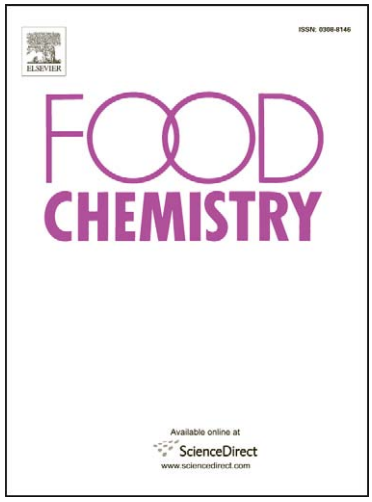

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Liliana Silva, Mónica Fernández-Franzón, Guillermina Font, Angelina Pena, Irene Silveira, Celeste Linop, Jordi Mañes

PII: S0308-8146(08)00756-5
DOI: 10.1016/j.foodchem.2008.06.080
Reference: FOCH 7533

To appear in: *Food Chemistry*

Received Date: 24 July 2007
Revised Date: 3 June 2008
Accepted Date: 22 June 2008

Please cite this article as: Silva, L., Fernández-Franzón, M., Font, G., Pena, A., Silveira, I., Linop, C., Mañes, J., Analysis of fumonisins in corn-based food by liquid chromatography with fluorescence and mass spectrometry detectors, *Food Chemistry* (2008), doi: 10.1016/j.foodchem.2008.06.080

ANALYSIS OF FUMONISINS IN CORN-BASED FOOD BY LIQUID CHROMATOGRAPHY WITH FLUORESCENCE AND MASS SPECTROMETRY DETECTORS

Liliana Silva[a], Mónica Fernández-Franzón[b]*, Guillermina Font[b], Angelina Pena[a], Irene Silveira[a], Celeste Lino[a] and Jordi Mañes[b]

[a]Group of Bromatology – CEF, Faculty of Pharmacy, University of Coimbra, 3000-295 Coimbra, Portugal. [b]Laboratory of Bromatology and Toxicology, Faculty of Pharmacy, University of València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain.

* Corresponding author. Tel: +34-963543056; fax: +34-963544954
E-mail address: monica.fernandez@uv.es

ABSTRACT

The presented procedure involves an extraction with methanol-water, centrifugation and cleanup with immunoaffinity columns. A comparison study between fluorescence detector, mass spectrometry, and tandem mass spectrometry with a triple quadrupole (QqQ) analyzer using an electrospray ionisation interface for the determination of fumonisin B_1 and B_2 in corn-based products has been performed.

Limits of quantification obtained by the three detectors were lower than the maximum levels established by European Commission. Liquid chromatography coupled to tandem mass spectrometry provides higher sensitivity (12.5 μg $kg^{-1}$for fumonisins B_1 and B_2) when compared to mass spectrometry (40 μg $kg^{-1}$for both fumonisins), and fluorescence detection (20 μg kg^{-1} for fumonisin B_1 and 15 μg kg^{-1} for B_2), and also showed to be more precise. At 150 and 250 μg kg^{-1} spiking levels, the recovery rates for fumonisin B_1 and B_2 in corn products varied from 74% to 102%, with a relative standard deviation ranging from 9% to 17%. A critical assessment including advantages and drawbacks of each technique is presented. A total of 41 organic and non organic corn-based food samples from Valencia markets were analyzed. Seven samples were contaminated with levels ranging from 68 μg kg^{-1} to 922 μg $kg^{-1}$of fumonisin B_1 and 42 μg kg^{-1} to 640 μg $kg^{-1}$of fumonisin B_2. Only one sample exceeded the maximum level for the sum of fumonisin B_1 and B_2, proposed for corn products in a recent EU regulation. The contamination frequency of organic corn samples (40%) was higher than non-organic ones (3.7%), and contained higher levels of fumonisin B_1 and B_2.

Keywords: fumonisins; fluorescence; mass detection; tandem mass detection; food analysis.

