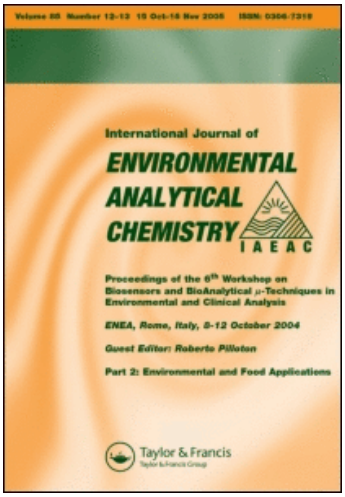

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Development of an LC-MS/MS method for the determination of aflatoxins B_1, B_2, G_1, and G_2 in Brazil nut

José Júnior Mendonça Xavier[a]; Vildes Maria Scussel[a]
[a] Food Science and Technology Department, Centre of Agricultural Sciences, Federal University of Santa Catarina, Brazil

To cite this Article Xavier, José Júnior Mendonça and Scussel, Vildes Maria(2008) 'Development of an LC-MS/MS method for the determination of aflatoxins B_1, B_2, G_1, and G_2 in Brazil nut', International Journal of Environmental Analytical Chemistry, 88: 6, 425 — 433
To link to this Article: DOI: 10.1080/03067310701836816
URL: http://dx.doi.org/10.1080/03067310701836816

Intern. J. Environ. Anal. Chem.
Vol. 88, No. 6, 15 May 2008, 425–433

Taylor & Francis
Taylor & Francis Group

Development of an LC-MS/MS method for the determination of aflatoxins B_1, B_2, G_1, and G_2 in Brazil nut

José Júnior Mendonça Xavier and Vildes Maria Scussel*

Food Science and Technology Department, Centre of Agricultural Sciences, Federal University of Santa Catarina, Florianopolis – SC, Brazil

(*Received 16 July 2007; final version received 30 November 2007*)

An LC-MS/MS method with atmospheric pressure chemical ionization in the positive mode $[M+H]^+$, for simultaneous determination of the aflatoxins (AFLs): AFB_1, AFB_2, AFG_1 and AFG_2 in Brazil nuts matrix is described. By means of multiple reaction monitoring it was possible to analyse the AFLs fragment ions to increase specificity and sensitivity. The separation of the toxins was carried out in a C_8 column with gradient mobile phase composed of water:methanol (25 mM ammonium acetate) in a total run time of 5.0 min. The toxin extraction solvent was acetonitrile : water (80 : 20) and no clean up was required for the Brazil nut samples. The method values of LOD and LOQ utilizing the Brazil nut matrix for AFB_1, AFB_2, AFG_1 and AFG_2 were 0.04; 0.045; 0.050 and 0.060 $\mu g\,kg^{-1}$; 0.08, 0.09, 0.10 and 0.12 $\mu g\,kg^{-1}$, respectively. The recoveries from the Brazil nuts were between 92 and 100% and ΣAFL levels obtained from the positive naturally contaminated Brazil nuts ranged from 1.2 to 11.5 $\mu g\,kg^{-1}$.

Keywords: aflatoxins; methodology; Brazil nuts; tandem mass spectrometry; LC-MS/MS; chromatography

1. Introduction

One of the major problems related to food safety is the presence of fungi able to produce toxic secondary metabolites – the mycotoxins. The most important group of toxins concerning toxicity and regulation worldwide are the aflatoxins (AFLs). These toxins can cause several damages to the health of animals and humans. Because of this, the presence of AFLs in food can lead to serious economic losses in countries exporting agricultural commodities, as mycotoxins-contaminated batches are increasingly rejected by food importing countries [1].

Due to the risk of mycotoxins presence in food, many countries have established regulations, mainly for AFLs. In the recent years an updating of international regulations has been carried out, especially by the European Union (EU), which has strongly reduced the AFL maximum residue levels (MRL) to 2 and 4 $\mu g\,kg^{-1}$ for AFB_1 and AFLs, respectively [2]. The EU has also increased the number of mycotoxins to be surveyed in different types of food [3], thus demanding higher sensitivity for the methodologies to detect and quantify lower levels of toxins in food.

