commonly separated by standard reversed-phase HPLC (RP-HPLC) systems in combination with a derivatisation of AFB₁ and AFG₁ and fluorescence detection (Arranz et al. 2006). The official method for OTA determination is based on liquid chromatography (LC) with fluorescence detection (Nesheim et al. 1992). The liquid chromatography–tandem mass spectrometry (LC-MS/MS) is rapidly gaining ground in routine laboratories as a method which enables the simultaneous detections of several mycotoxins in one run (Spanjer et al. 2008).

These methods were applied in the study of aflatoxins in several products as maize, peanuts, Brazil nuts, rice barley, cotton seeds, pistachio nuts, dried figs, spices and herbs (Arranz et al. 2006). Ochratoxin A occurs in cereals and cereal products, coffee, beans, pulses, grapes, wine and dried foods (Walker and Larsen 2005).

The present study aims to determine the occurrence of aflatoxin M_1 , aflatoxin B_1 and ochratoxin A in baby and infant foods available in the Portuguese market, both sold as conventional and organic origin. A total of 27 samples were collected in 2007 and analysed by a method previously applied to duplicate diet samples (Sizoo and van Egmond 2005). This method involves chloroform extraction, liquid–liquid partitioning, immunoaffinity cleanup and liquid chromatography with fluorescence detection after post-column derivatisation.

Results were compared with the maximum levels established in the EU and the available literature.

Materials and Methods

Samples

A total of 27 baby foods (15 sold as conventional and 12 as organic products) were randomly selected from big supermarkets, organic produce retail outlets and pharmacies in Lisbon city between May and June 2007. These samples were from nine market brands and include processed cereal-based foods. They were represented by acronyms A to I. They concern processed cereal-based foods (flours, biscuits) and infant milk and follow-on milk (powder) clearly labeled as being intended for infants or young children up to 3 years old. Cereal flours and milk powder are homogeneous samples. Therefore, no sample preparation was applied to the infant cereals due to their fine particle size and inherent consistency. A representative retail sample, in this case a minimum of 600 g in weight, was taken. According to EC 401/2006 (European Commission 2006a) the aggregate samples of infant milk and follow-on milk and processed cereal-based foods for infants and young children shall be at least 1 kg. Literature also reports the use of a representative sample of 600 g to survey the occurrence of mycotoxins in baby foods (<http://www.food.gov.uk/science/surveillance>).

When the product was sold in packs, a number of retail packs were purchased, ensuring that all came from the same batch, and these were mixed thoroughly, stored in a plastic container in a refrigerator until analysis and mixed again before removal of the analytical portion. A 10-gram sample was collected randomly before analysis. All samples were analysed within the shelf life of the product.

Further studies including the acquisition of data concerning infant foods lot size and consumer packages will allow a complete overview on mycotoxins contamination in baby foods in Portugal.

