ELSEVIER

Journal of Chromatography A, 708 (1995) 55–60

JOURNAL OF CHROMATOGRAPHY A

Determination of aflatoxins in airborne dust from feed factories by automated immunoaffinity column clean-up and liquid chromatography

Anders Kussak, Barbro Andersson*, Kurt Andersson

National Institute of Occupational Health, Analytical Chemistry Division, P.O. Box 7654, S-907 13 Umeå, Sweden

First received 2 December 1994; revised manuscript received 16 March 1995; accepted 16 March 1995

Abstract

A method for the determination of aflatoxins in airborne dust is described. The dust samples were extracted with acetone–water, cleaned up on immunoaffinity columns and analysed by HPLC, including a post-column derivatization reaction with bromine and fluorescence detection. The method was evaluated with filters spiked with 62–595 pg of aflatoxins B_1, B_2, G_1 and G_2, respectively. Average aflatoxin recoveries for B_1, B_2, G_1 and G_2 were 97, 101, 87 and 84%, respectively. The method was used for airborne dust collected at feed factories. The detection limit was 3.1 ng/g dust for aflatoxins B_1, G_1 and G_2 and 1.8 ng/g for aflatoxin B_2, for a 10-mg dust sample.

1. Introduction

Aflatoxins are toxic mycotoxins produced by several species of *Aspergillus* moulds. Four compounds produced by these moulds are aflatoxins B_1, B_2, G_1 and G_2 (Fig. 1). Of these, aflatoxin B_1 is the most carcinogenic and most commonly occurring variety. The aflatoxins have been found in various agricultural commodities–oilseeds, such as peanuts, cottonseed and copra, and grains, such as maize [1,2]. The effects of aflatoxin B_1 on several organ systems, including the respiratory system, have been reviewed [3], and it is concluded that inhalation of aflatoxin-containing dust may result in hepatic and extrahepatic tumours. In epidemiological studies, it has been observed that there is an elevated risk of liver cancer among employees at livestock feed processing companies in Denmark [4], and an increased risk of respiratory cancer among Dutch oil-press workers extracting oil from linseed and peanuts [5]. Aflatoxins have been

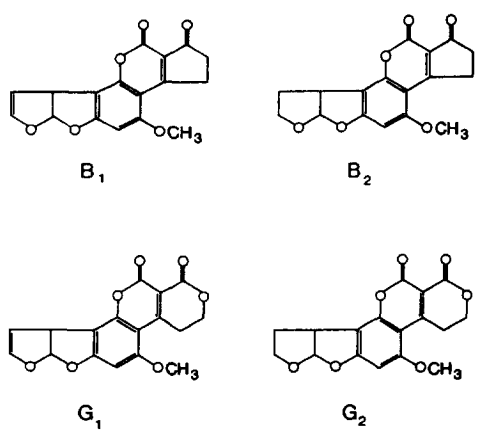

Fig. 1. Chemical structures of aflatoxins B_1, B_2, G_1 and G_2.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00365-7

shown to occur both in the mycelium and in the spores [6], and can thus be a health hazard to agricultural workers, for example, who may come into contact with contaminated products. Identified airborne mycotoxins, their effects on health, and their sources have been reviewed [7].

Methods for the determination of aflatoxins B_1, B_2, G_1 and G_2 in airborne dust have been developed by applying methods used by the Association of Official Analytical Chemists (AOAC), modified for samples of 1–10 g [8]. The TLC method employed in this work, was applied to the determination of aflatoxins in dust from various commodities, for example, maize during harvesting [9] and peanuts [10]. More recently, a method using supercritical fluid extraction has been developed for aflatoxin B_1 in 0.1–1.2 g of settled dust [11]. For the clean-up of aflatoxins in agricultural products, immunoaffinity columns have been widely used in recent years [12–16]. Various extraction methods have been employed in combination with the columns, such as methanol–water [12–14], acetonitrile–water [15] or acetone–water [16] extraction.