Introduction

Fumonisins (FBs) are worldwide distributed and produced by *Fusarium verticillioides* and *F. proliferatum*, mainly in corn and corn-based products (Soriano & Dragacci, 2007). Although several other fumonisin analogues have been characterized, fumonisin B1 (FB_1) remains the most abundant in naturally contaminated corn-based foods, followed by fumonisin B_2 (FB_2). Special attention has to be paid to these toxins because of the potential hazards for animal and human health. Consumption of fumonisin-contaminated corn has been associated with human oesophageal cancer in certain areas of South Africa and China. Based on their toxicity, FB_1 has been classified as a potential carcinogen for humans (Group 2B) by the International Agency for Research on Cancer (IARC, 2002).

Regarding this potential risk, the Scientific Committee for Food (SCF) from the European Commission has established a tolerable daily intake of 2 μg kg^{-1} body weight per day for the total FB_1, FB_2, and FB_3, alone or in combination. To reduce the intake of fumonisins, the European Commission has set action limits of 2000 μg fumonisin/kg for unprocessed corn, and 200 μg fumonisin/kg for processed corn-based foods and baby foods for infants and young children (Commission Directive 2007/1126/EC).

The problems and risks associated with fumonisin contamination have resulted in the development of precise, reliable and sensitive methods for its determination in corn and corn-based foods (Magan & Olsen, 2004). In this way, since its discovery and characterization in 1988, the analytical methods applied in their detection have been improved successfully (Duncan, Kruger, Zabe, Kohn & Prioli, 1998). Although gas chromatography determination, thin layer chromatography (Shephard & Sewra, 2004), capillary zone electrophoresis (Maragos et al. 1996), and enzyme-linked immunosorbent assay (Beg, Al-Mutairi, Beg, Al-Mazeedi, Ali & Saeed, 2006) have been reported, the most widely analysis technique used is liquid chromatography (Plattner, 1999).

FBs are usually extracted with mixtures of polar solvents, such as methanol, acetonitrile, and water in different combinations and proportions (Scudamore, Hetmanski, Nawaz, Naylor & Rainbird, 1997; Cortez-Rocha et al., 2003), and cleaned-up by solid phase extraction with reversed phase columns (Hinojo, Medina, Valle-Algarra, Gimeno-Adelantado, Jiménez & Mateo, 2006), strong anion exchange columns (SAX) (de Girolamo, Solfrizzo, von Holst & Visconti, 2001), and with higher specificity by using immunoaffinity columns (IAC) (de Castro, Shephard, Sewram, Vicente, Mendonca & Jordan, 2004).

Since fumonisins do not have any suitable chromophores, they must be derivatized for their fluorescence detection. The majority of the current methods use the technique of pre-column derivatization with ortho-phthalaldehyde (OPA) (Pagliuca, Zironi, Ceccolini, Matera, Serrazanetti & Piva, 2005) or naphthalene-2,3-dicarboxaldehyde (NDA) (Lino, Silva, Pena & Silveira, 2006; Lino, Silva, Pena, Fernández & Mañes, 2007). In recent years, significant improvements in coupling LC and mass spectrometry (MS) have resulted in the emerging availability of LC-MS (Plattner, 1999). Use of the atmospheric pressure ionization (API) techniques as electrospray (ESI), and atmospheric pressure chemical ionization (APCI) coupled with quadrupole mass analysers are well established for qualitative and quantitative LC-MS analysis of drugs and environmental contaminants. Thus, LC-MS methods have been successfully used for the quantification of FB_1 and also FB_2 in corn and corn-based foods, avoiding the need of derivatization (Cirillo, Ritieni, Visone & Cocchieri, 2003). The two-stage mass spectrometry process (MS/MS) provides even higher certainty, sensitivity, and selectivity in analyte quantification (Paepens, De Saeger, Van Poucke, Dumoulin, Van Calenbergh & Van Peteghem, 2005; Faberi, Foglia, Pastorini, Samperi & Lagana, 2005).

The present paper compares and discusses, for the first time, according to our knowledge, quality parameters in the analysis of FB_1 and FB_2 in corn-based products obtained with LC with FD, single quadrupole and triple quadrupole (QqQ), after adjusting the extraction process for each technique; fumonisins were extracted with methanol:water mixture, centrifugated and

clean-up with immunoaffinity columns. This comparison is of great importance in order to choose among the available detectors, taking in account aspects such as complexity and expensiveness versus quality parameters. Moreover, the selected method was employed to determine the occurrence and concentration of FB_1 and FB_2 in corn and corn-based food products, including organic and non-organic products from Valencia markets.

