Talanta 83 (2011) 1442–1446

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous determination of aflatoxins and ochratoxin A in baby foods and paprika by HPLC with fluorescence detection: A single-laboratory validation study

C. Brera*, F. Debegnach, B. De Santis, E. Pannunzi, C. Berdini, E. Prantera, E. Gregori, M. Miraglia

GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety, Italian National Institute for Health, Viale Regina Elena, 299–00161 Rome, Italy

ARTICLE INFO

Article history:
Received 16 June 2010
Received in revised form 8 November 2010
Accepted 14 November 2010
Available online 19 November 2010

Keywords:
Ochratoxin A
Aflatoxins
Baby foods
Paprika

ABSTRACT

Mycotoxins are toxic secondary metabolites of fungal origin, the major mycotoxins of food concern are aflatoxins and ochratoxin A. Due to the wide range of matrices susceptible to mycotoxin contamination, the possible co-occurrence, and the very wide range of concentration, validated versatile multi-mycotoxin and multi-matrix methods are strongly requested. A reversed phase HPLC method for the simultaneous determination of aflatoxins and ochratoxin A in baby foods and paprika was set up. Three bulk samples were prepared according to commercial availability, one for paprika and for baby foods, two different bulks were set, a corn based and a multi-cereal based baby food. A single-laboratory validation was performed, for each investigated level ten analyses were performed, relative standard deviations of repeatability (RSD_r) and recovery factors were calculated; RSD_r values ranged from 2% to 10% for AFB_1 and from 3% to 10% for OTA, while the recovery factors ranged from 86% to 96% for AFB_1 and from 77% to 96% for OTA. The checked compliance of the RSD_r and recovery with the values reported in the current EU Regulations confirmed the fitting for purpose of the method. Limit of detection and LoQ values of the method were respectively 0.002 and 0.020 μg/kg for AFB_1 and 0.012 and 0.080 μg/kg for OTA in baby foods; and 0.002 and 0.200 μg/kg for AFB_1 and 0.012 and 0.660 μg/kg for OTA in paprika. The current method represents a good example of the possibility of a multi-mycotoxin and/or a multi-matrix analysis depending on the laboratory research or official control purposes.

1. Introduction

Mycotoxins are toxic secondary metabolites of fungal origin, commonly present as contaminants in different relevant food crops, such as cereals, nuts, and spices. These contaminants show a wide spectrum of toxic effects, including carcinogenicity, immunotoxicity, neurotoxicity, and teratogenicity. To date, more than 300 mycotoxins are known, even if the public health concerns are mainly focused to less than twenty [1]. For this reason, regulations have been set in more than 100 countries worldwide [2]. Among mycotoxins, two of the most relevant mycotoxins of food concern are aflatoxins (AFs) and ochratoxin A (OTA).

Aflatoxins are produced by strains of *Aspergillus flavus*, *parasiticus* and *nomius* [3]. *A. flavus* produces B aflatoxins only, while the two other species produce both B and G ones. Toxic effects of aflatoxins include genotoxic (aflatoxin B_1), carcinogenic, mutagenic, teratogenic, and immunosuppressive activity [4]. Aflatoxin B_1 (AFB_1) is classified by the International Agency for Research on Cancer (IARC) as Group 1, carcinogenic to humans [5].

Ochratoxin A is mainly produced by some species of *Aspergillus* and *Penicillium*, particularly *A. ochraceus*, *A. carbonarius*, *A. niger* and *P. verrucosum*. Ochratoxin A is a potent nephrotoxin, immunotoxin, mutagen and teratogen [6]. The genotoxicity of OTA has been postulated *in vivo* and *in vitro* [7] but it has been revised by Mally and also stated by the European Food Safety Authority [8,9]. IARC has classified OTA in group 2B, possible human carcinogen [5]. OTA is also associated with Balkan Endemic Nephropathy, which is a fatal human kidney disease [10,7].