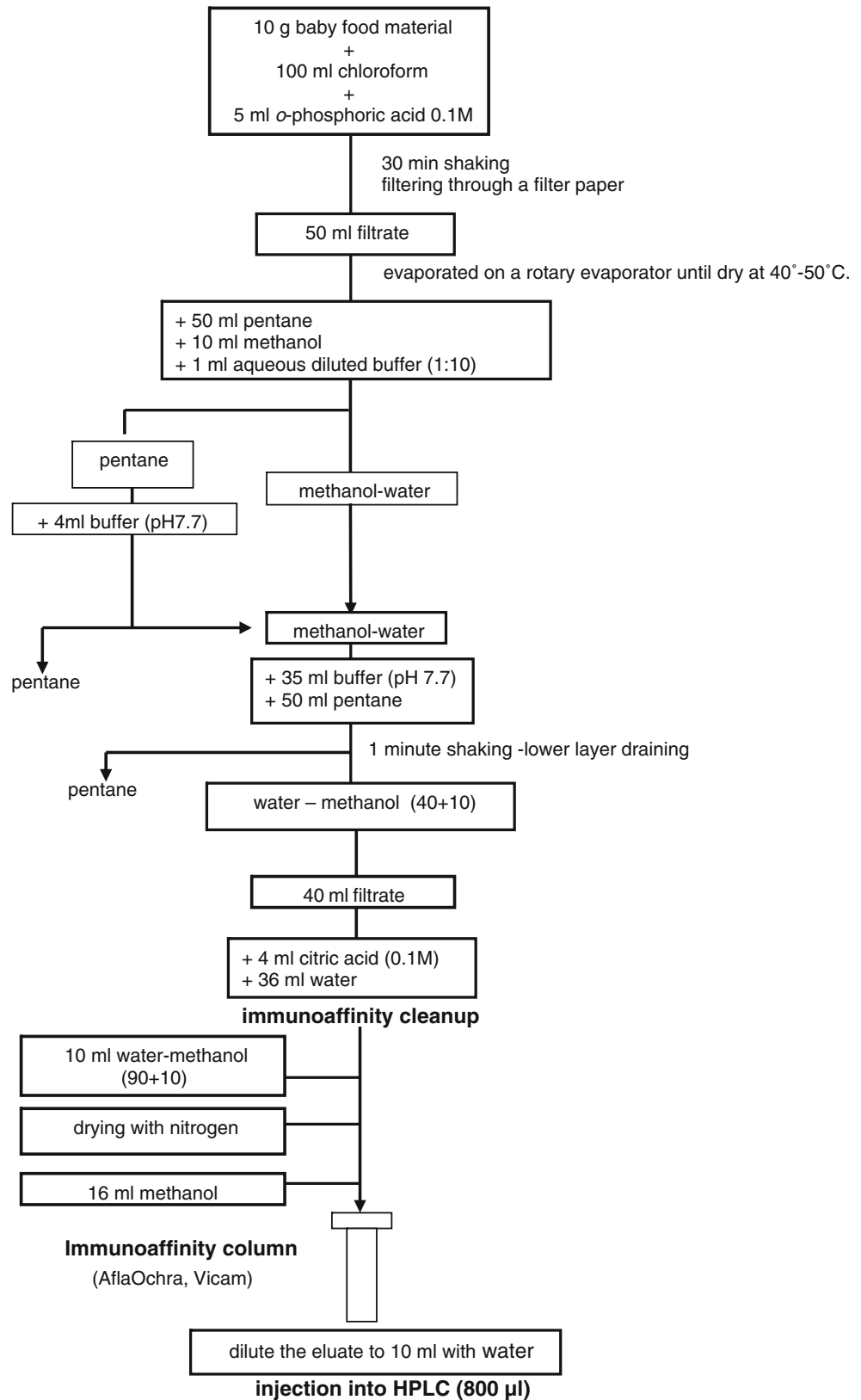
Chemicals and Reagents

Chloroform, methanol, ortho-phosphoric acid (85%), disodium hydrogen phosphate, citric acid and pentane (pro-analysis grade) were supplied by Merck (Darmstadt, Germany). Acetic acid, acetonitrile, methanol (HPLC grade) and potassium bromide (Suprapur grade) were supplied by Merck (Darmstadt, Germany). Phosphate–citrate buffer solution was prepared by mixing 0.2 mol l^{-1} di-sodium hydrogen phosphate with 0.1 mol l^{-1} citric acid in a 10:1 ratio (v/v), pH 7.7. Immunoaffinity columns Afla–Ochra were purchased from Vicam (MA, USA). Ultra-pure water was produced on a Milli Q Gradient A10 system, from Millipore (Molsheim, France). Preventive measures to avoid degradation of aflatoxins and ochratoxin A due to

laboratory glassware reaction or adsorption were undertaken. Laboratory glassware was soaked in dilute acid (e.g. sulphuric acid 2 mol l^{-1}) for several hours and rinsed with

ultra-pure water and measuring flasks were treated with Surfasil (Pierce, USA) treatment, before coming into contact with aqueous aflatoxin and ochratoxin A solutions.

Fig. 1 Scheme of the method for determination of aflatoxins and ochratoxin A in children foods (modified from E.A. Sizoo and H.P. van Egmond, 2005)



Standard Solutions

Standard solutions of AFM₁ (10 µg ml⁻¹, in 2.5 ml CHCl₃) and AFB₁ (10 µg ml⁻¹, in 2.5 ml CHCl₃) were from RIVM (The National Institute for Public Health and the Environment, Bilthoven, The Netherlands). A standard solution of OTA (10.07 µg ml⁻¹, in 5 ml acetonitrile) was from Biopure (Austria). From the three standard solutions, a multi mycotoxins stock solution was prepared in chloroform with 0.20 µg ml⁻¹ of AFM₁, 0.080 µg ml⁻¹ of AFB₁, and 0.40 µg ml⁻¹ of OTA. From this stock solution, a working stock solution was prepared in water–methanol (85+15, v/v), with concentrations of 0.20 ng ml⁻¹, 0.080 ng ml⁻¹ and 0.40 ng ml⁻¹ for AFM₁, AFB₁ and OTA, respectively. This working stock solution was further diluted to get several calibration solutions. This working stock solution was renewed every week.

