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Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B_1: development of a method for simultaneous extraction of ochratoxin A and citrinin

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Abstract

Crops may be contaminated by mycotoxins which can persist in the final products. Forty-five breakfast cereals were collected in French supermarkets. Ochratoxin A (OTA) and citrinin (CIT) were simultaneously extracted by a new method based on solvent partition validated in-house. The recoveries were over 80% for CIT and OTA. Fumonisin B_1 (FB_1) was analysed by an IUPAC method. The recoveries for FB_1 ranged from 50% to 70%, depending on the matrix. The losses were located at the step of immunoaffinity clean-up.

OTA was detected in 69% of the samples; 20% of them were above the EU limit of 3 μg/kg. Twenty percent contained CIT (1.5–42 μg/kg). FBs were detected, not only in cornflakes, but also in products containing oats or rice, in the range 1–1110 μg/kg. Some samples were contaminated by all three mycotoxins.

Keywords: Mycotoxins; Ochratoxin A; Citrinin; Fumonisins; Analytical method; Breakfast cereal

1. Introduction

Cereals and other crops are susceptible to fungal attack, either in the field or during storage. These fungi may produce mycotoxins (Pitt & Hocking, 1985; Prelusky, Rotter, & Rotter, 1994; Resnick, Costarrica, & Pacin, 1995; Resnick et al., 1996; Ross et al., 1990; Scott, 1990; see also Pfohl-Leszkowicz, 1999 for review). In the field, some *Fusarium* species growing on maize, oat or rice, but also on other cereals, such as wheat, millet and sorghum, produce fumonisin B_1 (Abbas et al., 1998; Shephard, Thiel, Stockenstrom, & Sydenham, 1996; Wilson & Maronpot, 1971; see also Pfohl-Leszkowicz, 1999 for review). During storage, Aspergilli (*Aspergillus ochraceus*, *carbonarius*, *niger*) and Penicillia (*Penicillium verrucosum*, *Penicillium griseofulvum*, Penicillium citrinum and *Penicillium expansum*) produce ochratoxin A (OTA) and citrinin (Abarca, Bragulat, Castellà, & Cabanes, 1994; Abarca, Bragulat, Castellà, Accensi, & Cabanes, 1997; Mantle & McHugh, 1993; Pitt, 1987; Wolff, 2000) in contaminated wheat, oat or barley. These mycotoxins can be very stable to food processing (Castellà, Katta, Sumner, Hanna, & Bullerman, 1998; Castellà, Sumner, & Bullerman, 1998; Katta, Jackson, Sumner, Hanna, & Bullerman, 1999; Osborne,

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1979; Osborne et al., 1996) and can be found in final products; e.g., fumonisin has been detected in numerous samples (Henningen et al., 2000; Kim, Scott, & Lau, 2003; Machinski & Valente-Soares, 2000; Scott, Lawrence, & Lombaert, 1999; Solfrizzo, Girolamo, & Visconti, 2001; Usleber & Märtlbauer, 1998). OTA has recently been reported in cereal-based products (Lombaert et al., 2003; Wolff, 2000), whereas CIT has not yet been reported in breakfast cereals. While AOAC International and EU have adopted a number of validated methods for the analysis of OTA and FB_1, these methods address only a few unprocessed food commodities. No EU validated method exists for CIT. Only a few methods were studied by AOAC International but none addressed complex mixtures. Until now, no methods are validated for analysis of mycotoxins in breakfast cereals. As expressed by Gilbert and Anklam (2002), "to be adopted as an official method, *any proposed method should be validated not only in a collaborative trial study, but also in the matrices of concern and at levels close to the regulatory limits* ". These restrictions are very important in European countries when analysing complex foods, such as breakfast cereals, pre-cooked meals and pastries. For such foods there are two alternatives: (1) analysis of individual components of the complex food before processing, by validated methods when they exist, or (2) analysis of the whole processed food by methods which will require validation. The first approach does not take into account interactions between the components and the toxins, nor the possible formation of these toxins during food processing. The most recent trend in analytical chemistry of mycotoxins is to use immunoaffinity columns for clean-up and enrichment.

The aim of this study was to evaluate the presence of fumonisin B_1 (FB_1), ochratoxin A (OTA) and citrinin (CIT) in some breakfast cereals collected from retail outlets in France. For the simultaneous analysis of OTA and CIT in some breakfast cereals, we developed a method using partition, since the AFNOR (*Agence française de normalisation*) conventional method (AFNOR, 1998a, 1998b), validated for OTA in barley, maize, wheat bran and flour, did not allow good and reproducible recoveries for CIT. Fumonisins were analysed by the method described by Visconti, Solfrizzo, and De Girolamo (2001).