Aflatoxins and OTA can occur in a wide range of raw commodities, including cereals, nuts, cocoa, coffee, dried fruit and spices and, due to their stability to the industrial processing, they can also occur in the derived products.

Infants are considered a vulnerable group of the population and they are more susceptible to mycotoxin exposure than adults since they have a restricted diet, rich in cereals, and consume more food on a body weight basis than adults. As a consequence, EU legal limits for mycotoxins in baby foods are much lower than the limits set for all other regulated matrices.

On the other hand, spices, despite their low intake from the population with the diet, are characterized from higher levels of contamination, and therefore legal limits are quite higher correspondingly.

* Corresponding author. Tel.: +39 06 49902377; fax: +39 06 49902363.
E-mail address: carlo.brera@iss.it (C. Brera).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.11.031

Due to the legislative provisions regulating mycotoxin levels in food and feed, a large amount of analyses for official control purposes is carried out to ensure the food safety. Due to the wide range of matrices susceptible to contamination, the possible co-occurrence, and the very wide range of mycotoxin concentrations, validated versatile multi-mycotoxin and multi-matrix methods are strongly necessary.

The aim of this study was to set up a RP-HPLC method for the simultaneous determination of aflatoxins and ochratoxin A in baby foods and paprika. These two matrices were chosen because of the co-occurrence of the selected mycotoxins. Moreover, the difference in legal limit values gave the opportunity to make available a method suitable in such a wide range of applicability. The limits for AFB_1 and OTA in baby foods are 0.10 and 0.50 μg/kg, respectively, while for paprika the limits are 5.0 and 10.0 μg/kg for AFB_1 and total aflatoxins, respectively, and 30 μg/kg for OTA [11,12]. Furthermore, these two matrices have different characteristics, since baby foods, conversely to paprika powder, have complex formulations that always include more than one ingredient; therefore, a method suitable for both these matrices has to be characterized by a satisfactory robustness.

Immunoaffinity column clean-up and HPLC determination were already employed for the simultaneous determination of AFs and OTA, but the methods described in literature are often focused on a single matrix [13–15]. A paper, recently published [16], reports a method for AFs and OTA determination but is characterized by a complex, and not environmental friendly, extraction step that involves chloroform and phosphoric acid. The work presented in this study is therefore aimed at reducing the analytical steps to obtain a high sensitivity, robust and time saving method.

2. Experimental

2.1. Apparatus

A Waring blender 700S, explosion proof, with 1 L jar and cover, operating at high speed (Waring Laboratory & Science®, Torrington, CT, USA), a Visiprep SPE vacuum manifold of Supelco (Supelco Inc., Bellefonte, PA, USA), and a centrifuge (Vittadini PK131, Milano, Italy) were used.

Chromatographic analyses were performed using a RP-HPLC equipped with a Jasco FP1520 fluorescence detector (Jasco Corporation, Tokyo, Japan). The injection in the HPLC system was performed in total loop mode, the injection volume was 150 μL. The excitation wavelengths were 365 nm for AFs and 333 nm for OTA, the emission wavelengths were 442 and 463 for AFs and OTA, respectively. The gradient was applied as follows: 0 min (λ_{ecc} 365 nm, λ_{em} 442 nm; 17 min (λ_{ecc} 333 nm, (λ_{em} 463 nm. The software for equipment control and data acquisition was Borwin version 1.5.

Aflatoxins (B_1, B_2, G_1 and G_2) and ochratoxin A were separated on a C18 column (Symmetry 150 × 4.6 mm, Waters). Two solvents were used: solvent A (40% methanol, 2% acetic acid in water) and solvent B (80% methanol, 2% acetic acid in water) at a flow rate of 1 mL/min. The gradient was applied as follows: 0 min 100% A; 14 min 100% A; 16 min 35% A; 30 min 35% A; 31 min 100% A; 40 min 100% A. AFs derivatization was performed with a 0.005% aqueous solution of Pyridine Hydrobromide Perbromide (PBPB) by using a post-column LC pump (zero-dead volume T-piece, reaction tubing minimum 450 × 0.5 mm id in PTFE) (LC pump Labflow 2000, Labservice Analytica, Bologna, Italy) at a flow rate of 0.4 mL/min.