Apparatus

HPLC analysis was performed using a Waters Alliance 2695 with fluorescence detector Waters 474 (Waters, USA) with Empower Chromatography Software. Post-column derivatisation was carried out with electrochemically generated bromine (Kobra cell) using a reaction tube of 120×0.25 mm id PTFE. The HPLC column was Prodigy ODS 100 Å (5 µm, 150×4.6 mm, Phenomenex, Torrance, CA) with a guard column ODS (4×3 mm id, Phenomenex, Torrance, CA).

Analytical Method

The levels of aflatoxin M₁, aflatoxin B₁ and ochratoxin A in baby foods were determined using a method previously

Table 1 Mobile phase, flow rate and fluorescence detection conditions (Sizoo and van Egmond 2005)

Gradient elution	Time (min)	Flow (ml min ⁻¹)	%A	%B
	0	1	100	0
	30	1	50	50
	42	1	50	50
	43	1	0	100
	44	1	0	100
	45	1	100	0
	59	0.2	100	0

Detection: Fluorescence detection with programmed wavelengths switch

Mobile phase A KBr (175 mg l⁻¹)–MeOH–ACN–HAC (1650:465:390:50 v/v/v/v), *Mobile phase B* KBr (175 mg l⁻¹)–MeOH–ACN–HAC (140:1283:1073:50 v/v/v/v)

Wavelength: switch for aflatoxins detection (start): λ_{exc}=365 nm, λ_{em}=435 nm, Switch after 22 min for OTA detection: λ_{exc}=332 nm, λ_{em}=468 nm

applied to duplicate diet samples (Sizoo and van Egmond 2005). This method, modified for baby food, is schematically depicted in Fig. 1 and involves chloroform extraction, liquid–liquid partitioning, immunoaffinity cleanup and liquid chromatography with fluorescence detection after post-column derivatisation. Briefly, a test portion of baby food sample was extracted with chloroform and ortho-phosphoric acid using a shaking machine (Edmund Bühler GmbH, Germany) and filtered through a filter paper (MN 617 1/4, 185 mm, Macherey Nagel). An aliquot of the filtrate (equivalent to 5 g test portion) was obtained and evaporated on a rotary evaporator (Büchi, Switzerland). The residue was redissolved in methanol and buffer solution, defatted by liquid–liquid cleanup with pentane and filtered through a glass microfibre filter (Whatman GF/A, 150 mm). A part of the filtrate (equivalent to 4 g test portion) was diluted with citric acid and water and the whole mixture passed through an AflaOchra immunoaffinity column. After the immunoaffinity cleanup, the eluate was diluted with water, and an aliquot was injected into the HPLC (equivalent to 0.32 g test portion).

The method was previously in-house validated for the analysis of these mycotoxins (Sizoo and van Egmond 2005). The chromatographic conditions like gradient elution, flow rate and fluorescence detection wavelengths are presented in Table 1. A mixture of potassium bromide (175 mg l⁻¹)–methanol–acetonitrile–acetic acid was used for mobile phases A and B in different proportions.

The linear range of the HPLC procedure was studied by analysis of six solutions containing the three target mycotoxins at different amounts (16–160 pg for AFM₁, 6–64 pg for AFB₁ and 32–320 pg for OTA). Linearity was evaluated after application of several statistical tests, such as residual analysis, determination coefficient, coefficient of variation of the method, RIKILT test (van Trijp and Roos 1991) and Mandel test (ISO 8466-1; ISO 1990). A six point calibration curve was prepared for AFM₁ (equivalent to 0.049–0.492 µg kg⁻¹), AFB₁ (equivalent to 0.020–0.197 µg kg⁻¹), and OTA (equivalent to 0.098–0.984 µg kg⁻¹).

