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Determination of aflatoxins in food by overpressured-layer chromatography

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Abstract

Legislative measures for monitoring and control of aflatoxin levels in foods and foodstuffs have been introduced in many countries. The aim of the present work was to make developments in the field of aflatoxin analysis, focusing upon the use of overpressured-layer chromatography (OPLC) for quantitative determination. OPLC methods have been developed for the determination of aflatoxins B_1, B_2, G_1, G_2 in different foods. These methods are suitable for sample clean-up and separation as well. Using OPLC we could analyze 10 samples simultaneously. The methods were investigated with fish, corn and wheat samples spiked with 2–10 ng/g aflatoxins. Quantitative evaluation of aflatoxins was accomplished by densitometry. Average recoveries from each food were greater than 73%. The OPLC technique seems to be a rapid, reproducible and cost-effective analysis for quantitative determination of aflatoxins in foods.

Keywords: Wheat; Food analysis; Overpressured layer chromatography; Fish; Aflatoxins

1. Introduction

Aflatoxins are toxic compounds produced by several species of *Aspergillus* moulds. These fungi can grow under diverse conditions but the rate of growth and aflatoxin production depends upon the prevailing physical, biological, biochemical and environmental conditions [1]. Several aflatoxins have been isolated and identified [2]; four of these – B_1, B_2, G_1, G_2 (Fig. 1) – are naturally prevalent. Aflatoxin B_1 is the most acutely toxic and carcinogenic aflatoxin.

Monitoring foods for the presence of aflatoxins is important to ensure consumer safety. During the past two decades several chromatographic methods have been developed for the quantification of aflatoxins in agricultural and food products [3].

Traditionally aflatoxin analysis has been performed using solvent extraction, followed by sample clean-up by solid-phase extraction (SPE) for purification prior to analysis [4]. The immunoaffinity technique enables a wide variety of food matrices to be analyzed using a one-step extraction protocol without the need to use halogenated hydrocarbon solvents for extraction [5].

Aflatoxins are still assayed using thin-layer chromatography (TLC) [6]. Improvements in TLC techniques have led to the development of high-performance thin-layer chromatography (HPTLC). The analysis of aflatoxins by HPTLC method in conjunction with the SPE clean-up was performed by Nawaz et al. [7,8]. However, due to its higher separation power and shorter analysis time, high-performance liquid chromatography (HPLC) using fluorescence

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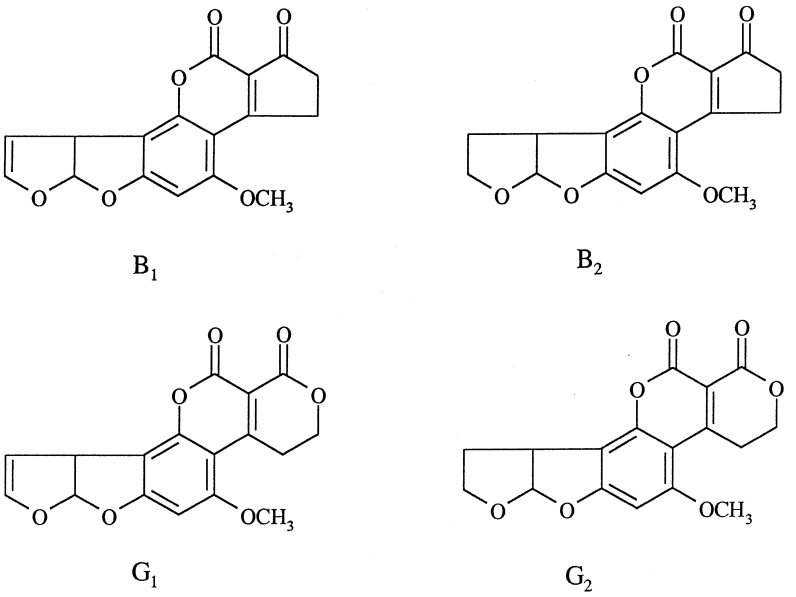

Fig. 1. Chemical structures of aflatoxins B_1, B_2, G_1 and G_2.

detection has already become the most accepted method for the determination of aflatoxins. Both normal- and reversed-phase HPLC can be utilized [3,9–12]. Reversed-phase methods using both UV and fluorescence detection have been developed. Sensitivity can also be improved by formation of derivatives [13–18].

Overpressured-layer chromatography (OPLC) developed by Tyihák and co-workers [19,20] unites the advantages of the HPLC and HPTLC methods. Aflatoxin analysis based on OPLC separation and densitometric quantitative evaluation was carried out by Gulyás [21].