Illustrative chromatograms of combined mycotoxins working standard solution, and baby foods and paprika samples are reported in Fig. 1.

2.2. Chemicals

Liquid chromatography and RPE grade methanol were purchased from Carlo Erba (Carlo Erba Reagenti, Milan, Italy). Potassium chloride, potassium dihydrogenphosphate, anhydrous disodium hydrogenphosphate, and sodium chloride analytical grade were obtained from J.T. Baker (Deventer, The Netherland). Water was purified by distillation and passage through a Milli-Q system (Millipore, Bedford, MA, USA).

Phosphate buffer solution (PBS) was prepared from potassium chloride (0.2 g), potassium dihydrogen phosphate (0.2 g), anhydrous disodium hydrogen phosphate (1.2 g), and sodium chloride (8 g) added to distilled water (900 mL). After dissolving, the pH was adjusted to 7.4 (with 0.1 M HCl or 0.1 M NaOH as appropriate), and the solution was made to 1 L.

Aflatoxins and OTA certified standard solutions were purchased from Biopure (Tulln, Austria). Ochratoxin A standard solution concentration was 10.15 ± 0.14 μg/mL; aflatoxins concentrations were AFB_1 2.00 μg/mL, AFB_2 0.500 ± 0.025 μg/mL, AFG_1 2.01 μg/mL, AFG_2 0.500 ± 0.025 μg/mL. The standard solutions were in 100% methanol.

For clean-up step immunoaffinity column (IAC) AFLAOCHRAPREP®, OCHRAPREP®, and AFLAPREP®, (R-Biopharm Rhône Ltd., Glasgow, UK) were used.

2.3. Samples

The method set up was performed on bulk samples. For baby foods, according to commercial availability, two different matrices were tested: the corn based baby foods (Pool 1), for which the formulation always includes tapioca, and the multi-cereal based baby foods (Pool 2), for which the composition always includes wheat as the main ingredient and other cereals such as barley, oats and spelt in percentages that vary depending on the product brand. All the information about the sample composition was obtained from the labels. Baby foods were purchased from specialized retailers in Rome. The bulks were prepared and homogenized by mixing different packs of different brands and lots, each bulk was about 3 kg.

The paprika bulk (Pool 3) was prepared and homogenized by mixing 15 different packs of different brands, of 100 g each, purchased from the market. The bulk size was about 1.5 kg.

2.4. Standard solutions preparation

Starting from the purchased AFs and OTA certified standards, stock solutions at 2.00 ng/mL and 10.00 ng/mL were prepared for aflatoxins (AFB_1 and AFG_1 2.00 ng/mL; AFB_2 and AFG_2 0.50 ng/mL) and ochratoxin A, respectively. The working standard solutions were prepared by appropriate dilutions. Due to the differences in mycotoxin contamination of the investigated matrices, two calibration curves were prepared. The calibration curve for determination of baby foods ranged from 0.020 to 0.320 ng/mL for AFB_1 and from 0.100 to 1.600 ng/mL for OTA; while for paprika determination the calibration curve ranged from 0.020 to 1.000 ng/mL for AFB_1 and AFG_1, from 0.005 to 0.250 ng/mL for AFB_2 and AFG_2, and from 0.060 to 3.015 for OTA.