2. Materials and methods

2.1. Sample collection

Forty-five samples were purchased from retail outlets, mainly by the "*Institut National de la consommation*" (Guibert & Victoria, 2003) from several shops, as would be done by a consumer. These included the following major ingredients, alone or mixed: maize, bran and fruits, cereals (wheat and/or rice), chocolate, rice and oats.

OTA and CIT were analysed in all samples whereas FBs were analysed only in the 32 samples containing maize, oats and rice.

2.2. Chemicals

All reagents (potassium chloride, sodium hydrogen carbonate, sulphuric acid, phosphoric acid, hydrochloric acid, acetic acid, sodium dihydrogen phosphate) were of normapur grade. All solvents (methanol, chloroform, acetonitrile, propan2-ol, *n*-hexane) were of HPLC grade from ICS (France). Deionised water was used for the preparation of all aqueous solutions and for HPLC. OTA, free from benzene, CIT, carboxypeptidase and ortho-phthalaldehyde (OPA) for the derivatisation of FBs, were from Sigma Chemicals (France). The immunoaffinity columns (Fumoniprep) were from Rhône Diagnostic technologies (France). Fumonisin B_1 was a generous gift from Dr. D. Miller (Carleton University, Ottawa, Canada). A qualitative FB_2 solution was obtained from the International Agency for Research on Cancer (Lyon, France).

2.3. Preparation of standard solutions

OTA or CIT standard solutions were prepared by dissolving 10 mg of OTA or CIT/ml of methanol. A series of working standards from 0.2 to 100 ng OTA or CIT/ml was prepared by dilution. They were used to calibrate the LC detector response. The concentration of the OTA stock solutions was determined by measuring the UV absorbance at 333 nm and calculated by using the molar extinction coefficient ε of 5500 $mol^{-1}\ cm^{-1}$. CIT stock solution was determined by measuring the UV absorbance at 321 nm and calculated by using the molar extinction coefficient ε of 5490 $mol^{-1}\ cm^{-1}$. Since FB_1 does not absorb under UV, two solutions were prepared by accurate weighing of the toxin, dissolution in acetonitrile/water (v/v: 50/50) and comparison of one against the other. The EU standards of FB_1, FB_2 and FB_3 were used as qualitative standards to identify retention times of the latter two toxins.

2.4. HPLC for the analysis of OTA and CIT

HPLC analysis used a Gilson 811B dynamic chromatography pump coupled to a Spectra Physics 2000 fluorescence spectrophotometer and an ICS auto sampler. In order to optimise the sensitivity for the analysis of OTA and CIT, which have different excitation and emission fluorescence parameters (OTA 335 and 465 nm, respectively; CIT 331 and 500 nm), the analysis was performed using HPLC conditions adapted for each toxin.

A C18 spherisorb column (3 μm C18, 0.46 × 25 cm), preceded by a C18 pre-column from ICS, was used.

The system was run isocratically, with two different phases for the analysis of OTA, to reduce the risk of false positives.

Phase 1 was methanol/acetonitrile/sodium acetate (5 mM)/acetic acid (300/300/400/28); flow rate was 0.7 ml/min; elution time of OTA was about 9 min.

Phase 2 was H_3PO_4 (0.33 M)/acetonitrile/propan2-ol (600/400/50); flow rate was 0.7 ml/min, elution time of OTA was about 18 min. A third phase was used for determination of CIT: H_3PO_4 (0.33 M)/acetonitrile/propan2-ol (700/300/50); flow rate was 0.7 ml/min; elution time of CIT was about 19 min.

The chromatograms were analysed by a Normasoft software provided by ICS (France).

2.5. HPLC system for the analysis of FBs

The HPLC system used a Gilson 811B dynamic chromatography HPLC pump coupled to a fluorescence spectrophotometer (Lachrom) (excitation, 335 nm; emission, 440 nm) and a manual sampler.

A C18-nucleosil column, 150 × 4.6 mm, with an ultrasep C18 10 μm pre-column, 1 cm long from ICS, was used. The system was run isocratically, with methanol/0.1 M Na H_2PO_4 (80/20) adjusted to pH 3.35 with H_3PO_4; flow rate was 0.9 ml/min; elution time was 7.2 min.

The chromatograms were analysed by Normasoft software provided by ICS (France).

3. Method of analysis

FBs were analysed by the method of Sydenham, Shephard, and Thiel (1992) as validated by the AOAC International (Visconti et al., 2001).

OTA and CIT were analysed by a modified method of Lepom (1986) which was characterized in-house, as described in Section 6.

4. Detailed description of the optimised methodology for simultaneous OTA and CIT extraction/purification [1]

4.1. Homogenisation of samples

The entire content of one packet, as purchased, was ground to fine powder using a Waring Blendor at high speed (20,000 min^{-1}) for a short period of time to avoid heating of the sample. The size of the particles was less than 1 mm.