EXPERIMENTAL

Standards and chemicals

FB_1 and FB_2 standards were obtained commercially from Sigma Chemicals Co (St. Louis, USA). Stock solutions were made in 1 ml acetonitrile:water (50:50, v/v) at 1000 µg ml^{-1} as FBs are more stable in acetonitrile than in methanol for a long term storage (Cavaliere et al. 2005). Intermediate solutions were prepared at 50 µg ml^{-1} in acetonitrile:water (50:50). Standard working solutions were prepared with acetonitrile:water (50:50) at 25-0.1 µg ml^{-1} for both FBs, and used for accuracy, precision, and sensitivity tests. All solutions were kept in amber flasks at 2ºC.

NDA was obtained from Sigma Chemicals Co (St. Louis, USA). HPLC grade acetonitrile and methanol were purchased from Carlo Erba (Milan, Italy). Acetic acid, hydrochloride acid, sodium hydroxide, potassium chloride, potassium dihydrogenphosphate, anhydrous disodium hydrogenphosphate, sodium cyanide, sodium borate and sodium chloride were obtained from Merck (Darmstadt, Germany). Formic acid was from Scharlau Chemie (Barcelona, Spain). Immunoaffinity columns FumoniTestTM were from Vicam (Watertown, USA). Deionized water (<6 MΩ cm resistivity) from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA) was used.

Phosphate buffer solution (PBS) was prepared from 0.2 g potassium chloride, 0.2 g potassium dihydrogen-phosphate, 1.2 g anhydrous disodium hydrogen-phosphate, and 8.0 g sodium

chloride to 990 mL deionized, adjusted to pH 7.0 with 25% HCl, and the solution was made to 1L.

Samples and sample procedure

A total of 41 samples of corn and corn based foods from Spanish markets were purchased in commercially available size from shops, health food stores, and supermarkets located in Valencia (Spain) during 2006. Fifteen samples were from organic origin. When needed, the samples were finely milled using a Bapitaurus food chopper, and analysed as quickly as possible after their purchase. Ground samples (25 g) were extracted with 40 ml methanol:water (80:20, v/v), and centrifuged for 15 min at 2500 g. The remaining solid was extracted twice with 30 ml methanol:water (80:20, v/v) each time and the obtained extracts were combined and filtrated (Whatman Nº 1 paper). For cleanup, 10 ml of filtrate diluted with 40 ml PBS were filtrated through glass microfiber. An aliquot of 20 ml was added to a FumoniTest TM IAC attached onto a vacuum manifold. The column was washed with 10 ml PBS, and FBs were eluted twice with 1.5 ml methanol, and evaporated under one gentle nitrogen stream at 60ºC.

Instrumentation and chromatographic conditions for LC-FD

For LC-FD analysis, determination and quantification were carried out on the NDA-derivatives of fumonisins. The residue was reconstituted in 50 µl methanol:water (50:50, v/v), thereafter 500 µl 0.05M sodium borate buffer (pH 9.5), 500 µl sodium cyanide reagent, and 150 µl NDA reagent (0.5 mg ml^{-1} in acetonitrile) were added to the reconstituted residue. The mixture was heated for 15 min at 60ºC in a heating bath and cooled to room temperature.

LC apparatus used consisted of a 307 Gilson (Gilson Medical Electronics, Villiers-le-Bel, France) pump model, Rheodyne 7125 injector (Cotati, CA, USA), a C18-5 µm Nucleosil 120 KS (30 mm x 4 mm i.d.) guard column, and a C18-5 µm Nucleosil 120 (250 mm x 4.6 mm i.d.) column. A Perkin Elmer LS45 spectrofluorimeter (Perkin Elmer, Beaconsfield, UK) operated at an excitation wavelength of 420 nm, and an emission wavelength of 500 nm was used.

The results were recorded on a 3390 integrator (Hewllet-Packard, Philadelphia, PA). The mobile phase acetronitrile/water/acetic acid (61:38:1 v/v/v) was maintained at a flow rate of 1 ml min^{-1}. The injection volume was set to 50 and 25 µl, for standards and samples injections, respectively.