2.5. Extraction and clean-up

The method for simultaneous determination of aflatoxins and ochratoxin A in baby foods and paprika was assessed starting from the method for aflatoxin B_1 determination in corn samples described by Brera et al. [17]. Only slight modifications were requested, and the obtained method, for baby foods and paprika, went through a single-laboratory validation to confirm the perfor-

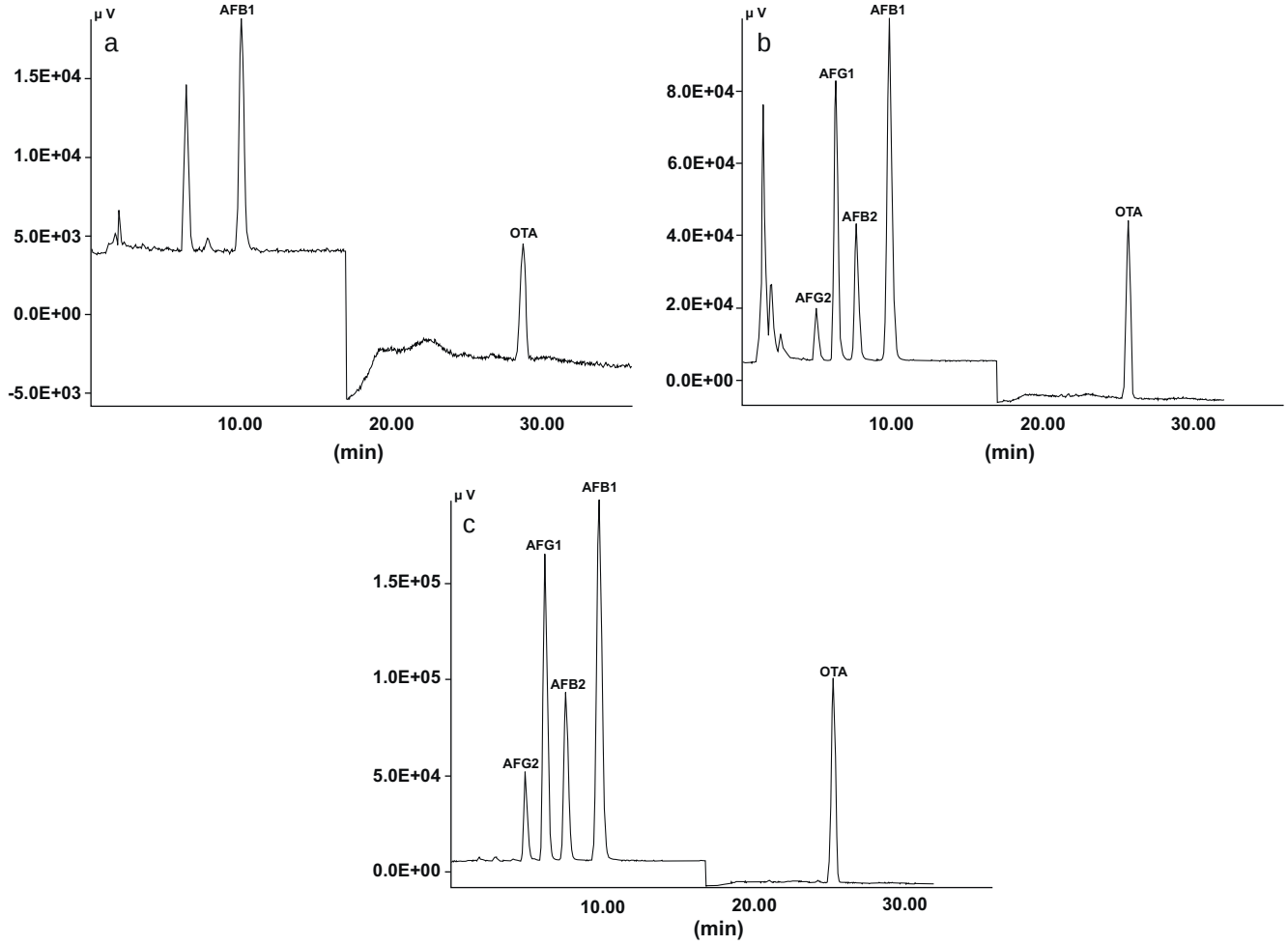

Fig. 1. Chromatograms of: (a) baby foods sample with 0.19 μg/kg AFB_1 and 0.89 μg/kg OTA; (b) paprika sample with 5.07 μg/kg AFB_1, 1.07 μg/kg AFB_2, 4.60 μg/kg AFG_1, 0.80 μg/kg AFG_2, and 14.47 μg/kg OTA; (c) working standard solution with 1.00 ng/mL AFB_1 and AFG_1, 0.25 ng/mL AFB_2 and AFG_2, and 3.02 ng/mL OTA.

mance characteristics of the starting method. A brief description of the modified extraction and purification steps for the investigated matrices is given below.

