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Short Communication

Automated aflatoxin analysis of foods and animal feeds using immunoaffinity column clean-up and high-performance liquid chromatographic determination

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ABSTRACT

A commercially available system is described for the fully automated clean-up and high-performance liquid chromatographic (HPLC) analysis of aflatoxins in foods and animal feeds. The system marketed primarily for handling solid-phase extraction columns has modified software to facilitate use with immunoaffinity columns. Sample extract clean-up followed by injection onto an HPLC column with post-column iodination and fluorescence detection is carried out completely unattended. A coefficient of variation of 5.1% for aflatoxin B_1 analysis was obtained, and the accuracy of the system was demonstrated by the analysis of peanut butter certified reference material.

INTRODUCTION

Contamination of foods and animal feeds with aflatoxins B_1, B_2, G_1 and G_2 is controlled by tolerance limits in at least 50 countries worldwide [1]. Monitoring for these mycotoxins is carried out not only by enforcement authorities but also for purposes of commercial trade and for quality control of foods and animal feed constituents. There is thus a considerable requirement for large numbers of aflatoxin determinations, this demand being further exacerbated by difficulties of sampling which means that it is often necessary to analyse large numbers of replicate samples from the same batch or consignment of material. Although rapid immunologically-based screening tests and enzyme-linked immunosorbent assay (ELISA) methods are available for determining aflatoxins, they do not normally allow for simultaneous monitoring of both individual and total aflatoxin levels [2,3]. There is therefore some advantage in adopting the approach of instrumental analysis by high-performance liquid chromatography (HPLC) where aflatoxins are separated and then individually quantified. This approach becomes even more attractive if the procedure can be automated.

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Immunoaffinity columns comprise an anti-aflatoxin antibody bound to a gel material contained in a small plastic cartridge. Crude extract is forced through the column and the aflatoxins are left bound to the recognition site of the immunoglobulin. Extraneous material can be washed off the column with water and the aflatoxins can be recovered in purified form by liberating the bound analyte from the antibody with an elution solvent such as methanol or acetonitrile. Immunoaffinity columns are commercially available and have been routinely employed for determining aflatoxins B_1, B_2, G_1 and G_2 in nuts, nut products and dried fruit [4,5], as well as for determining aflatoxin M_1 in milk [6] and cheese [7]. These columns have the advantages of speed and simplicity compared to conventional clean-up and of high specificity thereby producing extracts free of interferences. A disadvantage can be the need to push the sample extract through the column at a slow but steady rate, which is tedious when carried out manually and can be a source of variable recoveries when not properly controlled [4].

The simplicity of analysis using immunoaffinity columns makes this approach particularly amenable to automation, which in turn overcomes any problems associated with sample loading onto the column. In this paper we describe modification to the design of the plastic immunoaffinity cartridges to fit the rack of a commercially available automated sample preparation system (ASPEC). Changes to the software of the system have also been made to permit the operation of conditioning, loading, washing and eluting from the columns. The system has been fully intregrated with the HPLC analysis, using post-column iodination [8] with fluorescence detection to determine aflatoxins. The ruggedness of the system has been proved over several months in routine unattended operation in surveillance work monitoring aflatoxins in imported dried figs and pistachio nuts.

EXPERIMENTAL

Materials

Total aflatoxin immunoaffinity columns ASPEC type TD110 were obtained from Biocode (York, U.K.). Acetonitrile, methanol and chloroform were purchased from Rathburn (Walkerburn, U.K.). All water was deionised and distilled. Aflatoxins B_1, B_2, G_1 and G_2 standards were purchased individually from Sigma (Poole, U.K.) and buffer salts were from BDH (Poole, U.K.). Peanut butter certified reference materials (CRMs) [9] containing aflatoxins B_1, B_2, G_1 and G_2 (CRMs 385 and 401) were purchased from the Community Bureau of Reference (BCR) of the Commission of the European Communities (Brussels, Belgium).