4.2. Extraction

Twenty ml of a 4% aqueous solution of potassium chloride acidified to pH 1.5 with undiluted sulphuric acid was added to each 20 g aliquot of sample. The mixture was homogenised and extracted with 180 ml acetonitrile on an orbital shaker for 20 min. The extract was then passed through a Whatman No. 4 paper in a porcelain filter under vacuum, collecting in a measuring cylinder.

4.3. Purification of the extract

One hundred ml of *n*-hexane was added to the filtrate and shaken for 1 min. After separation, the upper phase (*n*-hexane) was discarded. This step was repeated. To the combined lower phase, 50 ml deionised water and 100 ml chloroform were added. The solution was shaken for 10 min. After separation, the lower phase (chloroform) was collected. The upper phase was re-extracted three times with 20 ml of chloroform using the above conditions. The chloroform extracts were pooled.

To the pooled chloroform extract, 50 ml sodium bicarbonate were added and shaken for 10 min. The upper phase (bicarbonate) was collected, acidified to pH 1.5 with concentrated hydrochloric acid and allowed to stand about 20 min. The acidified solution was extracted three times with chloroform (100, 50 and 50 ml). The chloroform phases were pooled.

Then the chloroform was evaporated to near dryness under vacuum using a rotatory evaporator in a 40 °C water bath at low speed, protecting the flask from light. The vial, which contained the pooled extracts, was rinsed with 10 ml chloroform and the chloroform added to the evaporator flask and evaporated to near dryness. Two ml of methanol were added and the solution was sonicated and filtered through cartridges (Spartan 0.2 μ, Schleicher and Schuell, Germany) and finally evaporated to dryness under nitrogen. For analysis in the different HPLC systems, 500 μl of methanol were added.

5. Confirmation of the presence of OTA

The confirmation of the presence of OTA in samples detected at 2 μg/kg was performed by two techniques:

1. *The carboxypeptidase technique*, for producing α-ochratoxin (α-Ot). Briefly, an aliquot taken from the purified extract of a sample where OTA was detected by the two HPLC methods was dried, and residue dissolved in 0.975 ml of a buffer solution of 0.04 M Tris–

[1] *Caution:* in view of the toxicity of some of the solvents, all the extractions/purifications were carries out in a well ventilated fume cupboard. The preparation of the standard solutions was also performed using all precautions related to the handling of potentially carcinogenic compounds.

HCl, 1 M NaCl, pH 7.5. Twenty-five μl of carboxypeptidase in water (100 U/ml) was added and the solution was incubated at room temperature overnight. The samples were analysed by the HPLC chromatographic conditions used above for the analysis of OTA. The peak of OTA disappeared whereas the peak of α-OT appeared.

2. *Identification by mass spectrometry*. The LC–MS/MS consisted of an Agilent 1100 G1312A pump connected to a triple stage quadrupole mass spectrometer (MDS Sciex API 3000 with a Turbo Ion spray source, Applied Biosystems, Darmstadt, Germany). Samples were injected using an autosampler (Agilent 1100). Nitrogen was produced using a generator (Parker Blaston). Data were recorded and analysed using PE Siex Analyst Software VI.I. The dried extracts were dissolved in 500 μl water by ultrasound and 10 μl were injected into the LC–MS/MS. The samples were analysed by gradient elution. Solvent A was purified water, solvent B acetonitrile. Using a Phenomenex Nucleosil C18 column (150 × 2.0 nm, 5 μm, 100 Å) gradient conditions were applied at a flow rate of 300 μl/min. The column was first equilibrated with 100% of solvent A. Then 5 min with 40% solvent B in water, 10 min with 50% solvent B in water, then return to solvent A for 10 min. OTA was quantified in the negative-ion mode and spectral data were recorded with N_2 as collision gas (CAD = 4) in the multiple reaction monitoring mode (MRM).Turbospray parameters were: NEB 10;CUR 15; IS-4000V; TEM 400 °C. For quantification of OTA, the following parameters were used for the MS–MS ion transitions Q1: 402/Q3:167 (500 ms) DP-36V; FP-300V; EP 10V; CE-32V; CXP-15V and (used as quantifier) DP-36V; FP-300V; EP 10V; CE-32V; CXP-7V.

6. Results

6.1. General

In the first instance, the new methodology for simultaneous extraction/purification of OTA and CIT was validated in house for breakfast cereals. Then several tests for the efficiency of purification of complex matrices such as breakfast cereals, were performed by immunoaffinity columns for the analysis of fumonisins.

6.2. Method development for simultaneous analysis of OTA and CIT

6.2.1. Extraction/recovery

The solubility in an organic solvents, of both toxins, is dependent on pH. In order to optimise their simultaneous extraction, acidification of the matrix before the extraction by acetonitrile was tested at two pH values (1.5 and 4). The efficiencies and repeatabilities of extraction from 10 analyses were measured. The recovery of CIT increases from 23% to 80.3% and that of OTA from 65.5% to 79.0%, when the pH is lowered. The variability was also improved, from ±15% to ±5% for CIT and from ±10% to ±3% for OTA. For all further work, the extraction was thus performed at pH 1.5.