In our laboratory an OPLC method was developed for the analysis of aflatoxin content in foods [22]. The aim of our work was to determine the aflatoxin content of different agricultural and food samples by OPLC. The investigated products can occur in different feedstuffs. The aflatoxin content of the feedstuff can accumulate in animals. We investigated the aflatoxin content of fish nourished on feedstuff and the aflatoxin content of those agricultural products (corn and wheat) which can be components of the feedstuff.

2. Experimental

2.1. Materials

Aflatoxins B_1, B_2, G_1 and G_2 were purchased from Serva (Heidelberg, Germany) in crystalline form. The chemicals used were HPLC-grade; acetonitrile, methanol, dichloromethane, acetone, diethyl ether, toluene, chloroform, ethyl acetate, tetrahydrofuran, hexane from Merck (Darmstadt, Germany). The water used was purified with a Milli-Q system (Millipore, Milford, MA, USA)

TLC, HPTLC silica gel 60 plates (Merck), 20×20 cm with impregnated edges (sealed by OPLC NIT, Budapest, Hungary) were used.

2.2. Preparation of standards

Aflatoxin solutions were prepared in a glove box. A 1 mg amount of aflatoxin in a septum cap vial was diluted with 4 ml of methanol by means of an injection syringe. The standards for spiking the samples were prepared from aliquots of these solutions diluting them with methanol.

2.3. Equipment

Chromatographic plates were developed by means of an OPLC system (Chrompress 25 and POPLC BS 50). The Personal OPLC BS-50 system (OPLC NIT) includes the hydraulic unit, the separation chamber and the liquid-delivery system. The cassette containing the analytical or preparative layer was inserted into the chamber. In automatic development pressurization, the development parameters can be stored by the software of the delivery system. External pressure (max. 5 MPa), eluent volume and flow-rate were stored in this manner, and development time is calculated automatically.

2.4. The steps of the analysis method

The complete analysis method consists of the following steps: (1) extraction, (2) filtration, (3) liquid–liquid extraction, (4) OPLC prewash, (5) OPLC separation and (6) densitometry.

2.4.1. Sample preparation

Ground samples (20 g) were extracted with a methanol–water (7:3) mixture (80 ml) in the case of fish and an acetonitrile–water (9:1) mixture (80 ml) in the case of corn and wheat for 15–30 min on a vibrating shaker. The solution was filtered, a portion (4 ml) was evaporated to dryness and the residue was dissolved in dichloromethane–acetone (9:1) (100 μl). In the case of fish sample the filtrate was extracted with dichloromethane (2×3 ml) before evaporation. The impurities were washed from the chromatographic layers by methanol–acetone (1:1). Sample extracts (50 μl) were applied with a syringe to the activated chromatographic plates (105°C, 10 min) as 5-mm bands (10 bands per plate).

2.4.2. Chromatography

2.4.2.1. Chromatography of fish and corn samples

Some of the impurities were removed by prewashing the plate with a mixture of diethyl ether–toluene (1:1, v/v). The separation of aflatoxins was performed using a mobile phase consisting of chloroform–ethyl acetate–tetrahydrofuran (10:15:1, v/v).

2.4.2.2. Chromatography of wheat samples

In the case of wheat samples we had to use another chromatographic method for the determination of aflatoxin content. Most of the impurities were removed by prewashing with a mixture of diethyl ether–hexane (1:1, v/v) but at this step the plate was developed in the reverse direction. After removing the undesirable coextractives by prewashing from the outlet side (sample application were applied at 173 mm from the outlet trough, 10 mm from the inlet channel) the layer was dried and stored for 20–30 min at room temperature. The separation of aflatoxins was performed using a mobile phase consisting of chloroform–toluene–tetrahydrofuran (15:15:1, v/v).

2.4.2.3. Quantitation of the aflatoxin content

Quantitative densitometric evaluation of aflatoxins was accomplished by means of an OPTON KM 3 densitometer (slit length 14 mm, slit width 0.71 mm, scanning speed 50 mm/min, excitation: 365 nm, emission: 436 nm).

3. Results and discussion

In our laboratories we investigated OPLC for the determination of the aflatoxin content in foods. We examined the aflatoxin content (B_1, B_2, G_1, G_2) of spiked fish, corn and wheat samples.

Using OPLC after the solid–liquid and liquid–liquid extraction steps we evaporated the solvent to dryness and applied aliquots of the samples dissolved in dichloromethane–acetone (9:1) to the chromatographic plate as bands. For different sample matrices different OPLC methods were developed for the separation of the aflatoxins and other materials which often interfere in the determination of target analytes. By using OPLC methods we could eliminate other expensive clean-up steps.

The OPLC measurement of fish and corn samples was performed using a two-step gradient. Firstly some of the impurities were removed by prewashing and this step was followed by the separation of aflatoxins.