*Corresponding author. Fax: +55 048 3338 0027. Email: vildescussel_2000@yahoo.co.uk

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310701836816
http://www.informaworld.com

The current methods used on AFL analysis are mainly by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) with fluorescence detector (FLD) and immunoassay [4,5]. However they have some limitations such as, being time consuming, as far as the extraction and clean up steps are concerned; use large volumes of solvents; some of which are toxic, and need additional confirmation tests to release the final results. Nowadays, the use of methods with tandem mass spectrometer (MS/MS) seems to be one of the best techniques to detect and quantify toxins. They have the ability to analyse toxins from different groups and from different types of food matrix with no, or quite simple, clean up steps, reducing the solvent used, and the total run time, as well as being able to release self-confirmatory results [1,6–11].

Most of the LC-MS and LC-MS/MS methods developed to date for mycotoxins are for the trichotecenes [6–8,12,13] patulin [14], zearalenone metabolites [15], fumonisins [11,16] and for Alternaria toxins [17,18]. There are some LC-MS/MS methods for AFLs (AFB_1, AFB_2, AFG_1, AFG_2) and they use, in most cases, electrospray (ESI) as the ionization source [10,11,19,20]. A paper published in 2002 [21] reported an application of APCI for AFLs, however its total run time with C_{18} was 30 min, with RT for AFB_1 and AFB_2 of 15.46 and 14.11 min, respectively.

Economically, Brazil nut is a very important commodity to the South American countries that comprise the Amazon forest. In recent years the Brazil nut export market to the EU has been greatly reduced [2] as the AFL contamination levels did not reach its very low MRLs.

Considering that the low EU MRL has restricted the Brazil nuts export, there is a need for a highly sensitive, self-confirmatory and faster method for measuring AFLs to comply with that regulation. Therefore, a study on the development of a methodology by LC-MS/MS that can be able to detect and quantify the AFLs: AFB_1, AFB_2, AFG_1, AFG_2 at lower levels than the current methodologies for Brazil nut, was carried out.

2. Method

2.1 *Materials*

(a) *Chemicals*: methanol, acetonitrile, benzene (HPLC grade), Carlo Erba, Rodano, Italy. Ultrapure water (MilliQ system, Millipore, Billerica, USA). Amonium acetate (analytical grade), Vetc, Rio de Janeiro, Brazil.

(b) *Aflatoxin standards*: AFB_1, AFB_2, AFG_1 and AFG_2, Sigma (Saint Louis, USA).

(c) *Matrix*: 40 (30 kg) samples of dry in-shell Brazil nuts for export provided by the CIEX factory, from Manaus city (Amazon State, Brazil).

(d) *Equipments*: liquid chromatograph, 1100, Agilent (Santa Clara, USA) with a quaternary pump, degasser, auto sampler and a 20 µl loop. Reverse phase columns studied: three C_{18} [(4.5 mm id), 150 (5 µm) from Hichrom (Theale, UK) and 250 (5 and 10 µm) mm lengths] from Phenomenex (Torrance, USA) and one C_8 [4.6 mm id, 150 mm length (5 µm)] from Agilent (Santa Clara, USA). Tandem mass spectrometer, API 4000 triple-quadrupole, Applied Biosystems MDS SCIEX (Foster City, USA), equipped with atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interface in positive mode and infusion pump, Harvard Apparatus (Holliston, USA). Spectrophotometer, U2010 Hitachi (Tokyo, Japan). Mill, Romer (Union, USA). Industrial nut-cracker from CIEX (Manaus, Brazil).