6.2.2. OTA recoveries

Five different breakfast cereals spiked with 3 μg/kg (corresponding to the EU legislation value) or 10 μg/kg were analysed, on the same day, by the same operator and with the same HPLC system. The average recoveries were 76.1 ± 5.7% and 75.1 ± 11.9%, respectively, for 3 and 10 μg/kg.

6.2.3. Repeatability test for OTA analysis

Samples spiked with 3 μg/kg (corresponding to EU legislation) were analysed on five successive days, by the same operator and with the same HPLC system. The average OTA concentration was 2.5 ± 0.17 μg/kg. In a naturally contaminated sample, the average from five analyses was 3.06 ± 0.55 μg/kg.

6.2.4. Dose–response curve for OTA analysis

Solutions containing from 0.5 to 1 mg/l were measured by HPLC (corresponding to 12.5 ng/kg to 24.9 μg/kg of cereal). The coefficient of linearity (R^2) was 0.997. The LOD was 0.05 μg/kg and the LOQ was 0.2 μg/kg at nine times background.

6.2.5. Citrinin recoveries

Five different samples were spiked with 3 or 10 μg/kg and analysed, on the same day, by the same operator with the same HPLC. The average recovery was 80.3 ± 5%.

6.2.6. Repeatability test for CIT analysis

Samples spiked with 3 μg/kg were analysed on five successive days, by the same operator with the same HPLC. The average CIT concentration was 2.43 ± 0.30 μg/kg. In a naturally contaminated sample the average level of five analyses was 2.5 ± 0.37 μg/kg.

6.2.7. Dose–response curve for CIT analysis

Solutions containing from 50 μg/l to 2.25 mg/l were measured by HPLC (corresponding to 1.12–56.25 μg/kg of cereal). The coefficient of linearity (R^2) was 0.98. The LOD was 0.5 μg/kg and the LOQ was 1.5 μg/kg at seven times background.

6.2.8. Recovery tests of both toxins simultaneously

Five different samples were spiked with each toxins at 3 μg/kg and analysed, on the same day, by the same operator with the same HPLC system. The average

recovery was 81.3 ± 4.2% for OTA and 80.1 ± 5% for CIT.

6.3. Study of the applicability of the fumonisin AOAC international method to breakfast cereals

6.3.1. Dose–response curve for fumonisin analysis

Solutions containing from 25 μg/l to 1 mg/l were measured by HPLC (corresponding to 10–400 μg/kg of cereal). The coefficient of linearity (R^2) was 0.99. The LOD was 1 μg/kg and the LOQ was 2 μg/kg.

6.3.2. Study of recoveries on these matrices

In four different samples spiked with 200 μg/kg of FB_1 recoveries ranged from 40% to 74.9%. In order to elucidate the reason for some low recoveries, the equivalent of 300 μg/kg of FB_1 was added to the extract of nine different samples just before the immunoaffinity column. The non-enriched and spiked samples were then analysed from the immunoaffinity column step, in parallel, and the non-enriched results subtracted from the enriched one. Results corresponding to the recoveries of the immunoaffinity columns are presented in Table 1. These recoveries varied from 54.4% to 79%.

Table 1
Test on the recovery of FB_1 in various samples spiked just before the immunoaffinity column

Sample	Recovery (%)
3 (maize and barley)	74.9
5 (maize)	69.5
7 (maize and barley)	74.8
19 (rice, wheat flour, wheat bran)	54.4
41 (maize)	78.4
42 (cocoa, rice, maize, oats, wheat flour)	61.7
43 (fruits, oats, wheat bran, wheat flour)	79.1
44 (fruits, oats, wheat flour)	59
45 (oats)	67

6.3.3. Study of repeatability of the analysis

Seven naturally contaminated samples, analysed in duplicate on two successive days, showed that near the limit of quantification, repeatability was less than 40% whereas, at 10 times higher concentrations, results varied by only 8%.

6.4. Results of the sample analysis

6.4.1. General

Representative chromatograms are presented in Figs. 1–3. The overall results, uncorrected for recoveries, are presented in Table 2.