2.5.1. Baby foods

Weigh a test portion of 50 g into a high-speed blender jar. Add 5 g of sodium chloride and 250 mL of methanol:water extraction solvent (80:20, v/v). Stop up the jar and blend at high speed for 3 min. Filter the extract through pre-folded filter paper. Pipette 30 mL of filtrate and dilute with 30 mL of PBS. Mix thoroughly, and centrifuge the diluted sample for 10 min at 10,000 rpm. Apply 40 mL of the diluted sample to the conditioned immunoaffinity column and wash with 10 mL of PBS. Elute mycotoxins in a 2-step procedure. First, apply 1.0 mL methanol to the IAC and let it flow through under gravity. Collect eluate in calibrated 5 mL volumetric flask. Wait 1 min and apply a second portion of 1.0 mL methanol. Use a 10 mL syringe to pass air through the column to collect the remaining few drops. Fill the 5 mL volumetric flask to the mark with water, shake well, and store the sample at +4 °C prior to analysis.

2.5.2. Paprika

Weigh a test portion of 25 g into a high-speed blender jar. Add 2.5 g of sodium chloride and 100 mL of methanol:water extraction solvent (80:20, v/v). Stop up the jar and blend at high speed for 3 min. Filter the extract through pre-folded filter paper. Pipette 4 mL of filtrate and dilute with 36 mL of PBS. Mix thoroughly, and centrifuge the diluted sample for 10 min at 10,000 rpm. Apply 20 mL of the diluted sample to the conditioned immunoaffinity column and wash with 10 mL of PBS. Elute mycotoxins in a 2-step procedure. First, apply 1.0 mL methanol to the IAC and let it flow through under gravity. Collect eluate in calibrated 5 mL volumetric flask. Wait 1 min and apply a second portion of 1.0 mL methanol. Use a 10 mL syringe to pass air through the column to collect the remaining few drops. Fill the 5 mL volumetric flask to the mark with water, shake well, and store the sample at +4 °C prior to analysis.

2.6. Single laboratory validation

The method was validated according to Eurachem and IUPAC guidelines [18,19].

Limit of detection (LoD) was calculated by the "$b + 3s$" approach; for this purpose a blank sample standard deviation "s" (10 injections) was calculated, "b" being the blank signal. Similarly the limit of quantification (LoQ) was calculated applying the formula "$b + 10s$". After the theoretical LoQ calculation, the value was verified by injecting 10 times a blank sample fortified at the LoQ level and the level of precision was evaluated. The blank sample used for the LoD and LoQ evaluation was a sample that showed, for the two investigated mycotoxins, the presence of a small toxin amount that was possible to detect but not to quantify.

Applicability and linearity of the method were assessed from the calibration curves.

Table 1
Limit of quantification (LoQ), mean values (x), relative standard deviation of repeatability (RSD_r) and recovery factors resulted from the baby foods in-house validation.

Matrix	Level	Mycotoxin	x (μg/kg)	RSD_r (%)	Recovery (%)
Pool 1	LoQ	AFB_1	0.02	8	–
		OTA	0.08	7	–
	Level 1 (spiked)	AFB_1	0.10	10	96
		OTA	0.46	4	93
	Level 2 (spiked)	AFB_1	0.19	9	93
		OTA	0.89	6	88
Pool 2	LoQ	AFB_1	0.02	6	–
		OTA	0.08	10	–
	Level 1 (spiked)	AFB_1	0.09	0	90
		OTA	0.43	2	86
	Level 2 (spiked)	AFB_1	0.17	6	86
		OTA	0.78	3	77

Precision was assessed by analysing ten times each sample level and evaluating the relative standard deviation of repeatability (RSD_r).

