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Use of high-performance liquid chromatography to assess airborne mycotoxins☆ Aflatoxins and ochratoxin A

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Abstract

An HPLC analytical method combining methanol-deionised water (80:20, v/v) extraction, methanol–acetonitrile (50:50, v/v) extraction and fluorescence detection was implanted to analyse ochratoxin A and aflatoxins B1, B2, G1 and G2 of air samples collected during the usual production process in a number of workplaces of a coffee factory to assess the occupational exposure of the engaged workers. The average levels of airborne ochratoxin A and aflatoxins were less than 1.2 and 0.4 ng/m^3, respectively, using 50 L air samples. When 150 L air samples were used, levels lower than 0.04 ng/m^3 ochratoxin A and 0.013 ng/m^3 for aflatoxins B1, B2, G1 and G2, could be detected.

Keywords: Air analysis; Mycotoxins; Aflatoxins; Ochratoxin A

1. Introduction

Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and ochratoxin A (OA), are metabolites that may be produced by moulds such as *Aspergillus* and *Penicillum.* Their biosynthesis depends on the temperature and humidity conditions.

Aflatoxins and ochratoxin A are to be found in agricultural products such as cereals, cacao, coffee, wine, fruits, peanuts, cotton seed, corn and rice as a consequence of unprosperous storage conditions (humidity of 70–90% and a minimum temperature of about 10 °C) [1].

As a matter of fact, components of heat, ventilation and air conditioning (HVAC systems) may be reservoirs of fungi and bacteria. In this regard, the occurrence of aflatoxins in homes and buildings has been reported, owing to the growth of mycotoxins producing moulds in the air conditioning systems [2,3].

AFB1, AFB2, AFG1, AFG2 and OA (Fig. 1) are mycotoxins whose absorption represents an important health hazard. Epidemiological studies have shown a correlation between liver cancer and the prevalence of aflatoxins in the food supply. Aflatoxin B1 is a powerful hepatocarcinogen and ochratoxin A is a well-recognised nephrotoxin [4–6].

The available information on airborne particulate aflatoxins and ochratoxin A occurrence is scarce [7,8], and the majority of the airborne particulate matter studies have been focused on aflatoxins during processing and handling of corn [9–17].

HPLC is a common method used for determination of aflatoxins in contaminated food [18,19]. To carry out this study, the method by Brera et al. [7] was modified to estimate the levels of aflatoxins and ochratoxin A in the air sampled in a coffee factory during the production process. The main modifications of Brera's method were as follows: inmunoaffinity columns were not used, and aflatoxins were extracted with 3 mL of methanol–acetonitile (50:50, v/v) rather than methanol-deionised water (80:20, v/v).

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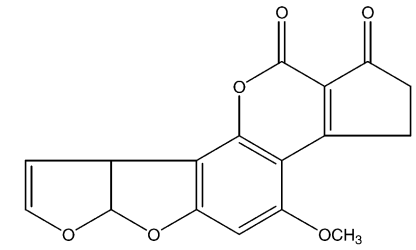
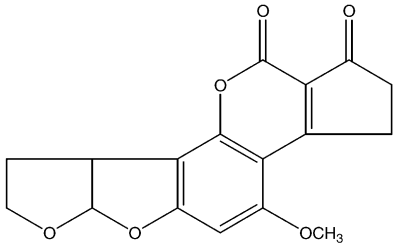


Aflatoxin B1 (AFB1)



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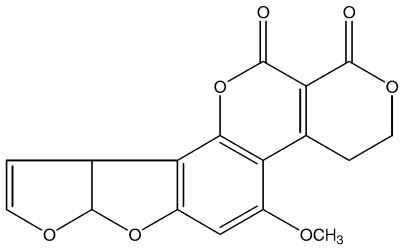
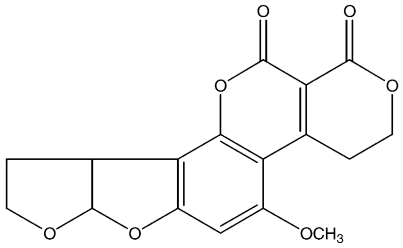
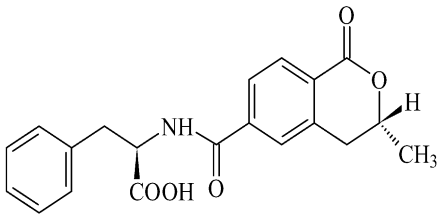


Aflatoxin G1 (AFG1)



Aflatoxin G2 (AFG2)



Ochratoxin A (OA)

Fig. 1. Chemical structures of aflatoxins and ochratoxin A.