We previously developed methods for the determination of aflatoxins B_1, B_2, G_1 and G_2 [17] and aflatoxin Q_1 in urine [18]. This paper describes a method for the determination of aflatoxins in airborne dust with acetone–water extraction, automated immunoaffinity column clean-up and liquid chromatography with on-line post-column derivatization. The method was used for airborne and settled dust from various agricultural commodities, collected from workplace atmospheres.

2. Experimental

2.1. Chemicals and reagents

Aflatoxins B_1, B_2, G_1 and G_2 were purchased from Sigma (St. Louis, MO, USA) in crystalline form. The chemicals used were HPLC-grade acetonitrile (Rathburn, Walkerburn, UK), potassium bromide p.a. from Riedel-de Haen (Hannover, Germany), methanol, acetone, chlorotrimethylsilane, hydrochloric acid and nitric acid all p.a. from Merck (Darmstadt, Germany) and analytical-grade toluene from May and Baker (Dagenham, UK). The water used was purified with a Milli-Q system (Millipore, Milford, MA, USA). Nitrogen and helium plus grade were obtained from AGA (Sundbyberg, Sweden). For sample clean-up, 25-mm Millex-hv filters from Millipore and Aflascan immunoaffinity columns from Rhône-Poulenc (Glasgow, UK), batch DM 186/1, were used for single use. For sampling, 37-mm Whatman GF/A glass microfibre filters (Maidstone, UK), placed on AP 10 filter supports (Millipore) in Millipore cassettes, were used.

2.2. Preparation of standards

Aflatoxin solutions were prepared in a glove box. One milligram of each aflatoxin in a septum cap vial was diluted with 2 ml of acetonitrile by means of an injection syringe in the vial in which it was delivered. The standards for spiking filters were prepared in acetone–water (85:15, v/v), in concentrations of 0.62, 3.05 and 5.95 pg/μl of each aflatoxin. Solutions for the calibration curve were prepared in acetonitrile–water (33:67, v/v) in the range 0.089–3.64 pg/μl.

2.3. Extraction

The filters with the sampled dust were folded by means of pincers and placed in centrifuge tubes. Filters intended for recovery studies were placed in centrifuge tubes and then spiked with the different spiking standards. The filters were then extracted in 4 ml of acetone–water (85:15, v/v) at 1200 rpm, using an IKA-Vortex-VXR (Janke and Kunkel, Staufen, Germany) for 10 min, and then centrifuged (Savant, Formingdale, NY, USA) at 300 *g* for 2 min. The extract was then transferred into a test-tube with a Pasteur pipette. The extraction procedure was repeated twice and the combined extracts were reduced in volume to about 1.5 ml, using a nitrogen flow of 120 ml/min at room temperature. Settled dust was treated with the same procedure as the sampled airborne dust. Pasteur pipettes, centrifuge tubes and test-tubes were all silanized

with chlorotrimethylsilane by the following method [17]: washed in 2 *M* hydrochloric acid, followed by water and acetone, air-dried and silanized with 2% chlorotrimethylsilane in toluene for 5 min, and finally washed in methanol and dried at 70°C for 20 min. The tubes were stored for up to three weeks in a desiccator with blue gel prior to use.

2.4. Automated filter and immunoaffinity column clean-up

The 1.5-ml extract was then diluted to 10 ml with water and the tube moved to a Millilab 1A work station (Waters, Milford, MA, USA), where it was first filtered automatically and then cleaned up on immunoaffinity columns. The diluted extract was first filtered on 0.45-μm Millipore filters at a flow-rate of 2 ml/min. The immunoaffinity column was then conditioned with 10 ml of water at 20 ml/min. The 10-ml sample was loaded onto the column at 2 ml/min and washed with 10 ml of water using the same flow-rate. The column was dried by nitrogen at a flow of about 1.2 l/min for 1 min. The aflatoxins were then eluted with 2 ml of acetonitrile at 2 ml/min into a silanized test-tube, with pauses of 30 s after 1 ml and 1.5 ml. Each sample was processed completely before the Millilab started with the next sample. The solvent in the tubes containing the eluted aflatoxins was then evaporated to dryness under a flow of nitrogen at 120 ml/min and then redissolved in 200 μl of acetonitrile–water (33:67, v/v) with the use of a Vortex-mixer (Bohemia, NY, USA) and transferred to 200-μl plastic microvials (Waters) for HPLC analysis.