The limits of detection (LOD) and quantification (LOQ) of the chromatographic method were determined with residual standard deviation (S_{x/y}) and slope (a) of calibration curve. These limits (LOD and LOQ) were also determined as the signal/noise ratio of 3 and 10, respectively, using the calibration solution with lowest toxin concentration level of the linear range and converted to microgram per kilogram. A random sample selection was carried out for recovery experiments using a spiking solution with concentrations close to the legal limits (0.25 µg AFM₁/kg, 0.10 µg AFB₁/kg and 0.50 µg OTA/kg).

Singular analyses of each of the coded samples were carried out.

A chemical blank (determination without test portion) was performed at regular intervals.

A check sample prepared at RIVM (mixture of milk powder, buckwheat, bambix) with contamination levels close to the legal limits was used as internal control sample in each series of experiments. Each check sample was performed in a routine quality control. For each set of samples, a check sample was made.

Food Analysis Performance Assessment Scheme (FAPAS) test materials (Ambifood, Portugal) with an assigned value and a satisfactory range for each toxin were purchased for milk powder (T0487: $0.218 \mu\text{g AFM}_1/\text{kg} \pm 0.096$) and baby foods (T0494: $0.145 \mu\text{g AFB}_1/\text{kg} \pm 0.064$; T1742: $0.600 \mu\text{g OTA}/\text{kg} \pm 0.26$) to study the accuracy performance of the method. Results were expressed in microgram per kilogram and were not corrected with the recovery percentage.

Results and Discussion

In Fig. 2, HPLC chromatograms are shown after injection of a solution of AFM_1 , AFB_1 and OTA standards, an extract of a cereal-based food (cereal flour) and a check (control) sample. The chromatogram showed a good resolution for target mycotoxins under the experimental HPLC conditions used.

The linear range, the determination coefficient, the PG values, the F value of Fisher/Snedecor (tabled value), the coefficient of variation of the method, the residuals analysis, the RIKILT test (van Trijp and Roos 1991) and limit of detection and limit of quantification for each compound are given in Table 2.

The determination coefficient (R^2) is a good indicator for correlation and not for linearity, which is shown by the Mandel test. If $\text{PG} \leq F$, the non-linear calibration function does not lead to a significantly better adjustment: the calibration function is linear. If $\text{PG} > F$, the working range should be reduced as far as possible to receive a linear calibration function; otherwise the information values of analysed samples must be evaluated using the non-linear calibration function.

All compounds showed linearity in the studied working range, with determination coefficient greater than 0.9993 and a $\text{PG} < F$ accordingly with Mandel's F -test. The PG values obtained vary between -0.90 and 1.2 which were lower than the F tabulated values for the corresponding degrees of freedom (Table 2).

Another good indicator of linearity is the coefficient of variation of the method (CVM), which values were between 0.75 and 2.6%.

Residual analysis showed residual values less or equal than 7.8% and in the RIKILT test (van Trijp and Roos 1991) all points are between the higher limit (110%) and lower limit (90%) with an acceptance criterion of 10%.

For all studied compounds, the chromatographic LOQ is lower than the lowest concentration level of the working range.

The limit of detection and the limit of quantification of the global method (extraction, partitioning, cleanup, chromatography and determination) and the results of recovery are reported in Table 3.

Quantification limits are $0.014 \mu\text{g kg}^{-1}$, $0.004 \mu\text{g kg}^{-1}$ and $0.028 \mu\text{g kg}^{-1}$ for AFM_1 , AFB_1 and OTA, respectively, which allowed a toxin determination well below the regulated limits.