The aim of this paper is to describe an HPLC-fluorescence detection (FLD) method for the determination of particulate aflatoxins and ochratoxin A in air samples intended to assess the exposure of the workers to these substances by inhalation at their workplaces.

2. Experimental

2.1. Sampling of airborne particulates

Eleven air samples were collected using aircheck pumps at a flow rate of 1.0 L/min during the coffee production process while workers were handling raw materials, tools and machinery in the factory. The sampling device through which the airflow passed was provided with a microfibre filter (Whatman 3–7 cm GF/C), in order to retain the particulate matter. The sampling time at each of the eleven workplaces in the factory was set in accordance with the length of the specific handling activity, between 50 and 150 min, approximately.

2.2. Reagents

Acetonitrile, acid acetic (glacial), *n*-hexane, methanol and trifluoroacetic acid (HPLC grade) were supplied by J.T. Baker (Barcelona, Spain).

Deionised water was obtained using a Milli-Q purification system (Waters Millipore QTUM 000EX).

Aflatoxin primary standards to check the linearity were provided by Tecnova (Madrid, Spain). Six milliliters vial of a methanol solution with the following specifications: 1000 ng/mL total aflatoxin, 250 ng/mL of AFB1, AFB2, AFG1 and AFG2.

Ochratoxin A primary standard for linearity was provided by Tecnova (Madrid, Spain). Six milliliters vial of methanol solution with the following specifications: 1000 ng/mL.

Aflatoxins and ochratoxin A primary standards for a recovery appraisal were provided by Sigma (Spain). Solid primary standards with the following specifications: 5 mg of AFB1, 5 mg of AFB2 and 5 mg of OA.

2.3. Working standard solutions

Working standard solutions of 0.25, 0.5, 1.25 and 2.5 ng/mL off each aflatoxin (AFB1, AFB2, AFG1, AFG2) were prepared by diluting the primary standard solution with methanol–acetonitrile (50:50, v/v). Ochratoxin A working standard solutions of 0.04, 0.2 and 2 ng/mL were prepared by diluting the primary standard solution with methanol-deionised water (80:20, v/v).

2.4. Instrumental analysis

Experiments were performed on a Waters 717 plus autosampler liquid chromatograph coupled to a Waters 474 scanning fluorescence detector, equipped with isocratic pump

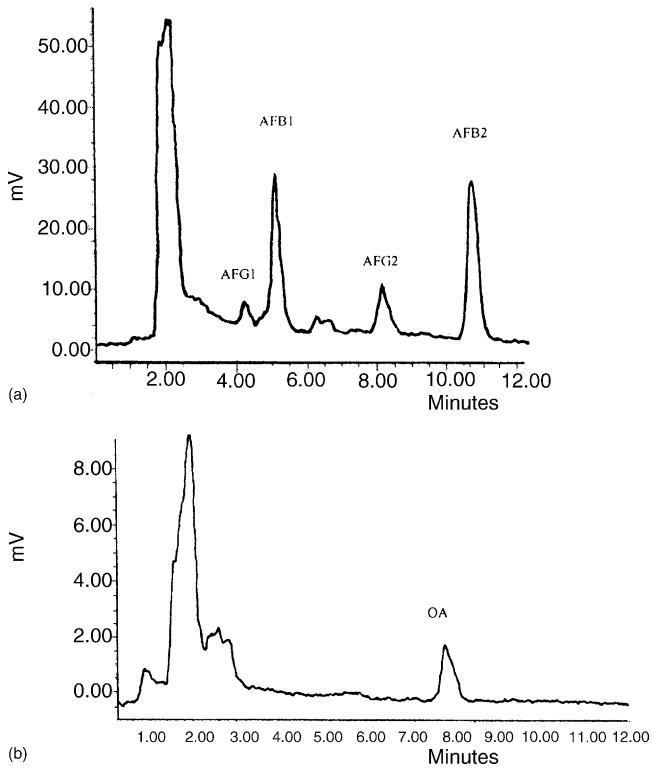


Fig. 2. HPLC-fluorescence chromatogram of (a) hemiacetal forms of AFB1, AFG1 and aflatoxins AFB2, AFG2 at 0.25 ng/mL, (b) ochratoxin A (OA) at 0.1 ng/mL.