Sample preparation

Nuts and nut products (peanut butter). A finely ground sample of the product (30 g) was weighed accurately into a 400 ml beaker to which was added acetonitrile–water (6:4, v/v; 90 ml). The mixture was homogenised with an IKA Ultra Turrax blender at high speed for 3–5 min. Water (135 ml) was added and the sample was homogenised for a further 2 min to produce a slurry which was subsequently filtered through Whatman 113V filter paper into a conical flask (250 ml). A portion of the filtrate (15 ml) was diluted with phosphate-buffered saline (PBS) (135 ml, pH 7.4). An aliquot (*ca.* 52 ml) of this solution was transferred into a plastic Falcon tube No. 2070 (Philip Harris, London, U.K.) and placed in the rack (code 24) of the ASPEC system.

Dried fruit (figs). Samples were prepared by passing the dried fruit through a meat mincer (Butcher Boy TM22) and then slurrying with water (5:3, w/w) in a pilot-plant electric food mixer (Crypto Peerless EB60) for 0.5 h. A sample of the slurry (80 g) was weighed into a 400-ml beaker to which was added acetonitrile–water (68.75:31.25, v/v; 120 ml). The mixture was homogenised with an IKA Ultra Turrax blender at high speed for 3–5 min to produce a slurry which was subsequently filtered through Whatman 113V filter paper into a conical flask (250 ml). A portion from the top layer of the filtrate (5 ml) was diluted with PBS (120 ml, pH 7.4). An aliquot (*ca.* 52 ml) of this solution was transferred into a plastic Falcon tube and placed in the rack (code 24) of the ASPEC system.

Animal feedingstuffs. A sample of the feed (30 g) was weighed accurately into a 250-ml beaker to which was added chloroform (150 ml) and water (5 ml). The mixture was homogenised with an IKA Ultra Turrax blender at high speed for 3–5 min to produce a slurry which was subsequently filtered through Whatman 113V filter paper into a conical flask (250 ml). A portion of this filtrate was transferred to a round bottomed flask (500 ml) and the chloroform was removed under vacuum at 30°C. The residue was redissolved by the addition of methanol (5 ml) and PBS (145 ml) followed by gentle swirling. After the addition of hexane (*ca.* 50 ml) and further gentle swirling the mixture was transferred to a separating funnel and shaken gently for 10 s. The lower layer was collected and an aliquot (*ca.* 52 ml) of this solution was transferred into a plastic Falcon tube and placed in the rack (code 24) of the ASPEC system.

Automatic clean-up and chromatography

The automated HPLC system (from Anachem, Luton, U.K.) consisted of a Gilson 307 isocratic pump, a Gilson ASPEC (an automatic solid phase extraction system fitted with a Rheodyne 7010 injector and a Gilson 401 dilutor), an LKB 2150 isocratic pump (Pharmacia, Milton Keynes, U.K.) a Colora water bath thermostatted at 80°C, and a Perkin-Elmer (Beaconsfield, U.K.) LC240 fluorescence detector set at 364 nm excitation and 434 nm emission wavelengths. The detector, the Gilson pump and the ASPEC were linked via a Gilson 506B system interface module to an IBM Model 30 PC. The use of Gilson 712 HPLC software allowed both collection of data and the control of the interfaced equipment.

A Spherisorb ODS1 analytical column (5 μm particle size, 250 × 4.6 mm I.D.) was employed and this was protected by an inline filter (A315, Upchurch) fitted with a 2-μm frit (A101, Upchurch) and a C_{18} guard column (C752, Upchurch). The mobile phase of water–acetonitrile–methanol (58:30:12) was pumped at 0.86 ml/min. Post-column iodination as described elsewhere [8], employed a 5 m × 0.3 mm I.D. reaction coil maintained at 80°C. Eluent from the HPLC column was mixed in the reaction coil with a saturated solution of iodine in water pumped at 0.7 ml/min through a Valco T-piece (Chrompack, No. 22458).

RESULTS AND DISCUSSION

The Gilson ASPEC system was primarily designed and marketed for the use of solid-phase extraction columns for the automated clean-up of samples. The system as supplied comprised the following units: (1) A rack containing 250-ml plastic bottles of water, PBS and acetonitrile. (2) Two racks (code 24) for holding up to 24 Falcon tubes

(50 ml maximum volume containing diluted extracts) and up to four standards in 20-ml vials. (3) A rack (code 30) for holding 2-ml amber crimp cap vials (2CVA, Chromacol) which are used to retain a portion of the sample extract for future reference purposes if required. (4) A DEC rack designed to hold 500 mg/3 ml Bond Elut solid-phase extraction columns (Analytichem).