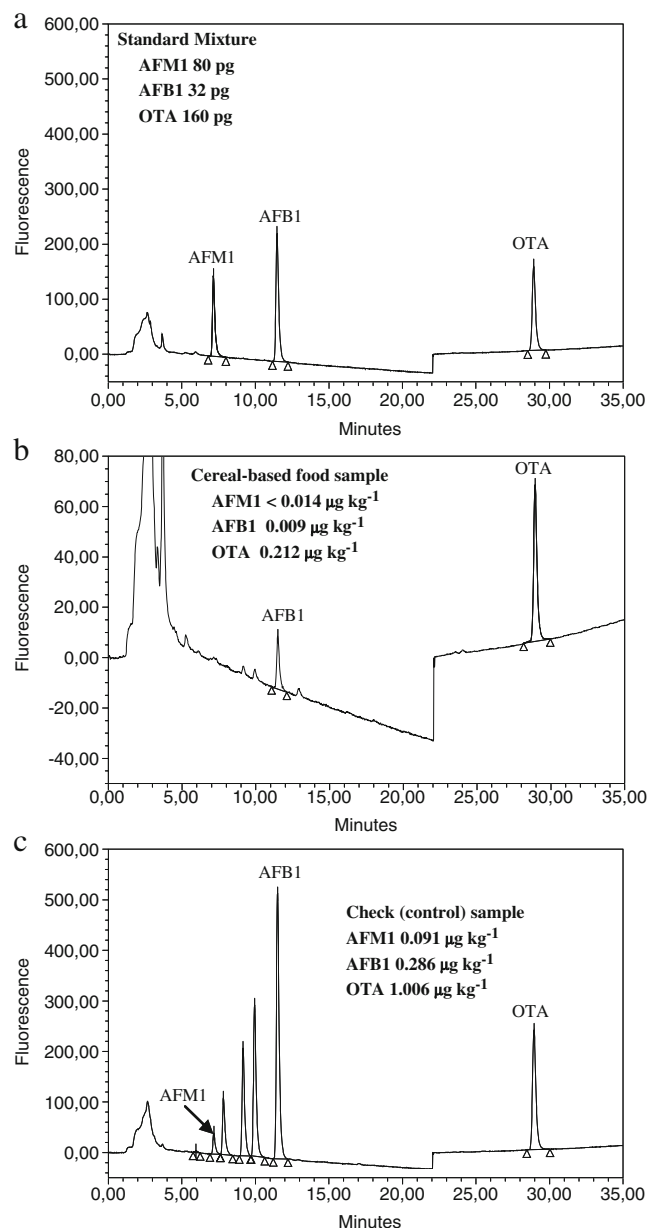


Fig. 2 HPLC chromatograms of a solution of AFM_1 , AFB_1 and OTA standards (a), an extract of a cereal-based food (b) and a check (control) sample (c)

Table 2 Regression data and detection and quantification limits for AFM₁, AFB₁ and OTA by HPLC method with fluorescence detection after post-column derivatisation

Compound	N	Linearity range (pg)	R ²	a	CVm (%)	PG	F	Residual analysis [-15, 15](%)	RIKILT test [90,110](%)	LOD (pg)	LOQ (pg)
AFM ₁	6	16–160	0.9999	1793.80	0.81	-0.90	7.7	[-0.26, 7.8]	[98, 106]	1.4	4.6
AFB ₁	6	6.4–64	0.9999	7155.90	0.75	0.19	7.7	[-0.38, 6.7]	[98, 105]	0.52	1.7
OTA	6	32–320	0.9993	849.45	2.6	1.2	7.7	[-4.5, 5.8]	[95, 106]	8.8	29

R² Determination coefficient, N the number of data points, a slope, CVm coefficient of variation of the method, PG test value PG required for the Mandel F-test must be lower than the tabulated F value of Fisher/Snedecor distribution. In this case, the calibration function is linear, leading to a significantly better adjustment of the calibration points than the non-linear calibration function, F Value of Fisher/Snedecor (tableted value); F(1, N-3,95%), LOD (pg) limit of detection of the chromatographic method. The value was expressed as the amount of each mycotoxin corresponding to three times the ratio between residual standard deviation (S_{x/y}) and slope of calibration curve (LOD = 3 × S_{x/y}/a), LOQ (pg) limit of quantification of the chromatographic method. The value was expressed as the amount of each mycotoxin corresponding to ten times the ratio between residual standard deviation (S_{x/y}) and slope of calibration curve (LOQ = 10 × S_{x/y}/a)

All recovery values, ranging between 85 and 93%, are within the requirements of the Regulation (EC) 401/2006 (European Commission 2006a) for these toxins that states recovery values of 50–120% for AFB₁ and OTA and 70–110% for AFM₁. The obtained values are in the same range as those reported by Sizoo and van Egmond (2005) for duplicate diet (64% for AFM₁—0.18 µg kg⁻¹ spiking level; 91% for AFB₁—0.090 µg kg⁻¹ spiking level; and 81% for OTA—0.45 µg kg⁻¹ spiking level).