6.4.2. OTA and CIT contamination

OTA was detected in 69% of samples, measurable (47%) at 0.2–8.8 μg/kg. The highest concentration was in a sample containing dry fruit and bran. Sixteen

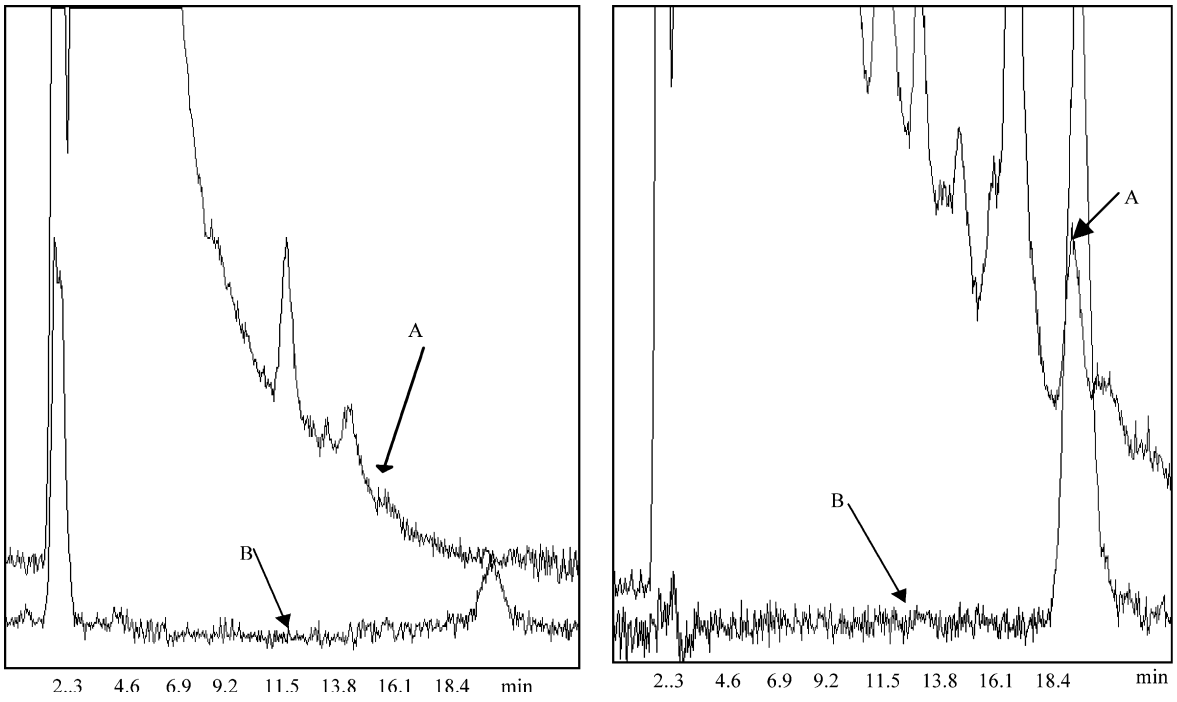

Fig. 1. Typical chromatogram of CIT: (A) chromatogram of breakfast cereal; (B) chromatogram of CIT standard solution. On left, absence of CIT; on right presence of CIT.