Instrumentation and chromatographic conditions for LC-MS

For LC-MS analysis, the residue was reconstituted to 500 µL methanol-water (50:50, v/v). A Hewlett Packard (Palo Alto, CA, USA) HP-1100 Series LC-MS system equipped with a binary solvent pump, an autosampler, and a MS detector coupled with an analytical work station were used. The MS detector consisted of a Standard API source that can be configured as APCI (atmospheric pressure chemical ionization) or ESI (electospray ionization). The LC separation was carried out on a Luna C18 column (250 mm×4.6 mm i.d., 5 µm) protected by a Securityguard cartridge C18 (4 cm×2 mm i.d.), both from Phenomenex (Madrid, Spain).

The analytical separation for LC-MS was performed using gradient elution with water as mobile phase A, and methanol as phase B, both containing 0.5% formic acid. After an isocratic step of 65% B during 4 min, it was gradually increased to 95% B in 4 min and held constantly for 7 min. Flow rate was maintained at 0.5 ml min^{-1}. The injection volume was set to 10 µl.

The ESI-MS interface was operated in positive ion mode under the conditions: gas temperature, 350ºC; drying gas flow rate, 13.0 L min^{-1}; nebulizer gas pressure, 30 psi and capillary voltage, 4000 V. Mass spectra were obtained by scanning from *m/z* 300 to 800. Selected ion monitoring (SIM) was carried out for the most abundant ion of FB_1 and FB_2 (using high-resolution settings and a dwell time of 400 ms).

Instrumentation and chromatographic conditions for LC-MS/MS

As for LC-MS, LC-MS/MS analysis was performed after reconstituting the residue to 500 µL methanol-water (50:50, v/v). LC analysis was carried out with a 2695 Waters system, equipped with a 4 channels pump and an autoinjector (Milford, MA, USA). The autoinjector was

programmed to inject 10 µL into the X Bridge TM C18 column (100 x 2.1 mm, 3.5 µm) (Waters, Ireland) maintained at 30ºC. The analytical separation for LC-MS/MS was performed using gradient elution with water as mobile phase A, and methanol as mobile phase B, both containing 0.5% formic acid. After an isocratic step of 65% B for 3 min, it was linearly increased to 75% B in 4 min and held constantly for 3 min. Flow rate was maintained at 0.3 ml min^{-1}.

A TQ mass spectrometer Quattro LC from Micromass (Manchester, U.K.), equipped with an LC Alliance 2690 system (Waters, Milford, MA) consisted of an autosampler and a quaternary pump, a pneumatically assisted electrospray probe, a Z-spray interface, and a Mass Lynx NT software. 4.1 was used for data acquisition and processing. Analysis was performed in positive ion modes. The ESI source values were as follows: capillary voltage, 3.20 kV; source temperature, 125 °C; desolvation temperature, 300 °C; desolvation gas (nitrogen, 99.99% purity) flow, 500 L/h. Ideal fragmentation conditions were accomplished varying the cone voltage and collision energies for each compound.

RESULTS AND DISCUSSION

LC-FD

The derivatization with NDA was done accordingly to Chu and Li, 1994; and Silva, Lino, Pena and Moltó, 2007 as fumonisin derivatives obtained are less toxic and more stable compared to ortho-phthaldialdehyde derivatives. The elution of fumonisins from an LC column packed with reversed-phase silica based materials provided sharp and symetrical peaks using an acidified mobile phase. The mixture acetonitrile:water:acetic acid (61:38:1) was chosen for the determination and quantification of FBs. However, the presence of interferences in FD chromatograms could hinder the analysis.

LC-MS

In LC-MS, the abundance and sensitivity of both fumonisins were reduced when acetonitrile was chosen as mobile phase. Therefore, methanol was selected instead. For the determination of the FBs by LC-MS, it was considered the type of source, the ionization mode, and the conditions of the detector. Preliminary flow injection analysis (FIA) experiments were done to choose between electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) interfaces. ESI source provided greater sensitivity, and presents the advantage that samples can be directly ionized in the liquid phase at quasi-ambient temperature, minimizing the degradation of thermolabile compounds.