In absence of certified reference materials (CRMs), trueness was assessed through recovery evaluation, calculated by analysing blank samples after addition of known amount of AFs and OTA.

For recovery studies on baby foods, stock solutions at 100 ng/mL were prepared both for aflatoxins (AFB_1 and AFG_1 100 ng/mL; AFB_2 and AFG_2 25 ng/mL) and ochratoxin A. A 50.0 g test portion was added with appropriate amounts of AFs and OTA standard solutions to obtain the desired contamination. The investigated levels were 0.10 and 0.20 μg/kg for AFB_1; 0.50 and 1.00 μg/kg for OTA.

For paprika recovery purposes, a 25.0 g test portion was added with appropriate amounts of AFs and OTA standard solutions to obtain the desired contaminations. The investigated levels were 5.00 μg/kg for AFB_1 and AFG_1, 1.00 μg/kg for AFB_2 and AFG_2; 15.00 μg/kg for OTA.

3. Results and discussion

3.1. Method set up

The principle of the starting method [18] was not changed, and only slight modifications were requested to apply the method to different matrices and to include the ochratoxin A determination. The main difference between the baby foods and paprika determination is the amount of sample passed through IAC (namely 4.0 g for baby foods and 0.5 g for paprika). Since legal limits for AFB_1 and OTA in baby foods are quite low, the analytical method set up was aimed at reducing both the analytical steps and the time required for the purification without loss of sensitivity. At this scope it was chosen to pass a larger amount of diluted sample extract through the IAC (40 mL), avoiding to dry the sample after the elution step as other methods suggest [20]. Moreover, the extract was only slightly diluted to increase the equivalent sample amount in the IAC. Applying a dilution factor of 1:1, the percentage of methanol applied to the IAC was 40%; this value is rather high for total aflatoxin determination possibly preventing the antigen-antibody binding. Nevertheless, the tested IA columns showed good recovery performances when assessing only the aflatoxin B_1 content (Table 1) and, since EU Regulation express legal limit only for AFB_1 in baby foods, the method is fit for purpose.

On the other hand the ratio 1:4 for weighted sample/extraction solvent, the lower sample amount passed through the IAC and a dilution factor of 1:9 for paprika determination permits the total aflatoxin determination with good method performance (Table 2).

The variation in methanol percentage passed through the IAC seems not to affect the recovery factors for OTA in all the tested matrices (Tables 1 and 2).

As for the method setting up, test analyses were performed preliminarily on corn based baby foods where shaking techniques and different sample sizes were tested. The comparison between blender and orbital shaker extraction confirmed that the two techniques are equivalent. As for sample size, the best results were obtained for 50.0 g of sample, due to mechanical difficulty of the blender to homogenize small amounts of sample (Table 3).

The results obtained for corn based baby foods were transferred to multi-cereal based products since the related single-laboratory validation results were fully corresponding to the performance criteria reported in the reference norm [21].

For paprika analysis, 25.0 g sample size and the blender extraction resulted as the best choice. For the dilution step, different solvent mixtures were investigated; PBS solution and PBS plus different percentages of Tween 20, as suggested by the IAC manu-

Table 2
Limit of quantification (LoQ), mean values (x), relative standard deviation of repeatability (RSD_r) and recovery factors resulted from the paprika in-house validation.

Matrix	Level	Mycotoxin	x (μg/kg)	RSD_r (%)	Recovery (%)
Pool 3	LoQ	AFB_1	0.20	8	–
		AFB_2	0.07	6	–
		AFG_1	0.20	4	–
		AFG_2	0.07	8	–
		OTA	0.66	10	–
	Level 1 (naturally contaminated)	AFB_1	0.68	7	–
		AFB_2	–	–	–
		AFG_1	0.75	10	–
		AFG_2	–	–	–
		OTA	–	–	–
	Level 2 (spiked)	AFB_1	5.08	3	89
		AFB_2	1.08	6	87
		AFG_1	4.55	6	76
		AFG_2	0.80	10	70
		OTA	14.55	4	96

Table 3
Relative standard deviation of repeatability (RSD_r) and mean recoveries obtained ($n = 3$) for different sample sizes for baby foods analysis.