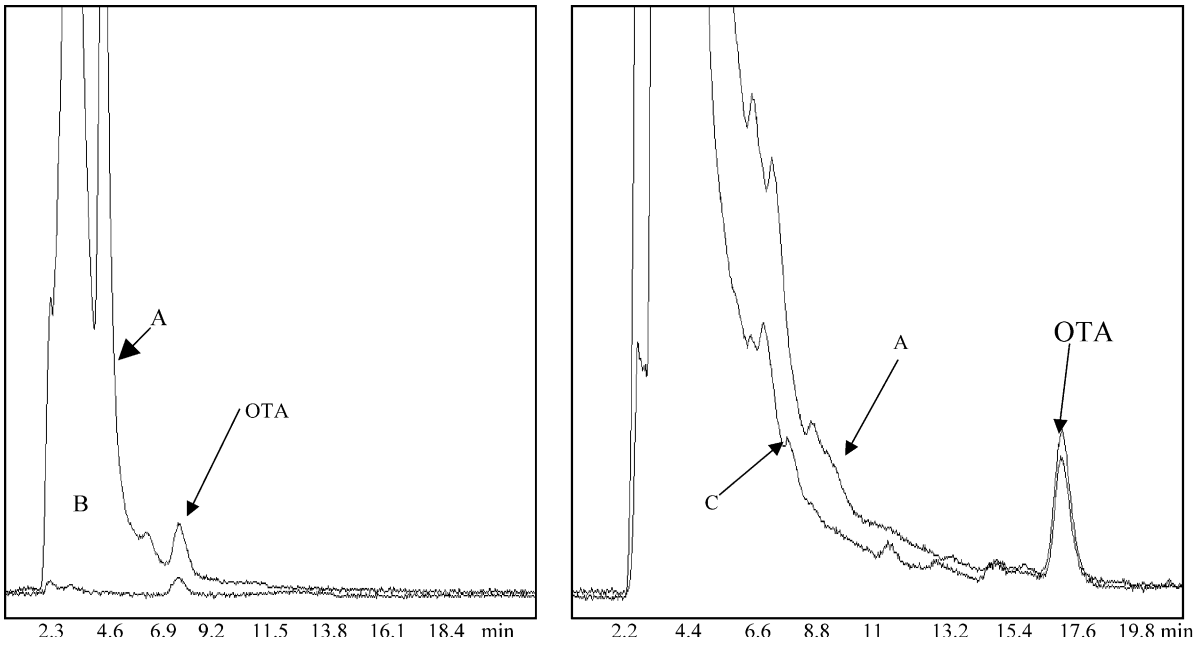

Fig. 2. Typical chromatograms of OTA: (A) chromatogram of breakfast cereal; (B) chromatogram of OTA standard solution; (C) chromatogram of non contaminated breakfast cereal spiked with OTA. On left, mobile phase 1 (methanol/acetonitrile/sodium acetate (5 mM)/acetic acid (300/300/400/28); flow rate 0.7 ml/min); on right mobile phase 2 (H_3PO_4 (0.33 M)/acetonitrile/propanol-2 (600/400/50); flow rate 0.7 ml/min).

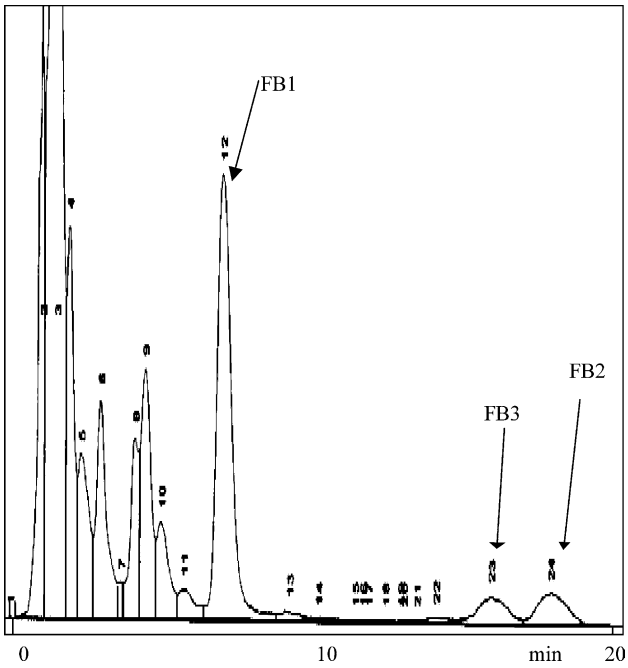

Fig. 3. Typical chromatogram of Fumonisins separation by HPLC. Example of cereal 16: peak 12, FB1; peak 23, FB3; peak 24, FB2.

percent of the samples contained OTA (uncorrected amount) above the EU limit of 3 μg/kg, 31% contained OTA between 0.2 and 2.99 μg/kg and, in 53%, OTA was non-detectable or non-quantifiable. Eighteen percent of them were also contaminated by CIT in the range 1.5–42 μg/kg.

6.4.3. Confirmation of the OTA

Disappearance of the OTA peak was observed, together with the appearance of the α-Ot peak, in all samples tested. Mass spectrometry data (Table 3) were confirmatory and close to the HPLC data.

6.4.4. FBI contamination

FB_1 was detected in 94% of samples containing maize, oats or rice at concentrations from 1 to 1110 μg/kg. The three highest concentrations were 120, 250 and 1113 μg/kg. Two samples, containing wheat and some rice, did not contain any FBs. Seventeen other samples containing rice were only slightly contaminated (10–50 μg/kg), except for two which reached 120 and 240 μg/kg. Among the rice contaminated samples, one in addition contained oats, another maize and two others oats and maize. Three samples contained only oats and were contaminated with 18, 35 or 45 μg/kg. One oat sample contained maize and had 46 μg/kg FB_1. All the other samples contained maize alone as cereal. The range of contamination was 1–1110 μg/kg FB_1. The most contaminated was made exclusively from maize flour. In two samples, FB_2 was also detected but not quantified. The most contaminated sample also contained FB_3. Thus, the data in Table 2 represent an underestimation of the actual contamination.