ESI is an ideal technique to detect and measure fumonisins, since they tend to be ionic and produce abundant signals. The most abundant ions of mass spectra were chosen for quantification porpouse. In positive ion (PI) mode, the protonated molecule for FB_1 was *m/z* 722, and for FB_2 was *m/z* 706, and in negative ion (NI) mode the $[M-H]^{-1}$ anion were *m/z* 720 for FB_1, and *m/z* 704 for FB_2. About 5 fold increases in detection sensitivity was obtained with PI mode compared to NI mode. Adduct formation with Na+ was observed in positive ion modes (Table 1). However, the addition of formic acid to the mobile phase turned the elution solvent system sufficiently acidic to exchange sodium adducts away. The best fragmentation voltage was 140V for both compounds. Figure 1 shows a LC-MS chromatogram and a SIM spectrum of a standard solution, and a spiked sample. The selectivity of the method was demonstrated by the absence of interfering peaks compared with those observed when LC-FD was used.

LC-MS/MS

Parameters were optimized by continuous infusion of a standard solution (10 µg/ml) via a syringe pump at a flow rate of 10 µl min^{-1}. In LC-MS/MS, data acquisition was performed in both, SIM and multiple reaction monitoring (MRM) modes. SIM conditions were the same as for the single quadrupole, [M+H]+ ions were mass-selected by the first quadrupole and fragmented, producing product ions corresponding to sequential losses of water and

tricarballylic acid (TCA) side chains from the alkylbackbone. From the MS/MS full-scan spectra, two suitable transition pairs were selected for acquisition in MRM mode.

Table 1 lists the precursor, product ions and the ratio of abundances among both ion transitions as well as the optimized cone voltages and collision energies used for MRM. For the detection of FB_1 the precursor ion was *m/z* 722, being the product ions selected *m/z* 352, and 334. For FB_2, the precursor ion was *m/z* 706, and the product ions *m/z* 318 and 336.

Based on the confirmation of parent ions, more than two product ions should be selected in accordance with relevant EU recommendation 2002/657/EC which corresponds to 4 identification points (one precursor ion and two product ions).

Figure 1c shows a LC-MS/MS chromatogram of an organic flour sample contaminated at 258 $\mu g\ kg^{-1}$ of FB_1 and 156 $\mu g\ kg^{-1}$ of FB_2. For FBs, the adducts observed in the single quadrupole spectra were not present in the MS-MS spectra obtained with the QqQ instrument. This fact can be explained by the absence of neutral molecules from the mobile phase inside the collision cell (Barceló-Barrachina, Moyano, Puignou & Galceran, 2004).

LC-FD, LC-MS, and LC-MS/MS comparison

Quality parameters such as limits of detection (LODs), limit of quantitation (LOQs) and precision of the three analytical techniques were studied and compared for the first time (Table 2 and 3). These parameters were established using different modes of data acquisition as SIM for LC-MS studies and MRM for LC-MS/MS.

LODs and LOQs were established as the amount of analyte that produces a signal-to-noise ratio of 3:1 and 10:1 respectively. The precision was calculated by run-to-run repeatibility (n=3) and day-to-day repeatability (3 different days). LODs for FB_1 and FB_2 achieved by the three techniques were different, being the lowest LODs obtained with LC-MS/MS (12.5 $\mu g\ kg^{-1}$), followed by LC-FD (20 and 15 $\mu g\ kg^{-1}$, for FB_1 and FB_2 respectively), and finally LC-MS (50 $\mu g\ kg^{-1}$), volume sample should be considered as 10 µL when injections were done in MS detectors and 25 µl in fluorescence detector. However, these LODs are all satisfactory

considering the maximum levels established by European Commission (Commission Directive 2007/1126/EC). The best relative standard deviation (R.S.D.) values were obtained when using triple quadrupole with MRM acquisition and ranged from 1.7% (FB_1) to 1.9% (FB_2) for run-to-run precision and from 8.3% (FB_1) to 9.6% (FB_2) for the day-to-day precision.

Average recovery of FB_1 and FB_2 by adding different spiking levels to analyte-free corn samples is presented in Table 3, which varied from 79% to 102% with a relative standard deviation from 9% to 15%. Similar results were obtained with the three methods, which are according to the values established by European Commission, recommended recoveries of 60-120% for individual FB methods (≤ 500 ng/g) (Commission Decision 2002/657/EC).

LC-MS/MS was the most precise, accurate, and sensitive method. LC-FD chromatograms, presented interfering peaks, and furthermore, this type of detection needs the extract to be derivatized before analysis, consuming time and bringing time dependence in what respects to the derivatizing reagent stability.