Densitograms of aflatoxin standards, spiked fish

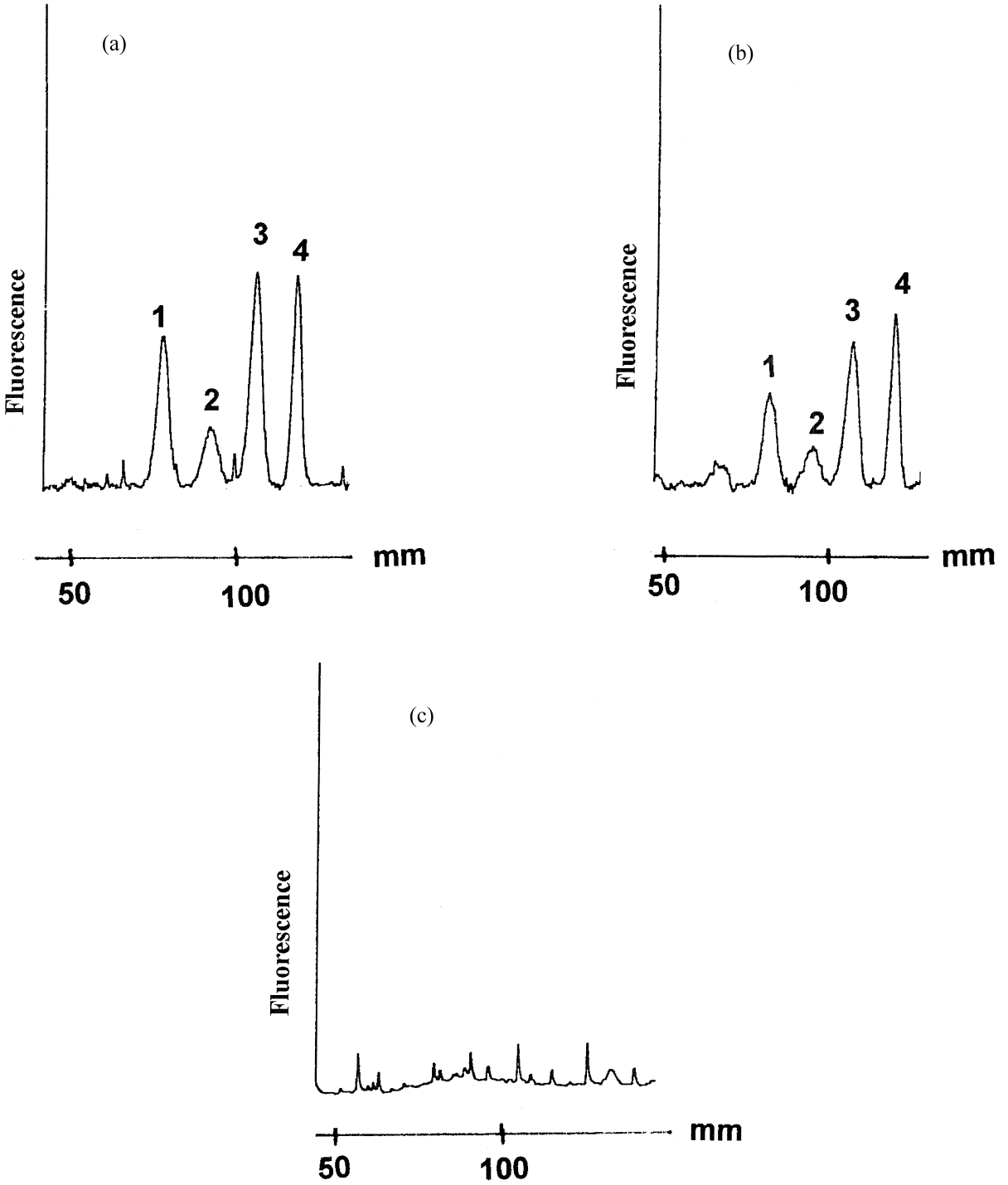

Fig. 2. Densitogram of (a) standard aflatoxins, (b) fish sample spiked with aflatoxins and (c) the blank fish sample recorded at the same attenuation: 1=G_2 1 ng, 2=G_1 1 ng, 3=B_2 2 ng, 4=B_1 2 ng.

sample and the blank fish sample are shown in Fig. 2.

This method was also used for the determination of the aflatoxin content of corn. The blank corn was free of aflatoxin.

In the case of wheat samples we had to modify the above-mentioned chromatographic method. From this sample we could eliminate the impurities by prewashing the plate in the reverse direction. After prewashing the undesirable coextractives, the layer was dried and the separation of the aflatoxins was carried out.

Densitograms of aflatoxin standards, spiked wheat sample and the blank wheat sample are shown in Fig. 3.

Using the above OPLC technique a level of 2 ng/g for each aflatoxin could be analysed. OPLC methods are suitable to determine the aflatoxin content within the allowed range set by international and Hungarian regulations.