Table 2
Summary of the results for OTA, CIT and FB_1 analyses

Sample number	CIT (μg/kg)	OTA (μg/kg)	FB1 (μg/kg)
1	N.D.	<LOQ	21
2[a,b]	N.D.	N.D.	36
3	N.D.	N.D.	8
4	N.D.	<LOQ	45
5	N.D.	N.D.	7
6	N.D.	N.D.	21
7	N.D.	N.D.	11
8[b,c]	<LOQ	0.9[d]	NA
9[a,c]	19	2.5[d]	10
10[c]	N.D.	2.8[d]	25
11[b]	N.D.	0.9[d]	N.D.
12[a,b]	42	4.1[d]	32
13[a,b]	N.D.	0.8[d]	48
14[a]	N.D.	4.6[d]	<LOQ
15[a]	N.D.	4.4[d]	NA
16[a]	<LOQ	3.4[d]	1113
17[a]	N.D.	2.5[d]	<LOQ
18[b,c]	N.D.	1.3[d]	NA
19[b]	N.D.	N.D.	240
20[b,c]	N.D.	1.3[d]	N.D.
21[b,c]	N.D.	0.4[d]	NA
22[b]	12	1.7[d]	NA
23[a,b]	N.D.	N.D.	NA
24[b]	N.D.	<LOQ	NA
25[a]	12	<LOQ	30
26[a]	N.D.	<LOQ	50
27[a]	N.D.	1.4[d]	65
28[b,c]	N.D.	8.8[d]	NA
29[a,b]	N.D.	1.9[d]	120
30[a]	N.D.	N.D.	NA
31[b,c]	N.D.	7.3[d]	NA
32[b,c]	N.D.	N.D.	NA
33[a]	N.D.	N.D.	40
34[a]	N.D.	N.D.	25
35[a,b]	5	<LOQ	28
36[a]	7	3.1[d]	45
37[a]	N.D.	<LOQ	50
38[b,c]	N.D.	<LOQ	NA
39[b,c]	N.D.	2[d]	NA
40[a,b]	N.D.	<LOQ	15
41	N.D.	N.D	25
42[a]	N.D.	N.D.	10
43[c]	N.D.	1[d]	46
44[c]	N.D.	N.D.	35
45	N.D.	<LOQ	18

NA, not analysed; N.D., below the LOD (limit of detection); LOQ, limit of quantification.

[a] Presence of chocolate.

[b] Presence of wheat bran.

[c] Dried raisins.

[d] Confirmed by carboxypeptidase.

Table 3
Analysis by LC–MS/MS

Breakfast cereals	OTA μg/kg of cereal
3 (maize and barley)	0.1
10 (rice, fruit, cocoa)	4.2
12 (rice, fruit, cocoa, wheat bran)	2.5
14 (rice, fruit, cocoa)	4.8
15 (cocoa and wheat flour)	3.8
16 (cocoa and maize)	3.1
17 (cocoa and rice)	2.3
28 (wheat bran and fruit)	8.4
31 (wheat bran and fruit)	11
36 (cocoa, fruit, oats)	3

Samples detected above 2 μg/kg by HPLC were analysed by LC–MS/MS. Sample 3 was used as negative control.

7. Discussion

The aim of this work was to analyse some breakfast cereals for their contents of OTA, CIT and FBs. Only one method has been proposed for the simultaneous analysis of OTA and CIT (Lepom, 1986) and separation techniques were investigated by Reinhard and Zimmerli (1999). For the analysis of OTA, traditionally the methods of isolation have used acidification of the matrix before extraction with an organic solvent. The recent trends avoid this acidification step (Entwisle, Williams, Man, Slack, & Gilbert, 2000) and some of them even use extraction with an alkaline medium (Entwisle et al., 2001). None of the published methods addressed breakfast cereals. The present work, demonstrated that greater variability in OTA analysis occurs when the medium is insufficiently acidified (65.5 ± 10% at pH 4 versus 79 ± 3% at pH 1.5). This is even more pronounced for CIT, for which low acidification gave even poorer extraction (23 ± 15% at pH 4), whereas acidifying to pH 1.5 improved both the recovery and the reproducibility (80.3 ± 5%).

In the EU legislation (Nos. 472/2002 and 26/2002), it is stated that, between 1 and 10 μg/kg, recoveries are acceptable in the range 70–110% and the RSD_r should be <20%. In the method developed for the simultaneous determination of OTA and CIT, recoveries exceed 80% for both toxins. The method can thus be qualified as "acceptable" according to the EU criteria.

For the analysis of FBs, we applied a method which proved acceptable for maize and cornflakes. When analysing cornflakes alone, recoveries were in the acceptable range (70–75%). With other types of samples (containing fruits, oats, rice, sugar and chocolate) recoveries varied and could be as low as 40%. On testing the possible causes of these results, it was clear that recoveries as low as 54.4% arose during purification through immunoaffinity columns. Some unknown co-extractants may block antibody sites and reduce the trapping efficiency for FBs. In view of the variability of recovery, the present values of FB_1 have not been corrected for recovery.