2.2 *Methods*

2.2.1 *AFB_1, AFB_2, AFG_1 and AFG_2 standards solution preparation*

The toxin solutions (stock, intermediary and work solutions) were prepared using benzene, acetonitrile and/or water according to the steps of the method development and calibrated [5]. The stock solutions were prepared with benzene:acetonitrile (99 : 1), and the concentrations were 8.48, 10.36, 9.21 and 9.83 $\mu g\,mL^{-1}$ for AFB_1, AFB_2, AFG_1 and AFG_2, respectively. The intermediary solutions were with acetonitrile at concentration of 1 $\mu g\,mL^{-1}$ for all the AFLs and the work solutions with water at concentration of 0.1 $\mu g\,mL^{-1}$ *(a.1) solutions for MS/MS tuning:* individual toxin standard solutions for the tuning were prepared at concentrations of 0.01 $\mu g\,mL^{-1}$ in acetonitrile with 25 mM ammonium acetate. *(a.2) solutions for LC-MS/MS system:* the AFLs mix standard solutions were prepared at concentration of 0.001 $\mu g\,mL^{-1}$ in water from the work solution, respectively.

2.2.2 *Optimisation of MS/MS parameters for AFB_1, AFB_2, AFG_1 and AFG_2*

The standards were injected individually directly into the tandem mass spectrometer with APCI as ionization source operating in the positive mode $[M+H]^+$, using an infusion pump and the following parameters were optimised: collision energy (CE), declustering potential (DP) and cell exit potential (CXP). With the optimisation of these parameters the parent and daughter ions were obtained for each AFL.

2.2.3 *Setting LC parameters for the AFLs in the LC-MS/MS and DW*

The LC was connected to the MS/MS detector and the AFLs mix solution, previously prepared, was injected (20 µL). The toxins separation was checked in each column (C_8 and C_{18}) using methanol : water (both with 25 mM of ammonium acetate) as mobile phase (MP). The MP gradient was developed to allow an optimal separation of the four toxins in the shortest time possible. The flow rate of MP was 1 $ml\,min^{-1}$, and its gradient was performed using water (A) and methanol (B) as solvents in a proportion of 45 (A) and 55 (B)% in the step zero (pre-run) for 3.5 min. In step 1 (3.0 min) the proportion of methanol increased to 70% and this gradient was maintained until the end of the run (7.0 min). This gradient showed an excellent separation of the four toxins. The toxins were successfully separated using the C_8 column and the first toxin to be eluted from the column was AFG_2. The t_r obtained increased from 3.23, 3.64, 4.06 to 4.45 min for AFG_2 AFG_1, AFB_2 and AFB_1, respectively. The ion mass chromatogram showing each toxin most sensitive and specific daughter ions are shown in Figure 1. Although the C_{18} columns, either 250 or 150 mm length and 5 or 10 µm particle size, presented reasonable resolution ($r > 3$) for the AFLs, using the chosen gradient MP; the C_8 (150 mm, 5 µm) showed, in this study, very good performance with the best peak shape, height and resolution (closer to 3). The DW was defined by injection of toxin mix standard solution to obtain the best chromatographic maximum and was expressed in milliseconds (see Table 1).

2.2.4 *LOD, LOQ, R^2, matrix effect and recovery*

Standard mix solution with the four AFLs at low concentration was injected in the LC-MS/MS system and the LOD defined by three times the signal/noise ratio (3 S/R) and the LOQ by six times the signal/noise ratio (6 S/R).

