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Determination of aflatoxins in food using LC/MS/MS

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Abstract A liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric method is described for the determination of aflatoxins B_1, B_2, G_1 and G_2 in food with the use of aflatoxin M_1 as an internal standard. The method works well with matrices such as those of figs and peanuts, but there are problems with spices, due to limitations of the clean-up method used.

Key words Aflatoxins · Food · Liquid chromatography · Tandem mass spectrometry

Introduction

The use of liquid chromatography (LC) coupled with mass spectrometry (MS) for the confirmation of polar contaminants, such as the mycotoxins aflatoxins, ochratoxin A and fumonisins, will probably become more common in the coming years in systems designed for the control of food quality.

Analysis of aflatoxins in figs and spices using traditional clean-up methods and liquid chromatography with fluorescence detection (LC/FD) can be difficult due to the presence of interfering peaks in the chromatograms. It is therefore often necessary to resolve the extracts on two columns of different polarity, for example a reverse-phase and a normal-phase LC column. The use of immunoaffinity columns can improve the efficiency of the clean-up step, but for some food matrices there are still analytical problems like low recoveries and interfering peaks for products such as spices [1].

A few papers on different kinds of mass spectrometric methods for the analysis of aflatoxins have been published [2–6].

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Tandem mass spectrometry (MS/MS) of collision-induced ion fragments is a very specific detection method, and it is reasonable to assume that chromatography using one type of LC column should be sufficient for the reliable determination of aflatoxins in food products. In this investigation we used our established clean-up method for the analysis of aflatoxins and compared the quantification of aflatoxins by fluorescence and MS/MS for a number of different food products. The clean-up method has been used in our laboratory for more than 10 years and FAPAS proficiency test results have been good.

Materials and methods

Chemicals. All solvents were analytical or HPLC grade. Aflatoxins B_1, B_2, G_1, G_2 and M_1 were obtained from Sigma. Stock standard solutions of aflatoxins were made in toluene:acetonitrile (98:2) for B_1, B_2, G_1 and G_2, and in chloroform for M_1. Concentrations were measured by spectrophotometry and stock solutions were diluted to standard solutions for LC/FD or LC/MS/MS calibration.

Extraction and clean-up. The extraction and clean-up procedure was based on that of Pons [7] with some modifications. Aflatoxins were extracted with methanol followed by precipitation of colour pigments using zinc acetate, extraction into dichloromethane and clean-up by column chromatography using cellulose and silica gel. The aflatoxins were finally dissolved in dichloromethane and, after evaporation, the aflatoxins were finally dissolved in the LC mobile phase with 0.1% acetic acid added in order to avoid their degradation [8].

LC/FD conditions. The LC/FD analysis was carried out using a Waters 510 chromatography pump and a Perkin-Elmer LC 240 fluorescence detector (excitation wavelength 360 nm and emission wavelength 440 nm). A 250 mm × 4 mm Spherisorb 5 ODS-1 column maintained at 35 °C was used. The mobile phase employed was water:acetonitrile:methanol (65:25:10) at a flow rate of 1.0 ml/min. Post-column derivatization with iodine-saturated water at 75 °C and a reaction coil of 3 m × 0.5 mm was used [9]. Sample volumes of 25 µl were injected with a Waters injector WISP 710B. External aflatoxin standards of B_1, B_2, G_1 and G_2 of in the range 0–50 ng/ml were used, and quantification was done using peak areas and standard curves.

LC/MS/MS conditions. MS was performed using a Micromass Quattro II instrument, a triple quadrupole mass spectrometer, and MassLynx software for control and data processing (Micromass, UK). Atmo-

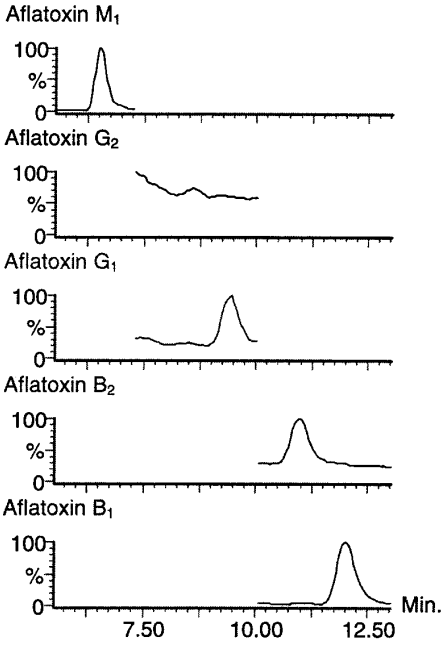

Fig. 1 Mass chromatograms of a fig sample naturally contaminated with 5 µg/kg aflatoxin B_1, 0.5 µg/kg aflatoxin B_2 and 1 µg/kg aflatoxin G_1. Aflatoxin M_1 was used as internal standard. Data were acquired in the multiple reaction monitoring mode, monitoring the most intense daughter ion of the pseudo-molecular aflatoxin ions

Table 1 Recovery of aflatoxins from 12 extracts of foods spiked with 2 µg/kg of all four aflatoxins immediately before analysis by liquid chromatography/tandem mass spectromes (LC/MS/MS)