The accuracy of the method was evaluated through FAPAS test materials and through the analysis of a check sample used as in-house reference material.

Figure 3 shows the values obtained for the reference materials, in comparison with the FAPAS reference values. Toxin contents are close to the assigned values and within the reported range (T0487—0.238 µg AFM₁/kg, T0494—0.116 µg AFB₁/kg and T1742—0.556 µg OTA/kg).

The relative errors (%) obtained in the FAPAS test material for the toxins as compared to the reference values (9.1%, 20% and 4.7% for AFM₁, AFB₁ and OTA, respectively) suggest that this method has a good accuracy. The same conclusions are drawn when we compare the closeness of the experimental values with the assigned values of the RIVM check sample.

The determination of toxin content of the check sample used as in-house reference material showed good reproducibility (n=4) with coefficient of variation of 4.5%, 7.3% and 4.7% for AFM₁, AFB₁ and OTA, respectively.

The results concerning the contents on AFM₁, AFB₁ and OTA in all analysed samples are represented in Table 4.

All results are below the maximum levels established in EU legislation for processed cereal-based foods and baby foods for infants and young children and infant formulae and follow-on formulae, including infant milk and follow-on milk (European Commission 2006b). These toxins could only be quantified in 12 of 27 analysed samples (15 positive results): two samples with AFM₁, two samples with AFM₁ and OTA, one sample with AFB₁ and OTA and seven samples with OTA. Positive results concerned four for AFM₁ (26%), one for AFB₁ (7%) and ten for OTA (67%). For these samples, contents ranged between 0.017–0.041 µg AFM₁/kg, 0.034–0.212 µg OTA/kg and one sample had a value of 0.009 µg AFB₁/kg. For AFM₁, the 23 remaining samples showed values below the LOD (17 samples, 63%) and below the LOQ (six samples, 22%). For AFB₁, the 26 remaining samples showed values below the LOD (20 samples, 74%) and below the LOQ (six samples, 22%). For OTA, the 16 remaining samples showed values below the LOD (11 samples, 41%) and below the LOQ (six samples, 22%).

Aflatoxin B₁, the most toxic mycotoxin, was not detected in infant cereals analysed during the First French Total Diet Study (Leblanc et al. 2005; LOQ=1 µg kg⁻¹) and was detected in only one sample during the survey of baby foods undertaken by the Food Standards Agency (<http://www.food.gov.uk/science/surveillance>; LOQ=0.020 µg kg⁻¹). How-

Table 3 Performance of the analytical method for baby foods

Compound	LOD (S/N=3, in µg kg ⁻¹)	LOQ (S/N=10, in µg kg ⁻¹)	Spiking level (µg kg ⁻¹)	Recovery (n=3)	
				(%)	RSD%
AFM ₁	0.004	0.014	0.25	93	4.5
AFB ₁	0.001	0.004	0.10	92	3.7
OTA	0.009	0.028	0.50	85	12

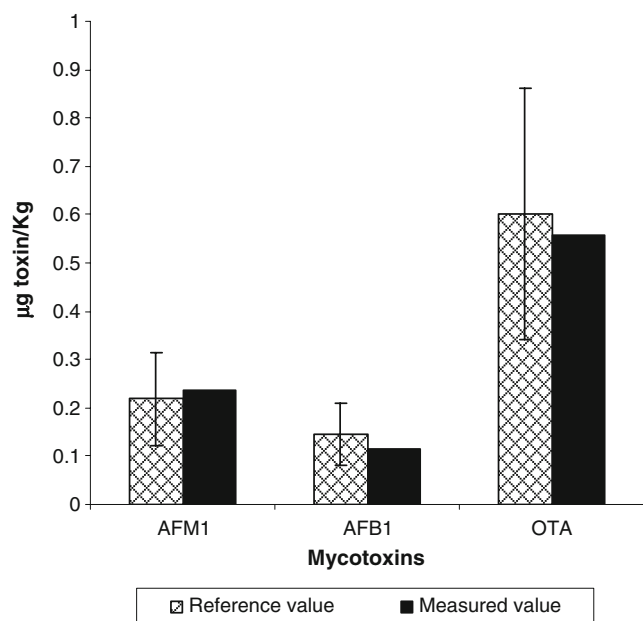


Fig. 3 Comparison of toxin contents found in the FAPAS test materials, with the reference values and respective satisfactory ranges