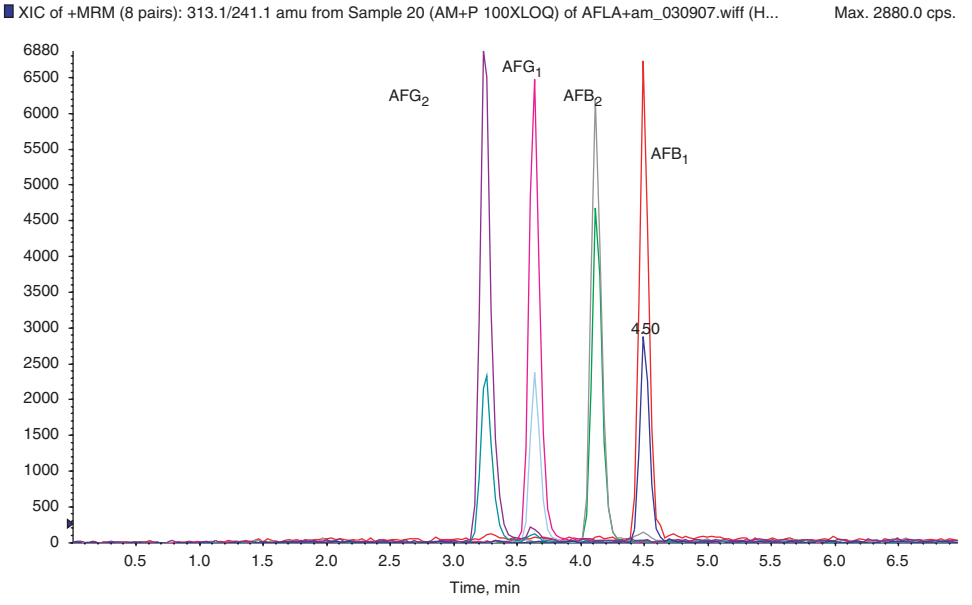

Figure 1. Ion mass MRM chromatogram of AFB_1, AFB_2, AFG_1 and AFG_2 using LC-MS/MS – Atmosphere Pressure Chemical Ionization $[M + H]^+$ with a concentration of $50 \times$ LOQ.

Table 1. Parent, daughter ions and the MS/MS optimised parameters for AFLs[a].

		Ion (m/z)					
Toxin	MW[b]	Parent	Daughter	DP[c] (V)	CE[d] (V)	CXP[e] (V)	DW[f] (milisec)
AFB_1	312	313.1	241.10*	91.00	53.00	42.00	250
			285.10**	91.00	33.00	24.00	250
AFB_2	314	315.0	259.09**	101.00	41.00	22.00	250
			287.20*	101.00	37.00	26.00	250
AFG_1	328	329.1	200.05**	96.00	27.00	34.00	250
			243.05*	96.00	37.00	40.00	250
AFG_2	330	331.2	245.07*	86.00	43.00	42.00	250
			313.20**	86.00	35.00	20.00	250

Notes: [a]Aflatoxins; [b]molecular weight; *quantifier product ions; **qualifier product ion; [c]declustering potential = potential required to unmake clusters formed among the analytes molecules; [d]collision energy = energy necessary to fragment the parent ion; [e]cell exit potential = potential demanded for fragments (daughter ions) to go from collision cell to detector; [f]dwell time.

2.2.4.1 *Calibration curves and R^2*. At this step AFLs calibrated mix solutions (5 concentration levels) were used. The concentration level of each solution was based on the LOQ values obtained previously, i.e., $1 \times$ LOQ, $5 \times$ LOQ, $10 \times$ LOQ, $20 \times$ LOQ and $100 \times$ LOQ. After the solution injection (triplicate), the R^2 was defined from the calibration curves. The matrix R^2 was obtained by analysing spiked Brazil nut at concentration of 1, 5, 10, 20 and 100 times the LOQ for each AFL in triplicate.

2.2.4.2 *Sample preparation*. Brazil nut samples were prepared for analysis by de-shelling them utilising an industrial nut-cracker, then finely grinding in a mill and weighing portions for AFL extraction. It is important to emphasize that, as the nuts were to be exported to the EU, after de-shelling all nuts, even the visually spoiled and deteriorated ones, were included into the final analytical sample.

2.2.4.3 *Extraction procedure*. Homogenised 25 g portions were taken for extraction by adding 100 ml acetonitrile : water (80 : 20 v/v) to the sample, mixed for 2 h and filtered. The filtered extract was diluted four times with water and an amount of 20 μL was injected into the LC-MS/MS.

2.2.4.4 *Matrix effect*. In order to check if the contamination of Brazil nuts could interfere on the AFL detection, nut extracts were spiked with AFLs mix standards with five different concentrations based on the instrumental LOQ and injected into the LC-MS/MS. It was carried out in triplicate.

2.2.4.5 *Recovery*. Brazil nuts samples were grinded and spiked with AFLs at five concentrations ranging from 1 to 10 $\mu g\,kg^{-1}$ in triplicate and extracted as above.

2.2.4.6 *Application of the method in Brazil nuts naturally AFLs contaminated*. 40 samples were analysed using the method described above. Quantification was carried out by the analyst software based on the calibration curves.