2.5. HPLC analysis and post-column derivatization

The HPLC system consisted of a Waters Model 6000A pump, a Waters WISP 712 autoinjector and an RF-530 fluorescence detector (Shimadzu, Kyoto, Japan) with the excitation wavelength set at 365 nm and emission wavelength at 440 nm. The column was a 4 μm, 100 × 8 mm I.D. Nova-pak Phenyl column (Waters) and the mobile phase used was acetonitrile–water (33:67, v/v). The water contained 1 m*M* potassium bromide and 1 m*M* nitric acid. The flow-rate was 1.5 ml/min, and the injection volume 150 μl. The mobile phase was continuously degassed by helium. The post-column derivatization of aflatoxins B_1 and G_1, was performed with bromine at room temperature (21°C), using a Kobra cell from Lamers and Pleuger (Den Bosch, Netherlands) at 20 μA. The post-column reactor consisted of PTFE tubing, 500 × 0.55 mm I.D. The length of the reaction tube, the current for bromine generation and the amount of potassium bromide in mobile phase were those that gave optimum fluorescence in a previously studied factorial design [17]. The chromatogram was recorded with a Spectra Physics integrator (San Jose, CA, USA).

2.6. Determination of recoveries

The overall method was validated by spiking blank filters with 100 μl of the different spiking standards containing a mixture of 62, 305 or 595 pg of each aflatoxin. The spiked filters were extracted and cleaned up together with unspiked filters according to the method described above.

2.7. Sampling

Airborne dust samples were collected at two feed factories during unloading of copra, cottonseed, soya beans, maize gluten and sugar beets, used for feed production. The dust samples were collected on glass microfibre filters using GAST pumps (Benton Harbor, MI, USA) at flow rates of 12–32 l/min, checked with a rotameter (Rota, Wehr, Germany). The filters were placed in open-faced Millipore cassettes about 1.5 m from the ground and about 2–3 m from the dust source. The collected volumes were in the range 0.15–1.3 m^3. Before and after sampling, the filters were placed in a thermostated room overnight, and weighed for the gravimeteric determination of the sampled airborne dust. Settled dust

Table 1
Recoveries of aflatoxins B_1, B_2, G_1 and G_2 from spiked glass microfibre filter

Aflatoxin	Sample added (pg/filter)	Recovery (%)	R.S.D. (%)
B_1	62	108	10
	305	97	9
	595	86	4
B_2	62	113	6
	305	99	6
	595	91	1
G_1	62	89	13
	305	89	27
	595	83	2
G_2	62	84	16
	305	87	22
	595	81	5

$n = 5$.

was collected on aluminium sheets on the floor during the air sampling. Filters and settled dust were stored in a refrigerator at 8°C prior to analysis.

3. Results and discussion

The recoveries from spiked filters that passed all the analysis steps are presented in Table 1. The average recoveries for aflatoxins B_1, B_2, G_1 and G_2 were 97%, 101%, 87% and 84%, respectively, with relative standard deviations in the range of 4–10% for B_1, 1–6% for B_2, 2–27% for G_1 and 5–22% for G_2. The recovery for aflatoxins B_1 and B_2 are higher at lower levels, but the R.S.D. values are at the same time larger. The relative standard deviations agree with the results obtained with other methods using immunoaffinity columns [12–15]. The recoveries were studied in the range 62–595 pg added per sample and compared with standards directly injected onto the liquid chromatographic system. No carry-over effect of aflatoxins was observed on blank filters with the use of the Millilab work station for filtering and immunoaffinity column clean-up.