Food product	Aflatoxin B_1 (% found)	Aflatoxin B_2 (% found)	Aflatoxin G_1 (% found)	Aflatoxin G_2 (% found)
Figs	58	80	75	73
	61	77	74	61
Peanut	63	81	88	112
butter	84	85	86	98
Peanuts	64	85	89	86
	83	92	88	63
Chilli	161	154	89	123
	144	133	95	100
Cayenne	172	163	91	93
pepper	276	270	201	147
Curry	42	86	83	49
	58	68	75	–

spheric pressure chemical ionisation in the positive mode was used. The probe and source temperatures were 375 °C and 130 °C, respectively; the corona voltage 3.5 kV and the cone voltage 20 V. Multiple reaction monitoring (MRM) mode of operation was used. The $[M+H]^+$ ions of the aflatoxins were used as parent ions. The most intense daughter ions, resulting from collision-induced dissociation with argon, were used. The argon pressure was set at 2×10^{-3} mbar and the collision energy at 30 eV. The daughter ions detected were: *m/z* 241 for aflatoxin B_1, *m/z* 259 for aflatoxin B_2, *m/z* 243 for aflatoxin G_1, *m/z* 257 for aflatoxin G_2, and *m/z* 273 for aflatoxin M_1.

The LC separation was carried out using a Jasco PU980 chromatography pump, and a 150 mm × 2 mm Spherisorb 5 ODS-1 column at ambient temperature. The mobile phase employed was water:acetonitrile:methanol (60:28:12) at a flow rate of 200 µl/min. Sample volumes of 20 µl were injected with a Hewlett Packard 1050 injector. Aflatoxin M_1 was used as the internal standard and quantification was done using peak areas and standard curves relative to aflatoxin M_1.

Table 2 Quantification of aflatoxins in 11 samples of foods using *MS/MS* and fluorescence detection (*FD*). *n.d.* Not detected; for other abbreviations, see Table 1

Food product	Aflatoxin B_1 (µg kg^{-1})		Aflatoxin B_2 (µg kg^{-1})		Aflatoxin G_1 (µg kg^{-1})		Aflatoxin G_2 (µg kg^{-1})	
	MS/MS	FD	MS/MS	FD	MS/MS	FD	MS/MS	FD
Figs	1.1	1.6	1.1	1.7	1.1	(2.4)[a]	0.7	(2.3)[a]
	4.1	5.9	0.5	0.6	0.9	(3.0)[a]	n.d.	(2.3)[a]
	4.4	5.5	0.5	0.5	1.3	(2.7)[a]	n.d.	(2.3)[a]
	4.3	5.5	0.6	0.5	0.9	(2.9)[a]	n.d.	(2.4)[a]
Peanut butter	1.8	2.1	0.4	0.6	0.6	0.7	0.2	0.4
	5.5	8.8	1.0	1.5	1.2	1.8	0.2	0.5
Chilli	2.7	1.5	2.2	1.2	1.2	1.6	1.0	1.4
	2.5	2.3	1.7	1.5	0.6	1.8	0.7	1.7
Cayenne pepper	2.1	1.4	2.1	1.2	1.0	1.5	0.6	1.3
	5.0	1.7	3.9	1.5	2.4	2.1	1.5	1.7
Pistachio meal	61.0	62.5	5.6	11.6	n.d.	n.d.	n.d.	n.d.

[a] Interfering peaks in chromatogram

Results and discussion

Chromatograms of aflatoxins using MS/MS are shown in Fig. 1. The specificity of the MS/MS detection is illustrated by the absence of interfering peaks. The detection limit (signal/noise ratio = 3) for all four aflatoxins was 0.1 µg/kg or better, which is fully satisfactory for confirmation analysis in relation to the Danish maximum limits for aflatoxins in food products of 2 µg/kg for aflatoxin B_1 and 4 µg/kg for the sum of aflatoxins.

The quantification of the aflatoxins using MS in the selected ion recording mode, was unsatisfactory because of interference in the mass chromatograms. This is in agreement with results given by Kussak [6] for the determination of aflatoxins in copra-dust using an immuno-affinity clean-up procedure, but the interferences were much more intense in our experiments.

The percentage recovery of aflatoxins from extracts spiked with aflatoxins at a concentration of 2 µg/kg using MS/MS detection is shown in Table 1. Aflatoxin M_1 added to standards and final extracts was used as an internal standard. The results of the quantitative determination of aflatoxins in figs and peanut products by MS/MS were reasonable. The results for the spices were unsatisfactory, as the recoveries varied between 40% and 280%. Even in the same sample matrix the recoveries varied between 90% and 200% for individual aflatoxins.

Table 2 gives the results of the comparison of the two detection methods. The aflatoxin concentrations were similar for all aflatoxins in peanut butter and for aflatoxins B_1 and B_2 in figs. The results obtained for aflatoxins G_1 and G_2 in figs when using FD were too high due to interfering peaks in the chromatograms; these aflatoxins are normally determined using a normal-phase LC system. As expected

from the recovery experiments, the results obtained for the spices with MS/MS detection were generally higher than the results obtained with FD.

The result shows that aflatoxin M_1 is not a suitable internal standard for quantification of aflatoxins in spices, in fact no single compound could be used. The ionization conditions vary with retention time due to coeluting matrix constituents, which sometimes result in signal suppression and sometimes in signal enhancement. This demonstrates that samples must still undergo extensive clean-up before analysis as LC/MS/MS cannot act as an extensive clean-up step.

The use of isotope-labelled aflatoxins as internal standards (isotope dilution) would be the best analytical approach, but these compounds are not commercially available. Other possibilities to be investigated include the addition of standards for quantification, or, of course, a better clean-up method.

In conclusion, this study illustrates that the use of LC/MS/MS is a valuable confirmation technique for aflatoxins in food. However, more work is needed to improve the method so that it can be used for more food matrices.

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