In MS detectors, the matrix effect is usually caused by interfering matrix components in the extract, eluting at the same retention time as the analyte, and therefore competing in the ionisation process at the ion source. Then, the number of ions formed can be decreased or increased, resulting in a corresponding negative or positive matrix effect, respectively. Matrix effect was evaluated by comparison of the detector responses from standard solutions of the FBs in solvent with those from different matrix extracts at two concentration levels. From the calculated matrix effect results, it can be concluded, that the matrix effect for both FBs in positive mode is not significant or negligible.

Application to FB_1 and FB_2 determination corn-based foods

In order to evaluate the applicability of the optimized method, LC-MS/MS was applied to 41 corn based food from Valencia markets (Table 4, Fig. 33). Only 7 (17%) were contaminated. Fifteen samples were of organic origin (6 corn flour, 1 couscous, 3 corn bread, 4 corn flakes and 1 gofio). Gofio is a stone-ground flour made from roasted cereals typical from Canary

islands. Five flour samples were found to be contaminated with both fumonisins and a corn snack sample was contaminated with FB_1. Only one of the twenty six non-organic products was contaminated with both FBs, a flour sample. In flour, FB_1 was detected at concentration range from 258 μg kg^{-1} to 922 μg kg^{-1} with a mean value of 455 μg kg^{-1} and FB_2 was detected at concentration range from 156 μg kg^{-1} to 644 μg kg^{-1} with a mean value of 336 μg kg^{-1}, being a flour sample the most contaminated one.

The recommended limits established by the European Union were overlapped by one corn flour sample. In general, the occurrence and levels of fumonisins found in corn products is low, possibly because several food safety and quality standards are followed as good agricultural practices, good manufacturing practices and the hazard analysis and critical control point (HACCP) system.

In general, levels found from our study are in agreement with those of other surveillance studies from the Spanish market (Ariño, Estopañan, Juan, Herrera,. 2007; Ariño, Juan, Estopañan, González-Cabo, 2007) although percentage of positive samples was lower in our case, possibly because of the type of commercial corn product analyzed.

Only a few studies compare fumonisins in organic and non organic products. In our study percentage of contaminated organic samples (33%) was higher than non-organic ones (5%). These results are in contradiction with other reports. In Italian foodstuffs, occurrence contamination of FB_1 was 20% for organic food and 31% for conventional ones (Cirillo, Ritieni, Visone, Cocchieri, 2003). Ariño et al., 2007a, found that 13% of non organic corn samples and 10% of organic corn samples were contaminated with FBs, for this author the farming system is probably not of decisive importance for the contamination of agricultural products.

CONCLUSIONS

As demonstrated in the analytical procedure described herein, methanol:water extraction, centrifugation and purification through immunoaffinity columns allows the simultaneous, rapid

and sensitive detection and quantification of FB_1 and FB_2. A comparative study of the three LC detectors, FD, single quadrupole, QqQ for the analysis of fumonisins in corn samples has been performed. The response achieved by the three detectors was sensitive enough to study the maximum contents established by the EU legislation. These LC detectors would be appropriate for quantification purposes but the acquisition of at least two transitions achieved with QqQ provided a univocal identification.

These results reflected the situation of corn products on the Valencia market during 2006, the contamination level and occurrence of FB_1 and FB_2 in non organic food was lower than in organic food. To fully assess the differences in the quality of organic and conventional food it is required further study with a large number of food samples.

Acknowledgements

This work has been supported by the Spanish Ministry of Science and Technology (project No. AGL2006-04438) and of the integrated Actions Program between Spain and Portugal (project No. HI02-52 and IT962). The authors are also gratefully recognized to FCT for a PhD fellowship granted to Liliana J. G. Silva.

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Table 1. - Studied ions, cone voltages, and collision energies used in LC-MS/MS

Compound	*Mw*	Precursor ion (m/z)	Product ions (m/z)	MRM Ratio	Cone voltage (V)	Collision energy (eV)
Fumonisin B_1 ($C_{34}H_{59}NO_{15}$)	721.83	722 $[M+H]^+$ 744$[M+Na]^+$	352 - $[M+H-2TCA^1-H_2O]^+$ 334 - $[M+H-2TCA-2H_2O]^+$	1.37	50	40
Fumonisin B_2 ($C_{34}H_{59}NO_{14}$)	705.80	706 $[M+H]^+$ 728 $[M+Na]^+$	336 - $[M+H-2TCA-H_2O]^+$ 318 - $[M+H-2TCA-2H_2O]^+$	1.82	50	35

[1]TCA: tricarballylic acid

Table 2. -Results of the run-to-run and day-to-day precision study (both expressed as RSD%) obtained and calibration data for FB_1 and FB_2.