3. Results and discussion

3.1 *MS/MS spectrometer parameters for AFB_1, AFB_2, AFG_1 and AFG_2*

The APCI was the ionization source utilised for AFLs, producing protoned molecular ions in the positive mode. In the LC-MS/MS instrument used in this study, the ESI could not ionize the AFLs. Collisional induced MS/MS fragmentation is dominated by subsequent loss of small neutrals like water, carbon monoxide and carbon dioxide followed by cleavages within the polycyclic skeleton (Figure 2). For multi reaction monitoring (MRM), the most intensive daughter ions recorded are reported in Table 1, as are the other optimised parameters.

3.2 *LOD, LOQ, R^2, matrix effect and recovery*

The instrumental values of LOD, LOQ and R^2 were defined for all the daughter ions, however, for quantification, only the daughter ions with the highest sensitivity and best R^2 for each parent ion were used (see Table 2). The other daughter ions were used for the positive confirmation of the toxin presence. The method offered instrumental linearity over the range of 0.005, 0.02, 0.25 and 0.04 to 0.5, 2.0, 25 and 4.0 $\mu g\,kg^{-1}$ for AFB_1, AFB_2, AFG_1 and AFG_2, respectively with R^2 values closer to one.

The LOD and LOQ obtained from the application of the LC-MS/MS method for AFLs quantification utilising Brazil nuts were excellent as were the method's recovery rates: between 92 and 100% (spiking concentrations: 1 to 10 $\mu g\,kg^{-1}$). The values of LOD

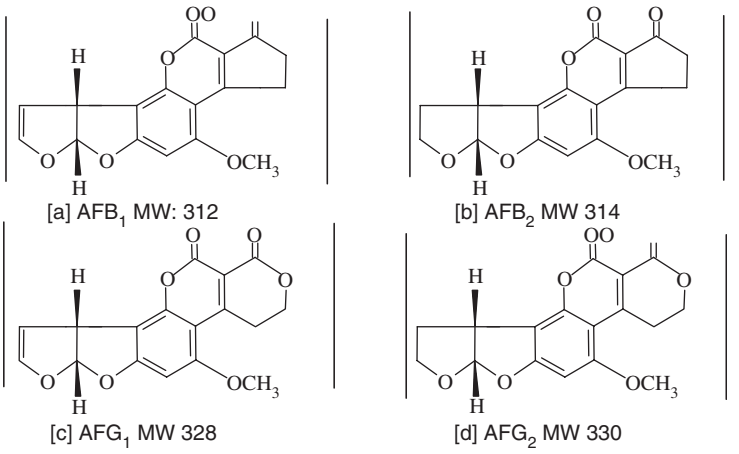

Figure 2. The aflatoxin chemical structures with their respective molecular weight: [a] AFB_1 (312), [b] AFB_2 (314), [c] AFG_1 (328) and [d] AFG_2 (330).

Table 2. Values of instrumental LOD[a], LOQ[b] and R^{2c}.

Toxin	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	R^2
AFB_1	0.0025	0.005	0.9995
AFB_2	0.01	0.02	0.9996
AFG_1	0.0125	0.025	0.9995
AFG_2	0.02	0.04	0.9994

Note: [a]Limit of detection; [b]limit of quantification; [c]correlation coefficient.