Seven of the 45 samples had OTA concentrations above the regulatory limit of 3 μg/kg. If the data had been corrected for recoveries, an additional three samples would have exceeded this limit. Eight of the samples over the EU limit contained chocolate. Nevertheless, none with the most mycotoxin contamination contained chocolate (samples #28 and 31). Three of the most

contaminated had dried raisins. This may point to these two ingredients (raisins and chocolate) as risk factors for OTA contamination. Another point should also be mentioned. The three samples having the highest mycotoxin content had wheat bran. All samples containing wheat bran had detectable OTA. Thus, the present study confirms wheat bran as another risk factor. However, of the samples in which OTA was undetectable, seven had chocolate, and two had raisins. This points to the fact that if there is good quality control of ingredients, even components theoretically having a higher risk, can give breakfast cereal products which conform to EU regulations.

Our results are in line with Wolff (2000) who found OTA up to 2.13 μg/kg in breakfast cereals. Very recently, Lombaert et al. (2003) reported OTA up to 6.9 μg/kg in 10/47 samples of infant barley based cereal foods.

When CIT was found in a sample, it always occurred with OTA. These samples contained oats (3/8), wheat (5/8), maize (2/8) or rice (4/8). The presence of OTA in oat, wheat and maize is well documented (for a review see Pfohl-Leszkowicz, 1999). Recently, Beretta et al. (2002) reported it in rice. As FBs are mainly found in maize, the samples containing wheat alone were not analysed for this mycotoxin. Some samples contained only one type of cereal but most of them were combinations of two or more. In this work, it was clear that contaminations by FBs are not only due to maize-based cereals but also to those containing oats or rice, as has already been reported for these ingredients (Abbas et al., 1998; Wilson & Maronpot, 1971). Our results are in line with those of the literature. Indeed, Lukacs, Schaper, Herderich, Schreier, and Humpf (1996) found 19 μg/kg FB_1 and 10 μg/kg FB_2 in ''baby menu'' containing maize. Scott (1999) reported 8 baby food samples positive for FB. Machinski and Valente-Soares (2000) found that one of two samples of maize flour baby cereal contained FB_1 at 440 μg/kg. Henningen et al. (2000) detected 447 μg/kg FB_1 and 158 μg/kg FB_2 in one of two samples of baby cereals. Solfrizzo et al. (2001) reported the presence of FB_1 in 17 out of 18 maize-based breakfast cereals up to 1 mg/kg. Recently, Kim et al. (2003) found FB_1 in 22 out of 25 samples, but did not take into account the FB_1 bound to proteins, which can be as much as 2.6-fold higher than that of the unbound toxin. Our results on FBs in maize-based breakfast cereals confirm those reported by these other teams.

Three of the samples have none of the three mycotoxins analysed. In contrast, several of them (#36, 35, 25, 16, 12, 9) are contaminated by all three. Sample 16 exceeded the regulatory limit for OTA and, moreover, contained FB_1 above the recommended upper limit for food contamination in Switzerland and well above the limit under consideration in European countries. OTA, CIT and FB_1 have the same target organ in animals, the kidney. From a toxicological point of view, the effect of exposure to several toxins can lead to additive or synergistic effects.

Considering the highest OTA contamination and the advice provided by the suppliers in terms of daily consumption of such products (~30 g), the OTA consumption from samples #28, 15, 14 and 12 would, respectively, be 264, 132, 138 and 123 ng per day. On the basis of the new European legislation, the tolerable daily intake (TDI) is 5.8 ng/kg b.w./day. This means less than 116 ng OTA per day for a child of 20 kg. One of the above products had twice the TDI contamination for OTA and three of them were just above this limit. This does not take into account correction for recovery and additional potential sources of contamination.

With regard to FBs, WHO (2002) proposed a provisional maximal tolerable daily intake (PMTDI) of 2 μg/kg b.w. for the sum of the three fumonisins (FB_1, FB_2, FB_3). According to this later recommendation, which does not take into account the results from the NTP carcinogenicity study on mice and rats, a child of 20 kg should not exceed a daily dose of 40 μg. Based on the carcinogenicity study, and the fact that IARC (2002) classified FB_1 as a possible human carcinogen (2B), the PMTDI would go down to 300 ng/kg bw and the consumption for the child should not exceed 6 μg. On the basis of the data obtained, with sample 16, a child would ingest 33 μg FB_1, thus close to the WHO limit for a child and five times higher than the potential new EU recommendation.

Finally, it is noteworthy that, during food processing, CIT and FBs can be converted into very toxic compounds (Hirota, Menta, Yoneyama, & Kitabatake, 2002; Saunders, Meredith, & Voss, 2001). They have not been analysed in this study.

8. Conclusions

Our results have demonstrated: (1) the danger of expecting that a method of analysis, validated for one substrate, can also apply to another substrate; (2) that OTA contamination can exceed the regulatory limit; (3) that FB_1 can occur not only in cornflakes, but also in food products containing oats or rice; (4) that some breakfast cereals can be contaminated simultaneously by the three mycotoxins OTA, CIT and/or FB_1. This is particularly important, since some studies indicate additive or synergistic toxic effects.

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