	Fumonisins	Correlation coefficient (r^2)	Calibration curve	Run-to-run precision (RSD%, n = 5)	Day-to-day precision (RSD%, n =5)
LC-FD	FB_1	0.984	y = 675254x + 299957	3.0	10.0
	FB_2	0.994	y = 608365x - 112296	2.7	15.1
LC-MS	FB_1	0.9995	y = 76748x - 23562	7.8	11.7
	FB_2	0.9998	y = 46347x - 13658	4.8	12
LC-MS/MS	FB_1	0.9994	y = 19073x + 22,963	1.7	8.3
	FB_2	0.9962	y = 13354x – 1240,8	1.9	9.6

Table 3. - Recovery, limits of quantification (LOQs) and limits of detection (LODs) obtained for FB_1 and FB_2 by LC-FD, LC-MS, and LC-MS/MS.

FBs	LODs (µg kg^{-1})			LOQs (µg kg^{-1})			Recovery mean (%) (n=3)			
	LC-FD	LC-MS	LC-MS/MS	LC-FD	LC-MS	LC-MS/MS	Fortification level (µg kg^{-1})	LC-FD	LC-MS	LC-MS/MS
FB_1	20	40	12	90	110	35	150	79±10	-	-
							200	-	98±11	97±9
							250	98±15	-	-
							400	-	94±10	102±10
FB_2	15	40	12	45	110	35	100	98±16	-	-
							200	99±17	99±13	81±10
							400	-	98±12	101±11

1

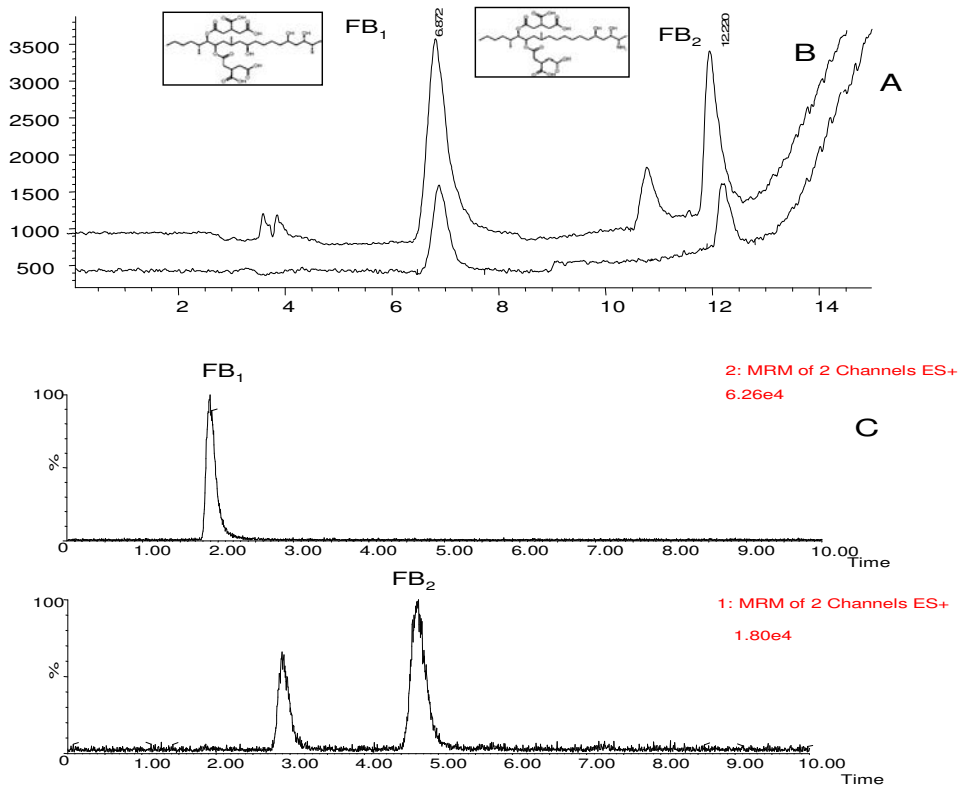
2 **Table 4.** Occurrence of the studied fumonisins in corn products from Valencia markets.