and LOQ for AFB_1, AFB_2, AFG_1 and AFG_2 were 0.04; 0.045; 0.050 and 0.060 $\mu g\,kg^{-1}$; 0.08, 0.09, 0.10 and 0.12 $\mu g\,kg^{-1}$, respectively, with values of relative standard deviation (RSD) lower than 5%, which are reported in Table 3. Thus for ΣAFLs they were 0.195 and 0.390 $\mu g\,kg^{-1}$. Using the Brazil nut matrix, the AFL linearity obtained was over a range of 0.08, 0.09, 0.10 and 0.12 to 8.0, 9.0, 10 and 12 $\mu g\,kg^{-1}$ and the values of R^2 were 0.9981, 0.9998, 0.9993 and 0.9997 for AFB_1, AFB_2, AFG_1 and AFG_2, respectively. The method LOD and LOQ values (Table 2) were far bellow the 1 $\mu g\,kg^{-1}$ level which is a reasonable approach to check for the MRL requested by the EU (2 $\mu g\,kg^{-1}$ for AFB_1). As far as Brazil nut matrix effect is concerned, it was observed a slight interference on the AFLs signals when injecting the spiked Brazil nuts extracts. That was supported by the data obtained from the 40 Brazil nut samples naturally contaminated (Figure 3) analysed with the current method. AFLs were detected in seven samples ranging from 1.2 to 11.5 $\mu g\,kg^{-1}$ and only four samples presented levels higher than the EU regulation for ΣAFLs (4–11.5 $\mu g\,kg^{-1}$). None were above the Canadian, Mercosur or USA regulation (15, 20, 20 $\mu g\,kg^{-1}$) [22]. Is is important to emphasize that all Brazil nuts after de-shelling were ground, as they were to be exported in-shelled, inclusive some spoiled/deteriorated ones, thus leading to the levels detected. However, the consumers reject/discard the spoiled nuts after de-shelling, as they are visually of low quality as reported by Makindler *et al.* (2005) [24].

Table 3. Aflatoxin detection and repeatability in Brazil nut matrix spiked at different concentrations.

	Aflatoxin detection ($\mu g\,kg^{-1}$)									
	100 × LOQ[a]		20 × LOQ		10 × LOQ		5 × LOQ		1 × LOQ[a]	
Toxin	Mean ($\mu g\,kg^{-1}$)	RSD[b] (%)	Mean ($\mu g\,kg^{-1}$)	RSD (%)	Mean ($\mu g\,kg^{-1}$)	RSD (%)	Mean ($\mu g\,kg^{-1}$)	RSD (%)	Mean ($\mu g\,kg^{-1}$)	RSD (%)
AFB_1	7.99	0.75	1.69	4.73	0.79	5.10	0.41	1.46	0.075	4.00
AFB_2	8.98	1.78	1.89	1.05	0.90	3.33	0.46	4.34	0.082	2.43
AFG_1	9.97	0.30	2.19	5.47	1.00	5.00	0.48	1.04	0.094	3.20
AFG_2	11.94	1.84	2.64	7.90	1.18	4.23	0.60	1.46	0.099	5.05

Note: [a]Limit of quantification; [b]Relative standard deviation; $n = 5$.