Recoveries of aflatoxins from spiked samples are shown in Table 1. Known amounts of standard solutions were added to the investigated fish, corn and wheat samples. All samples were analyzed at least three times for aflatoxins.

Table 1
Recovery of aflatoxins from spiked samples[a]

Sample	Added aflatoxin (ng/g)	Average recoveries (%)			
		G_2	G_1	B_2	B_1
Fish	10	86.3	84.6	83.0	94.1
	4	86.5	92.7	77.4	84.9
	2	92.3	85.9	74.9	84.7
Corn	2	76.8	74.4	103.9	98.6
Wheat	5	73.0	92.5	82.0	94.0
	2	80.0	91.0	92.0	101.0

[a] The investigation of samples was performed at different levels of each aflatoxin: (number of replicates was ≥3).

The aflatoxin recoveries from each food were greater than 73%.

Repeatability of the methods was checked using fish and wheat samples. Seven portions of each food were spiked with aflatoxins, respectively. The results are shown in Table 2.

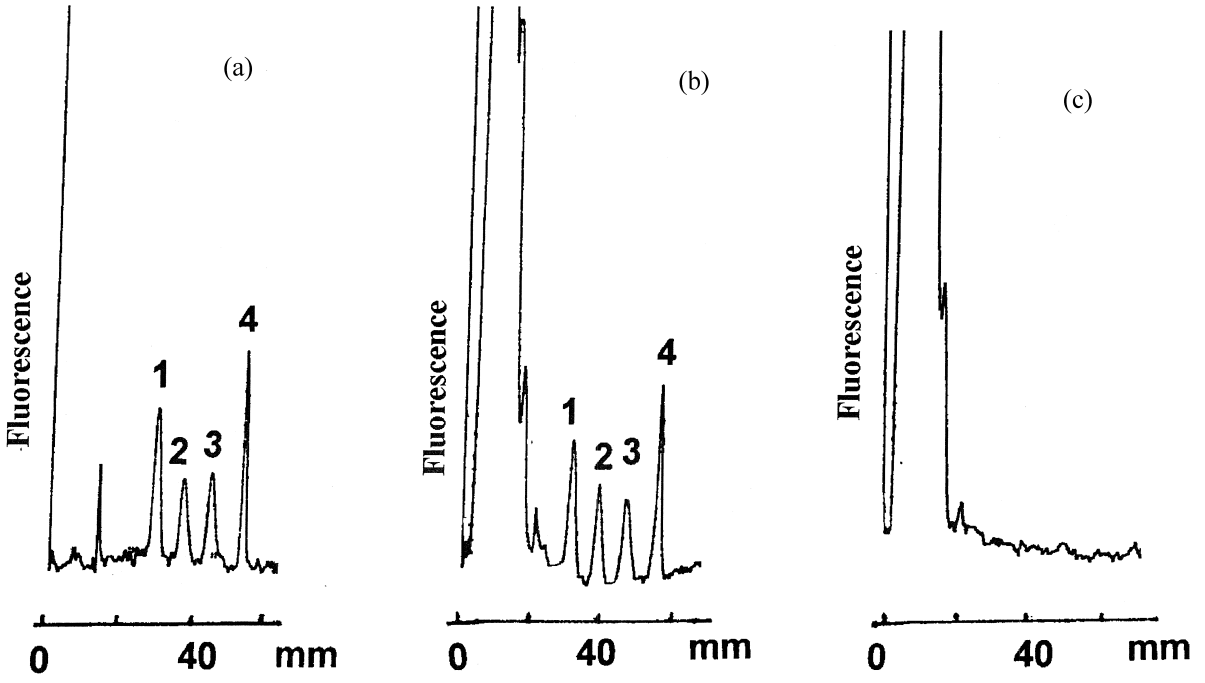

Fig. 3. Densitogram of (a) standard aflatoxins, (b) wheat sample spiked with aflatoxins and (c) the blank wheat sample recorded at the same attenuation: 1=G_2 1 ng, 2=G_1 1 ng, 3=B_2 1 ng, 4=B_1 1 ng.

Table 2
Repeatability test on fish and wheat samples ($n=7$)[a]

	Fish		Wheat	
	SD	RSD (%)	SD	RSD (%)
G_2	9.29	10.77	5.02	6.87
G_1	10.90	12.89	5.98	6.46
B_2	8.88	10.70	8.63	10.50
B_1	9.72	10.33	7.75	8.26

[a] Levels of aflatoxins used were 5 ng/g for each aflatoxins.

These data characterizing the repeatability of the method are in good agreement with data found in the literature.

The use of OPLC for the determination of the aflatoxin content ensured high sample throughput and low operating costs. The OPLC method is ideally suited to the analysis of a large number of samples and only the positive samples have to be controlled by means of the more expensive HPLC methods.

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