3

Sample	FB_1			FB_2			Maximum levels ($\mu g\ kg^{-1}$) FB_1+FB_2	Nº samples > Maximum levels FB_1+FB_2
	Positive/total (%)	Mean value ($\mu g\ kg^{-1}$)	Range (min-max)	Positive/total (%)	Mean value ($\mu g\ kg^{-1}$)	Range (min-max)		
Flour	5/9 (55%)	455	258-922	5/9	336	156-644	1000	1
Sweet corn	0/6	-	-	0/6	-	-	400	-
Corn snacks	1/9 (11%)	68	68	0/9	-	-	400	-
Cornflakes	0/11	-	-	0/11	-	-	400	-
Bread	0/3	-	-	0/3	-	-	400	-
Others	1/3 (33%)	71	71	1/3	42	42	400	-
TOTAL	7/41 (17%)	345	68-922	7/41	287	42-640	400-1000	1

1 Fig 1 –LC-MS chromatogram in SIM mode of: (a) a standard solution at 0.4 µgmL^{-1} FB_1 and FB_2. and (b) positive flour sample contaminated
2 with 922 µgkg^{-1} of FB_1 and 644 µgkg^{-1} of FB_2. (C) QqQ MRM chromatogram of an organic flour sample contaminated at 258 µg kg^{-1} of FB_1 and
3 156 µg kg^{-1} of FB_2.

4 Fig 2 – Results obtained of corn based food from Valencia markets during 2006.



1

2 Fig.1

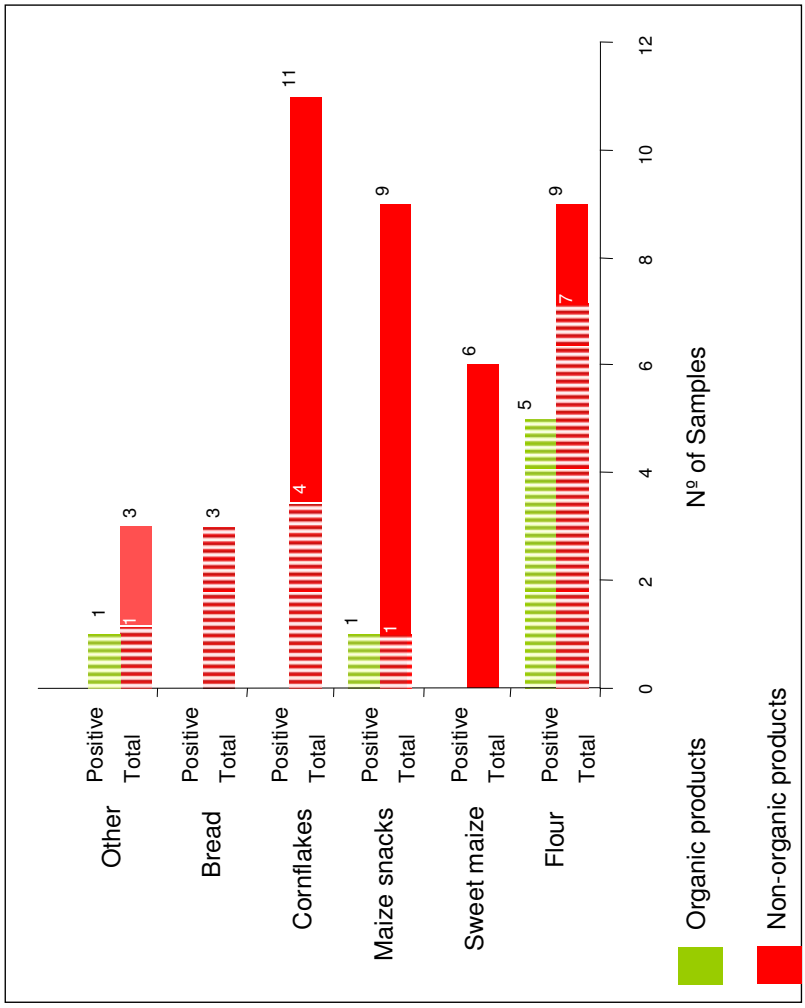


Fig.2