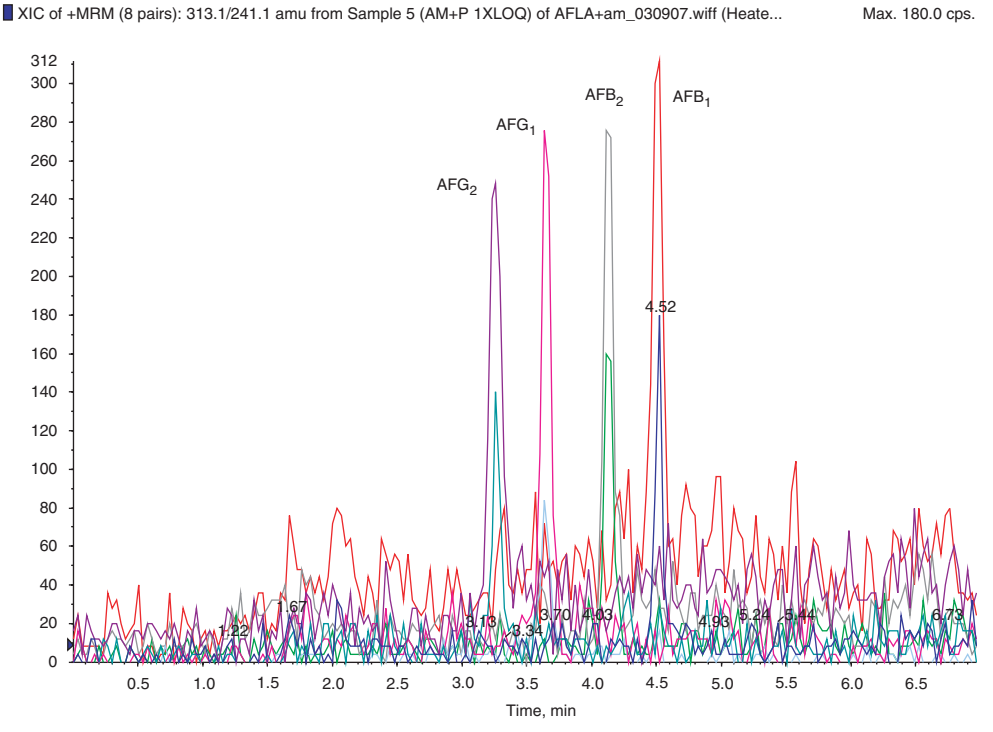

Figure 3. Ion mass MRM chromatogram of a contaminated sample using LC-MS/MS – Atmosphere Pressure Chemical Ionization $[M+H]^+$ with a concentration of 0.1, 0.13, 0.15 and 0.12 $\mu g\,kg^{-1}$ for AFB_1, AFB_2, AFG_1 and AFG_2, respectively.

3.3 *General discussion*

Although some AFL methods on tandem mass spectrometry have been developed, the ones reported in the literature that include those toxins [10,11,20,23] have in common the ionization source. They utilise the ESI as the ionization source, C_{18} column for separation and some include a clean-up step. Spanjer *et al.* (2006) did not use any clean up and also

scanned several other mycotoxins. On the other hand, Cavalieri *et al*. (2007) analysed the AFL group in olive oil samples, to separate the four toxins, however reaching a quite long total run time of 16 min for each run. A paper that applied APCI for AFLs reported in the literature by Abbas *et al*. [21], had a rather long run time also, reaching for AFB_1 and AFB_2 15.46 and 14.11 min in a C_{18} column. Contrary to these methods, our method utilizes APCI as ionization source, C_8 column for separation and the total run time was slightly less than 5 min, being 3.23, 3.64, 4.06 to 4.45 min for each toxin, respectively. It can be an alternative to the laboratories that have LC-MS/MS with that type of ionization source. If ultra performance liquid chromatography (UPLC) could be applied to the method, the total run time would be further reduced as reported by Ventura *et al*. (2006) that found a total t_r for AFLs of 3.1 min. In addition the AFL LC-MS/MS method developed can be very useful for releasing fast, reliable and self-confirmatory results for commodities that are due to be exported to the EU with its very strict AFL regulations.

From the current methodologies utilising HPLC-FLD and TLC [5], the advantages of the LC-MS/MS developed are the speed at which the result is obtained and the confidence of the result (no need of confirmation) unlike the other methods, where the final result still needs a confirmation step. The disadvantages of the current method are the cost of the instrument and maintenance. However, it can pay for itself with the time saved and the quality of analysis carried out. An equipment and method such the one developed can also help in detecting very low levels that – research wise – can gather epidemiological and exposure data to predict or avoid chronic AFL toxic effects such as cancer.

As far as matrixes are concerned, to date we have been able only to evaluate the Brazil nut. That commodity itself, can justify the use of a method like the one developed here, as large amounts of these nuts are exported annually, improving the economy of the Amazon region South American countries.

4. Conclusions

The LC-MS/MS method for AFL analysis in Brazil nuts was highly sensitive (low LOD and LOQ values), fast (total run time for AFLs < 5.0 min) and specific (two selected daughter ions for each AFL). APCI tends to give better sensitivity than ESI for less polar compound. The application of the method for AFLs quantification in Brazil nuts was excellent and able to comply with the detailed demand of the current EU regulations. The steps of extraction were fast and safe (low amounts of solvents) with no clean up needed. An improvement for the method should be the addition of toxins of different groups that may contaminate Brazil nuts, as well as other economically important Brazilian commodities.

Acknowledgements

The authors thank Mr H. Martins and Applied Biosystems SMD SCIEX – Brazilian branch, for providing support to carry out this study. Dr A.M. Pacheco and the CIEX Factory for providing Brazil nut samples.