Acetone–water was selected as extraction solvent because it has been used to extract aflatoxins, with recoveries exceeding 90%, from cattle feed with immunoaffinity column clean-up [16]. However, it was shown that less than 2%

Table 2
Determination of aflatoxins in airborne dust from two feed factories

Dust source	Dust weight (mg)	Sampling time (min)	Volume air (l)	Dust concentration (mg/m^3)	Aflatoxin in airborne dust (ng/g)				Total aflatoxin in air (ng/m^3)
					B_1	B_2	G_1	G_2	
Copra	10.9	18	576	18.9	48.8	2.2	6.1	nd	1.1
Copra	10.1	41	1271	7.9	53.0	2.9	3.1	nd	0.5
Cottonseed	17.6	11	154	114.3	8.3	1.4	nd	nd	1.1
Cottonseed	17.5	11	236	74.1	8.6	1.5	nd	nd	0.8
Maize gluten	18.6	29	377	49.4	4.1	nd	nd	6.4	0.5
Maize gluten	65.8	16	188	350.0	nd	nd	nd	nd	nd
Maize gluten	22.4	29	558	40.1	nd	nd	nd	1.8	0.1
Soya bean	99.2	36	729	136.0	nd	nd	nd	nd	nd
Soya bean	96.4	36	675	142.9	nd	nd	nd	nd	nd
Sugarbeet	12.0	36	459	26.2	nd	nd	nd	nd	nd
Sugarbeet	37.8	32	624	60.6	nd	nd	nd	nd	nd

nd = Not detected.

acetone is needed in the sample when the immunoaffinity columns are used. The combined extract was therefore evaporated from about 11 ml to 1.5 ml and then diluted to 10 ml with water before it was cleaned up on the column. The total analysis time was about 24 h for six samples, when extraction was performed on the first day, filtering and immunoaffinity processed unattended by the Millilab work station overnight, and LC analysis performed the morning after.

The method was used for dust suspected of containing aflatoxins, sampled at feed production. The samples of airborne dust were taken during one of the most dusty operations in the factories, i.e. the unloading of products from the various means of transports. The copra, cottonseed and soya beans used in the feed factories are in the form of press cakes remaining after the various products have been crushed for oil. The sugar beet used has been leached for its sugar content before being pressed into pellets, and the maize gluten is the residue obtained after starch fabrication. All products were unloaded into tip cavities, except soya bean pellets that were unloaded into a storeroom. Maize gluten and soya beans were unloaded from goods wagons, cottonseed from a tractor, copra and sugar-beet pellets from trucks and soya bean pellets from a conveyor belt.

The results of the analysis of filters sampled from the various dust sources are presented in Table 2. The detection limit (signal-to-noise ratio of 3) was 31 pg per dust sample of aflatoxins B_1, G_1 and G_2 and 18 pg of aflatoxin B_2. Aflatoxins B_1, B_2 and G_2 could be detected in copra, aflatoxins B_1 and B_2 in cottonseed, and aflatoxins B_1 and G_2 in maize gluten. No aflatoxins were detected in soya beans or sugar beets. These results agree with the report that copra, cottonseed and maize (corn) are often contaminated with aflatoxins, and that soya beans are less often contaminated [1]. Chromatograms of samples of various agricultural products are presented in Fig. 2. The method was also used with settled copra dust sampled from the floor. The results are presented in Table 3. The levels found in the settled dust are about the same as those found in the airborne dust.

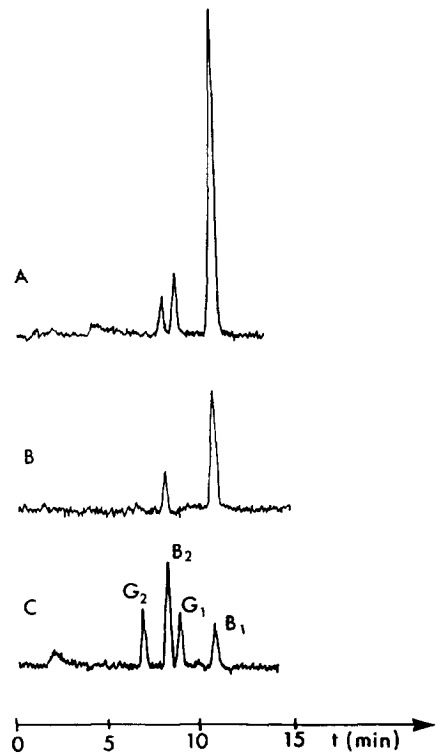

Fig. 2. Chromatograms (fluorescence detection) of dust samples from (A) copra naturally contaminated with aflatoxins B_1, B_2 and G_1, (B) cottonseed naturally contaminated with aflatoxins B_1 and B_2 and (C) filter spiked with 62 pg of each aflatoxin.