Mycotoxin	Sample size (g)	RSD_r (%)	Recovery (%)
AFB_1	25.0	42	53
	50.0	8	83
OTA	25.0	27	68
	50.0	6	86

Table 4
Relative standard deviation of repeatability (RSD_r) and mean recoveries obtained ($n = 3$) for different PBS/Tween 20 percentage as dilution solvent, for paprika analysis.

Solvent mixture	Mycotoxin	RSD_r (%)	Recovery (%)
PBS	AFB_1	2	91
	AFB_2	3	86
	AFG_1	9	78
	AFG_2	9	70
	OTA	9	111
PBS + Tween 20 10%	AFB_1	7	68
	AFB_2	15	65
	AFG_1	19	92
	AFG_2	6	48
	OTA	26	132
PBS + Tween 20 0.1%	AFB_1	5	81
	AFB_2	1	81
	AFG_1	21	95
	AFG_2	16	70
	OTA	21	124

facturer, were compared; and, as shown in Table 4, the PBS solution gave the best results.

Moreover, both, for baby foods and paprika, method performances were comparable also when a multi-mycotoxin IAC (AFs/OTA) or a single mycotoxin IAC (AFs and OTA) were used. For this purpose, a comparison study was conducted on baby foods (Pool 1) and paprika (Pool 3) samples evaluating the method performance (RSD_r and recovery factor) when a single- or a multi-mycotoxin IAC was used (Table 5).

3.2. Single-laboratory validation

Three different levels were investigated for each matrix. For corn based and multi-cereal baby foods, LoQ and two spiked levels were analyzed, while for paprika the values included the LoQ, a naturally contaminated and a spiked level (Tables 1 and 2).

Table 5
Relative standard deviation of repeatability (RSD_r) and mean recoveries obtained ($n = 3$) for single- and multi-mycotoxin IAC for baby foods and paprika analysis.

Matrix	Mycotoxin	IAC	RSD_r (%)	Recovery (%)
Baby foods (Pool 1)	AFB_1	AFLAPREP®	8	83
		AFLAOCHRAPREP®	9	93
	OTA	OCHRAPREP®	6	92
		AFLAOCHRAPREP®	4	93
Paprika (Pool 3)	AFB_1	AFLAPREP®	5	85
	AFB_2		5	85
	AFG_1		6	75
	AFG_2		10	70
	AFB_1	AFLAOCHRAPREP®	4	85
	AFB_2		5	84
	AFG_1		4	78
	AFG_2		10	70
	OTA	OCHRAPREP®	4	90
		AFLAOCHRAPREP®	5	96

Calculated LoD values were, for all the tested matrices, 0.002 and 0.012 μg/kg for aflatoxins and OTA, respectively. Limit of quantification, mean values, relative standard deviation of repeatability and recovery factors resulted from the single-laboratory validation are reported in Tables 1 and 2.

The method applicability in baby foods was from 0.02 to 0.19 μg/kg for AFB_1 and from 0.08 to 0.89 μg/kg for OTA. The application ranges for paprika determination are 0.20–5.08, 0.07–1.08, 0.20–4.55, 0.07–0.80 and 0.66–14.55 μg/kg for AFB_1, AFB_2, AFG_1, AFG_2 and OTA, respectively.

Standard curves were generated by linear regression of peak areas against concentration, for each calibration level triplicate injections were performed. The obtained calibration curves assured the linearity of the instrumental response in the investigated range and showed satisfactory coefficients of determination ($r^2 \geq 0.999$). After the calculation of LoD and LoQ, the limit of quantification values were stated at concentration levels with precision lower or equal to 10% of the RSD_r (Tables 1 and 2).