References

[1] P. Zöllner and B. Mayer-Helm, J. Chrom. A **1136**, 123 (2006).
[2] EC – Commission of the European Communities (Health and Consumer Protection Directorate-General), 2003. Final report of a mission carried out in Brazil from 27th January to 7th February 2003 to assess the facilities and measures in place for the control of aflatoxin levels in Brazil nuts intended for export into the European Union, DG(SANCO)/9027/2003 – MR – final. pp. 20.
[3] EU – European Union, Scientific Committee of Food, Brussels, http://europa.eu.int/comm/food/fs/sc/scf/opinions. Visited in September, 2007.
[4] A. Kussak, C.-A. Nilsson, B. Andersson, and J. Langridge, Rap. Comun. Mass Spec. **9**, 1234 (1995).
[5] AOAC – Association of Official Analytical Chemists, *Official Methods of Analysis of AOAC International*, 18th ed. H.W. Horwitz, G.W. Latimer Jr. (AOAC, Gaithersburg, MD, 2005).
[6] C. Cavalieri, G. D'Ascenzo, P. Foglia, E. Pastorini, R. Samperi, and A. Lagana, Food Chem. **92**, 559 (2005).
[7] L.K. Sorensen and T.H. Elbaek, J. Chrom. B **820**, 183 (2005).
[8] F. Berthiller, R. Schumacher, G. Buttinger, and R. Krska, J. Chrom. A **1062**, 209 (2005).
[9] S. Biselli and C. Hummert, Food Add. Cont. **22**, 752 (2005).
[10] C. Cavalieri, P. Fobia, C. Guarino, M. Nazzari, R. Sampiri, A. Lagana, Anal. Chem. Acta (2007), doi:10.1016/j.aca.05.055. 2007.
[11] M.C. Spanjer, J.M. Scholten, and P.M. Rensen, *Mycotoxins and Phycotoxins – Advances in Determination, Toxicology and Exposure Management*, edited by H Njapeu, S. Trujillo, H. van Egmond, and D. Park (Wageningen Academic Publishers, Wageningen, NL, 2006), pp. 117–124.
[12] E. Razzazi-Fazeli, J. Bohm, and W. Luf, J. Chrom. A **854**, 45 (1999).
[13] E. Razzazi-Fazeli, B. Rabos, B. Cecon, and J. Bohm, J. Chrom. A **968**, 129 (2002).
[14] V. Sewran, J.J. Nair, T.W. Nieuwoudt, N.L. Leggott, and G.S. Shephard, J. Chrom. A **897**, 365 (2000).
[15] E.O. van Bennekom, L. Brouwer, E.H.M Laurant, H. Hooijerink, and M.W.F. Nielen, Anal. Chim. Acta **473**, 151 (2002).
[16] Z. Lukacs, S. Schaper, M. Herderich, P. Schreier, and H. Humpf, Chromatographia **43**, 124 (1996).
[17] P.M. Scott, J. AOAC International **84**, 1809 (2001).
[18] P.M. Scott, G.A. Lawrence, and B.P.-Y. Lau, Mycotoxin Res. **22**, 142 (2006).
[19] P. Janssen and N. Franken, Technical Laboratory, Rotterdam The Netherlands. Personal Communiction (2000).
[20] M. Vezntura, A. Gómez, I. Anaya, J. Diaz, F. Broto, M. Agut, and L. Cornelias, J. Chrom. A **1048**, 25 (2004).
[21] H. Abbas, W. P. Williams, G. L. Windham, H. C. Pringle, W. Xie, and W. Shier, J. Agric. Food Chem. **50**, 5246 (2002).
[22] H.P. van Egmond and M.A. Jonker, *Mycotoxins in Food – Detection and Control*, edited by N Magan and M. Olsen (Woodhead Publishing, Cambridge, UK, 2004), pp. 49–68.
[23] J. Blesa, J.M. Soriano, J.C. Moitó, R. Marín, and J. Mañes, J. Chrom. A **1011**, 49 (2003).
[24] I. Marklinder, M. Linblad, A. Gidlund, and M. Olsen, Food Add. Contami. **22**, 56 (2005).