4. Conclusions

Acetone–water extraction, immunoaffinity column clean-up and liquid chromatography with post-column derivatization are described for the

Table 3
Concentration of aflatoxins in settled dust

Dust source	Dust weight (mg)	Aflatoxin in settled dust (ng/g)			
		B_1	B_2	G_1	G_2
Copra	13.6	31.4	1.5	7.8	nd
Copra	9.8	26.9	2.3	6.4	nd

nd = Not detected.

determination of aflatoxins in airborne dust. The method was studied by means of filters spiked with standards and used for airborne dust samples collected from commodities suspected of containing aflatoxins. For a 10-mg dust sample, the detection limit was 3.1 ng/g dust for aflatoxins B_1, G_1 and G_2, and 1.8 ng/g for aflatoxin B_2. Aflatoxins were detected in dust from copra, cottonseed and maize gluten by the described method. The highest level of aflatoxin B_1, was 53.0 ng/g, was found in copra dust.

Acknowledgement

We would like to thank Gunnel Bergman of the National Institute of Occupational Health in Solna, Stockholm, for the gravimetric determinations of the filters.

References

[1] L. Stoloff, in J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman (Editors), Mycotoxins in Human and Animal Health, Pathotox, Park Forest South, IL, 1977, p. 7.
[2] I. Dvorackova, Aflatoxins and Human Health, CRC Press, Boca Raton, FL, 1990.
[3] R.A. Coulombe, Jr., in D.L. Eaton and J.D. Groopman (Editors), The Toxicology of Aflatoxins, Academic Press, San Diego, CA, 1994, p. 89.
[4] J.H. Olsen, L. Dragsted and H. Autrup, Br. J. Cancer, 58 (1988) 392.
[5] R.B. Hayes, J.P. van Nieuwenhuize, J.W. Raatgever and F.J.W. ten Kate, Food. Chem. Toxicol., 22 (1984) 39.
[6] D.T. Wicklow and O.L. Shotwell, Can. J. Microbiol., 29 (1983) 1.
[7] K.M. Hendry and E.C. Cole, J. Toxicol. Environ. Health, 38 (1993) 183.
[8] O.L. Shotwell, W.R. Burg and T. Diller, J. Assoc. Off. Anal. Chem., 64 (1981) 1060.
[9] W.R. Burg and O.L. Shotwell, J. Assoc. Off. Anal. Chem., 67 (1984) 309.
[10] W.G. Sorenson, W. Jones, J. Simpson and J.I. Davidson, J. Toxicol. Environ. Health, 14 (1984) 525.
[11] M.I. Selim and M.-H. Tsuei, Am. Ind. Hyg. Assoc. J., 54 (1993) 135.
[12] M.W. Trucksess, M.E. Stack, S. Nesheim, S.W. Page, R.H. Albert, T.J. Hansen and K.F. Donahue, J. Assoc. Off. Anal. Chem., 74 (1991) 81.
[13] A.-L. Barmark and K. Larsson, J. Assoc. Off. Anal. Chem., 77 (1994) 46.
[14] G. Niedwetzki, G. Lach and K. Geschwill, J. Chromatogr. A, 661 (1994) 175.
[15] A.L. Patey, M. Sharman and J. Gilbert, J. Assoc. Off. Anal. Chem., 74 (1991) 76.
[16] G. Werner, Agribiol. Res., 44 (1991) 289.
[17] A. Kussak, B. Andersson and K. Andersson, J. Chromatogr., 616 (1993) 235.
[18] A. Kussak, B. Andersson and K. Andersson, J. Chromatogr. B, 656 (1994) 329.