The operation of the ASPEC system is shown schematically in Fig. 1, and consists of initial washing of the immunoaffinity columns with 9.0 ml of PBS at 6.0 ml/min. The sample extract (50 ml) is then loaded from rack code 24 onto the immunoaffinity column at 6.0 ml/min. The column is washed with water (2.0 ml) at 6 ml/min and air dried (2.0 ml) prior to the elution of the aflatoxins into a plastic collection tube. The elution step is carried out by application of 0.5 ml of acetonitrile onto the column at 1.5 ml/min followed by a wait period of 12 s and a further addition and elution with 1.0 ml of acetonitrile. Air (1.0 ml) is used to recover any solvent remaining on the column and the eluted sample is mixed by bubbling air (3.0 ml) through the solution in the collection vial. A portion of this solution (0.5 ml) is transferred to a sealed amber vial in rack code 30 for retention. The remaining acetonitrile solution (1.0 ml) is diluted with water (2.0 ml) and mixed with air bubbling (3.0 ml). Sample extract (400 μl) is injected using partial loop fill into a 800-μl loop.

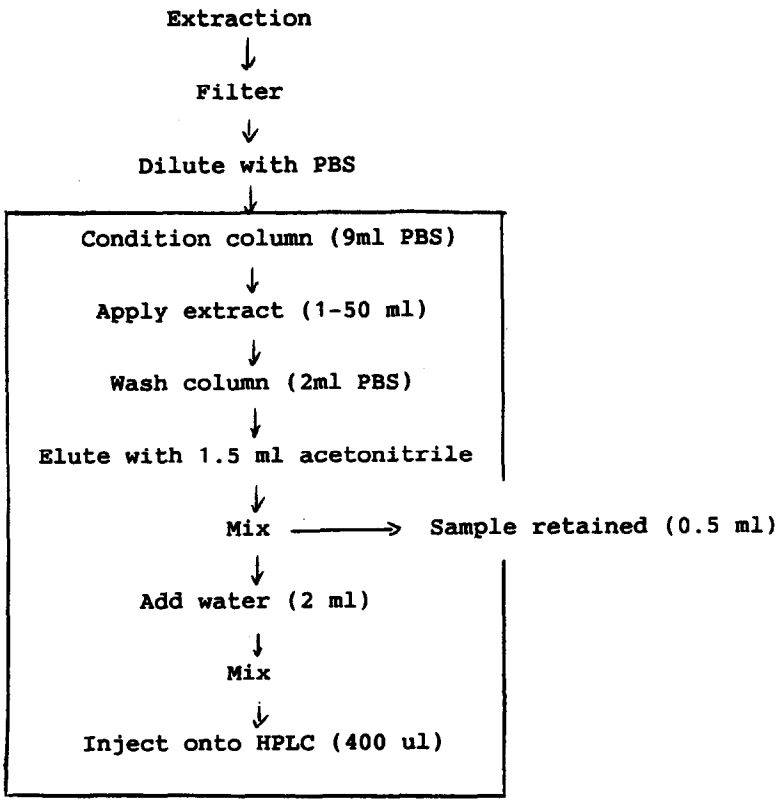

Fig. 1. Schematic illustrating the automated analysis of aflatoxins. Operations within the box are fully automated.

The running sequence for the ASPEC includes the injection of four standards to construct a calibration curve. During the chromatography of the last standard the first sample is prepared and preparation of all subsequent samples is then carried out whilst the previous sample is being separated on the HPLC system. This allows a sample to be injected and quantified every 22 min.

Quality assurance checks in relation to instrumental stability are programmed into the system by running a standard after every fourth sample extract has been prepared and injected. The ASPEC system also has considerable flexibility and will respond to highly contaminated extracts which are found to be above the working range of the detector/integrator by reinjection of 100 μl of the prepared solution directly from the plastic collection tubes in the DEC rack.