ever, it was detected in infant cereals from the Canadian market (Tam et al. 2006; LOQ=0.050 $\mu\text{g kg}^{-1}$) as well as OTA (Lombaert et al. 2003; Roscoe et al. 2008; LOQ=0.200 $\mu\text{g kg}^{-1}$). OTA was also detected in milk-based infant formula from the Canadian market (Lombaert et al. 2007). AFM₁ was reported in dry milk for infant formula marketed in Italy (Galvano et al. 2001). Spanjer et al. (2008) referred a LOQ of 0.011 $\mu\text{g kg}^{-1}$ for AFB₁ and 0.173 $\mu\text{g kg}^{-1}$ for OTA in a crisp-bread sample.

Conclusions

A good resolution was achieved for target mycotoxins under the experimental HPLC conditions used.

All compounds showed linearity in the studied working range, with determination coefficient (R^2) greater than 0.9993, a $PG < F$ accordingly with Mandel's F -test. The PG values obtained vary between -0.90 and 1.2 (lower than F tabulated values for the corresponding degrees of freedom) and the coefficient of variation of the method was between 0.75 and 2.6%.

Residual analysis showed values lower or equal to 7.8% and in the RIKILT test all values are within the acceptance criterion.

The method showed recovery percentages from 85–93% for these analytes.

Toxin contents are close to the FAPAS assigned values with relative errors of 9.1%, 20% and 4.7% for AFM₁,

AFB₁ and OTA, respectively. These results agree well with the closeness of the experimental values with the assigned values of the RIVM check sample. The determination of toxin content of the check sample used as in-house reference material showed good reproducibility with coefficient of variation of 4.5%, 7.3% and 4.7% for AFM₁, AFB₁ and OTA, respectively.

These toxins could only be quantified in 12 of 27 analysed samples (15 positive results): two samples with AFM₁, two samples with AFM₁ and OTA, one sample with AFB₁ and OTA and seven samples with OTA. Positive results concerned four for AFM₁ (26%), one for AFB₁ (7%) and ten for OTA (67%). For these samples, contents ranged between 0.017–0.041 $\mu\text{g AFM}_1 \text{ kg}^{-1}$, 0.034–0.21 $\mu\text{g OTA kg}^{-1}$ and one sample had a value of 0.009 $\mu\text{g AFB}_1 \text{ kg}^{-1}$. The limits of quantification obtained in this study were 0.014, 0.004 and 0.028 $\mu\text{g kg}^{-1}$ for AFM₁, AFB₁ and OTA, respectively. For the original method (Sizoo and van Egmond 2005), the quantification limits were estimated to be 0.024, 0.005 and 0.016 $\mu\text{g kg}^{-1}$ for aflatoxin M₁, aflatoxin B₁ and ochratoxin A, respectively. The LOQ values determined on this study for aflatoxins and OTA and those reported by other authors (Spanjer et al. 2008; Sizoo and van Egmond 2005; <http://www.food.gov.uk/science/surveillance>; Tam et al. 2006; Lombaert et al. 2003; Roscoe et al. 2008) are well below the maximum levels established in EU legislation. This method, applied to different matrices, allows detecting the lowest contamination values; therefore, it is recommended to perform determination of these mycotoxins in baby food samples. The selection of raw materials by the producers as well as the harvest time and storage conditions could influence the level of mycotoxins contamination in baby foods. In the present study and considering the analysed samples, the mycotoxins did not represent a problem in baby foods.

Considering the presented results, those reported from the literature and the potential negative health impact for the analysed toxins, more baby foods, both from conventional and organic origin have to be studied in order to contribute to a broader exposure assessment of babies and infants to mycotoxins. An optimisation step for the extraction procedure is in progress (to avoid the use of chloroform and reduce the time of extraction procedure).

Therefore, we could provisionally conclude that the presence of these mycotoxins in baby foods does not represent a public health problem. These data will be useful for future exposure estimates when conjugated with Portuguese data intakes of baby foods.

These are the first results of investigations on the occurrence of mycotoxins in baby foods marketed in Portugal and this is the first study using the HPLC method developed for duplicate diet investigations, applied to baby food sample analysis.