The results of precision of the method under repeatability conditions were good for each tested matrix and level, and quite satisfactory when the RSD_r was compared to the reference values reported in the EU Regulation 401/2006 [21].

In the absence of certified reference materials, trueness was evaluated by spiking procedure and evaluating recovery, the obtained values were compared with the recovery factors reported in the EU Regulation, the obtained results (Tables 1 and 2) were in compliance with the requirements.

In conclusion, the proposed method represents a useful analytical tool to perform a multi-mycotoxin and/or a multi-matrix analysis, depending on either the laboratory research or official control purposes.

Acknowledgement

The authors wish to thank R-Biopharm Rhône Ltd. for supporting this study by supplying the immunoaffinity columns.

References

[1] FAO (Food and Agriculture Organization), Manual on the Application of the HACCP System in Mycotoxin Prevention and Control, vol. 73, FAO Food and Nutrition Paper, Rome, 2001.
[2] FAO (Food and Agriculture Organization), Worldwide Regulations Mycotoxins in Food and Feed in 2003, vol. 81, FAO Food and Nutrition Paper, Rome, 2004.
[3] C.D. Kurtzman, B.W. Horn, C.W. Hessetline, J. Microbiol. 53 (1987) 158–174.
[4] D.L. Eaton, E.P. Gallagher, Annu. Rev. Pharmacol. Toxicol. 34 (1994) 135–172.
[5] IARC (International Agency for Research on Cancer), in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, some Mycotoxins, Naphthalene and Styrene 82 (2002) 171 IARC (Ed.), Geneve.
[6] C.A.R. Rosa, C.E. Magnoli, M.E. Fraga, A.M. Dalcero, D.M.N. Santana, Food Addit. Contam. 21 (2004) 358–364.
[7] E.E. Creppy, Toxicol. Lett. 127 (2002) 19–28.
[8] A. Mally, H. Zepnik, P. Wanek, E. Eder, K. Dingley, H. Ihmels, W. Völkel, W. Dekant, Chem. Res. Toxicol. 17 (2) (2004) 234–242.
[9] EFSA (European Food Safety Authority), EFSA J. 365 (2006) 1–56.
[10] J.W. Bennett, M. Klich, Mycotox. Clin. Microbiol. Rev. 16 (2003) 497–516.
[11] European Commission, Off. J. Eur. Union L364 (2006) 5–24.
[12] European Commission, Off. J. Eur. Union L35 (2010) 7–8.
[13] J.M. Hernandez Hierro, R.J. Garcia-Villanova, P. Rodriguez Torrero, I.M. Toruño Fonseca, J. Agric. Food Chem. 56 (2008) 751–756.
[14] R.J. Garcia-Villanova, C. Cordon, A.M. Gonzalez Paramas, P. Apapricio, M.E. Garcia Rosales, J. Agric. Food Chem. 52 (2004) 7235–7239.
[15] D. Chan, S.J. MacDonald, V. Boughtflower, P. Brereton, J. Chromatogr. A 1059 (2004) 13–16.
[16] P.C. Alvito, E.A. Sizoo, C.M.M. Almeida, H.P. van Egmond, Food Anal. Methods 3 (2010) 22–30.
[17] C. Brera, F. Debegnach, V. Minardi, E. Pannunzi, B. De Santis, M. Miraglia, J. AOAC Int. 90 (3) (2007) 763–772.
[18] Eurachem, The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics (1998) LGC Ltd., Teddington, UK.
[19] M. Thompson, S.L.R. Ellison, R. Wood, Pure Appl. Chem. 74 (5) (2002) 835–855.
[20] J. Stroka, E. Anklam, U. Joerissen, J. Gilbert, J. AOAC Int. 84 (4) (2001) 1116–1123.
[21] European Commission, Off. J. Eur. Union L70 (2006) 12–34.