The performance of the system was evaluated by replicate analyses ($n = 20$) of a sample of well homogenised naturally contaminated dried fig slurry where the coefficient of variation (C.V.) for aflatoxin B_1 was = 5.1% (8.5 μg/kg), for aflatoxin B_2 = 6.2% (1.6 μg/kg), for aflatoxin G_1 = 10.3% (4.7 μg/kg) and for aflatoxin G_2 = 15.0% (1.5 μg/kg). The accuracy of the system was tested by examining peanut butter reference materials where it can be seen from Table I that the results were found to be in good agreement with the certified values for the aflatoxins.

The system has proved to be rugged and reliable in routine survey work where in a period of 6 months some 2000 samples were analysed (4000 determinations including standards and replicates). There were no instrumentation failures in that period or software difficulties, the only problem encountered being the failure of needle seals which did not lead to loss of samples. At present with a run time of 22 min and a sample carousel capacity for 20 sample tubes, in unattended operation the system runs for about 11 h. For the future a possible improvement would be a larger sample carousel to extend the running time during unattended operation.

TABLE I

RESULTS OF AUTOMATED AFFINITY COLUMN HPLC ANALYSIS OF AFLATOXINS IN CERTIFIED REFERENCE MATERIALS

Certification data (μg/kg) from ref. 9.

	CRM 385				CRM 401			
	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Mean	7.1	1.4	1.9	0.4	<0.2	<0.1	<0.1	<0.1
Replicates (n)	6	6	6	6	6	6	6	6
S.D.	0.7	0.16	0.26	0.08	–	–	–	–
95% CL[a]	1.4	0.32	0.51	0.16	–	–	–	–
Certified level	7.0	1.1	1.7	0.3	<0.2	<0.2	<0.3	<0.2
S.D.	0.42	0.09	0.16	0.09	–	–	–	–
95% CL	0.80	0.20	0.30	0.20	–	–	–	–

[a] CL = Confidence limit.

CONCLUSIONS

An automated system for aflatoxin analysis using immunoaffinity columns for sample clean-up, has shown excellent accuracy and precision in routine operation. Although the system has been used primarily for the analysis of foods contaminated with aflatoxins, the ASPEC system is versatile and one attraction of this approach to automation is the ease with which the system can be readily tailored to other immunoaffinity column-based analyses. The conditioning of the immunoaffinity columns can be set from 1 to 20 ml, sample load volume from 1 to 250 ml, the transfer of reference sample from 0 to 2000 μl and the elution of the aflatoxin by acetonitrile from 100 to 3000 μl. The speed of aspiration/dispensing can be varied in the range 0.18 ml/min to 96 ml/min and the wait time for the elution of aflatoxins in steps of 0.01 min. Finally the injection volume can be varied from 50 μl to 800 μl. This flexibility means that the system can be readily adapted to the analysis of other mycotoxins such as zearalenone and ochratoxin A where immunoaffinity columns are already available, or can be used in other areas of contaminant analysis such as veterinary drug residues where there is a demand for automated analysis and this approach is becoming accepted.

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REFERENCES

1 H. P. van Egmond, *Food Add. Contam.*, 6 (1989) 139.
2 D. E. Koeltzow and S. N. Tanner, *J. Assoc. Off. Anal. Chem.*, 73 (1990) 584.
3 M. J. Shepherd, D. N. Mortimer and J. Gilbert, *J. Assoc. Publ. Anal.*, 25 (1987) 129.
4 A. L. Patey, M. Sharman and J. Gilbert, *Food Add. Contam.*, 7 (1990) 515.
5 M. Sharman, A. L. Patey, D. A. Bloomfield and J. Gilbert, *Food Add. Contam.*, 8 (1991) in press.
6 D. N. Mortimer, J. Gilbert and M. J. Shepherd, *J. Chromatogr.*, 407 (1987) 393.
7 M. Sharman, A. L. Patey and J. Gilbert, *J. Chromatogr.*, 474 (1989) 457.
8 M. J. Shepherd and J. Gilbert, *Food Add. Contam.*, 1 (1984) 325.
9 J. Gilbert, M. Sharman, G. M. Wood, A. Boenke and P. J. Wagstaffe, *Food Add. Contam.*, 8 (1991) in press.