Table 4 Content of AFM₁, AFB₁ and OTA in baby foods

Commodity	Brand	Production	Composition	Source	AFM ₁ ($\mu\text{g kg}^{-1}$)	AFB ₁ ($\mu\text{g kg}^{-1}$)	OTA ($\mu\text{g kg}^{-1}$)
Cereals							
Flour	A	O	Wheat	Bioshop	<LOD	0.002 ^a	0.013 ^a
Flour	A	O	Rice, wheat	Bioshop	<LOD	0.002 ^a	0.034
Flour	B	O	Oats flour	Bioshop	<LOD	<LOD	0.010 ^a
Flour	B	O	Maize flour	Bioshop	<LOD	<LOD	<LOD
Flour	B	O	Rice flour	Bioshop	<LOD	<LOD	<LOD
Flour	C	C	Milk, cereals 70% (starch maize, rice flour)	Pharmacy	0.018	0.003 ^a	0.142
Flour	C	C	Milk, apple 60%, cereals 35% (starch maize, rice flour)	Pharmacy	0.023	<LOD	0.010 ^a
Flour	D	C	Milk, wheat flour 40.3%, skimmed milk 29.9%	Supermarket	<LOD	<LOD	0.010 ^a
Flour	E	C	Rice flour and maize flour (79%)	Supermarket	0.008 ^a	0.002 ^a	<LOD
Flour	C	C	Milk, cereals 39% (wheat, rice, oats flour, barley, rye, maize)	Pharmacy	0.017	<LOD	0.150
Flour	F	C	Wheat flour (78.7%)	Supermarket	<LOD	<LOD	0.021 ^a
Flour	E	C	Milk, wheat flour 53%	Supermarket	<LOD	<LOD	<LOD
Flour	C	C	Rice flour 68%	Pharmacy	<LOD	<LOD	0.081
Flour	F	C	Flour 78% (wheat, hydrolysed wheat, maize, rye, barley, rice, oats)	Supermarket	<LOD	<LOD	0.077
Flour	C	C	Cereals 62% (wheat, barley, rye, rice, maize, oats flour), fruits	Pharmacy	<LOD	<LOD	<LOD
Flour	G	O	Rice flour 87.9%, quinoa flour 9.7%	Bioshop	<LOD	0.002 ^a	<LOD
Flour	A	O	Rice	Bioshop	<LOD	<LOD	<LOD
Flour	A	O	Rice, oats, barley, apple (5%), almonds, nuts	Bioshop	<LOD	0.009	0.212
Flour	B	O	Wheat flour	Bioshop	<LOD	<LOD	0.032
Biscuit	I	O	Cookies: wheat 60%, grape juice 28%, sunflower oil 2%, ginger 1%	Bioshop	<LOD	<LOD	0.052
Milk							
Powder	H	O	Soy proteins	Bioshop	<LOD	0.003 ^a	0.136
Powder	G	O	Milk, vegetable oils	Bioshop	0.011 ^a	<LOD	0.011 ^a
Powder	G	O	Milk, vegetal oils	Bioshop	0.007 ^a	<LOD	<LOD
Powder	F	C	Milky serum, vegetal fish oils	Supermarket	0.008 ^a	<LOD	<LOD
Powder	F	C	Milk, serum proteins, vegetal fish oils	Supermarket	0.041	<LOD	<LOD
Powder	E	C	Milk, hydrolysed serum proteins concentrate, vegetable oils, starch	Supermarket	0.013 ^a	<LOD	<LOD
Powder	E	C	Milk, hydrolysed serum proteins concentrate, vegetable oils, starch	Supermarket	0.005 ^a	<LOD	0.135

The LOD for AFM₁, AFB₁ and OTA are 0.004, 0.001, and 0.009 $\mu\text{g kg}^{-1}$, respectively. The LOQ for AFM₁, AFB₁ and OTA are 0.014, 0.004 and 0.028 $\mu\text{g kg}^{-1}$, respectively.

EU limits for AFM₁, AFB₁ and OTA in baby and infant foods are 0.25, 0.10 and 0.50 $\mu\text{g kg}^{-1}$, respectively

LOD Limit of detection $\mu\text{g kg}^{-1}$, LOQ limit of quantification $\mu\text{g kg}^{-1}$, Production mode: O (organic) and C (conventional)

^a<LOQ

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