(Waters 1525). The system was controlled by a Waters Millennium 32 chemstation. The analytical column was a 150 × 4.6 mm. i.d. Synergi Max-RP (Phenomenex, Spain), thermostatted at 27 °C.

For aflatoxins the fluorescence detector was set at 360 and 420 nm and the mobile phase consisted of a mixture of deionised water–acetonitrile–methanol (60:25:15, v/v). For ochratoxin A the detector was set at 333 and 470 nm and the mobile phase was acetonitrile–deionised water–glacial acetic acid (49.5:49.5:1, v/v).

The flow rate was 1 mL/min for each mobile phase and the injected volume of working standards and airborne samples was 50 μL. (Fig. 2).

2.5. Extraction aflatoxins B1, B2, G1, G2 and ochratoxin A procedure

Aflatoxins extractions from the glass fibre filter were carried out with 3 mL of methanol–acetonitrile (50:50, v/v) and, the solutions were kept in an ultrasonic bath for 10 min. The aflatoxins were then processed for derivatisation before the samples being injected into the HPLC.

Ochratoxin A was extracted from another glass fibre filter with 3 mL of methanol-deionised water (80:20, v/v) [7], the solution was kept in an ultrasonic bath for 10 min and immediately injected into the HPLC without derivatisation.

The aflatoxins and ochratoxin A extracts were not filtered before the analysis and the samples were analysed immediately after extraction to prevent solvent evaporation.

2.6. Aflatoxin derivatisation

An aliquot of 500 μL of the aflatoxins solution was dried by evaporation under gentle nitrogen stream. Hemiacetal forms of AFB1 and AFG1 (fluorescence active substances) were obtained by addition of a mixture of 50 μL of trifluoroacetic acid and 200 μL of *n*-hexane at 40 °C; the solution was mechanically shaken for 15 min [18]. The solution containing all four aflatoxins in its fluorescent form was subsequently dried by evaporation under gentle nitrogen stream, redissolved with 200 μL acetonitrile-deionised water (30:70, v/v) and it was finally injected in the HPLC system (Fig. 3).

3. Results and discussion

To check the quality of the proposed method several analytical parameters were determined.

3.1. Selectivity and specificity

The presence of any substance coming from the filter appearing at any of the retention times of ochratoxin A (8 min), aflatoxins AFB1 (5 min), AFB2 (10 min), AFG1 (4 min), AFG2 (8 min) (interfering substances) was ruled out (Fig. 4).

3.2. Linearity

Linearity was assessed using working standards at the following concentrations: 0.04, 0.1, 0.2, 0.4, 1, 2 ng/mL for ochratoxin A and 0.25, 0.5, 1.25, 2.5 ng/mL for each aflatoxin (AFB1, AFB2, AFG1, AFG2). Each concentration was injected three times (injection: 50 μL).

The regression lines calculated using least-squares method were:

$$\text{AFB1}: y = (-397708) + (5419351)x, \quad r^2 = 0.99990$$

$$\text{AFB2}: y = (-644297) + (7517284)x, \quad r^2 = 0.99999$$

$$\text{AFG1}: y = (-113444) + (771223)x, \quad r^2 = 0.99830$$

$$\text{AFG2}: y = (-220695) + (1931764)x, \quad r^2 = 0.99990$$

$$\text{OA}: y = (-578.6) + (420867)x, \quad r^2 = 0.99990$$

3.3. Limits of detection and quantitation

The limits of detection (LODs) were estimated as twice the signal-to-noise ratio (two times s/n). Thus, for the developed method, the above mentioned limits were 0.002 ng for

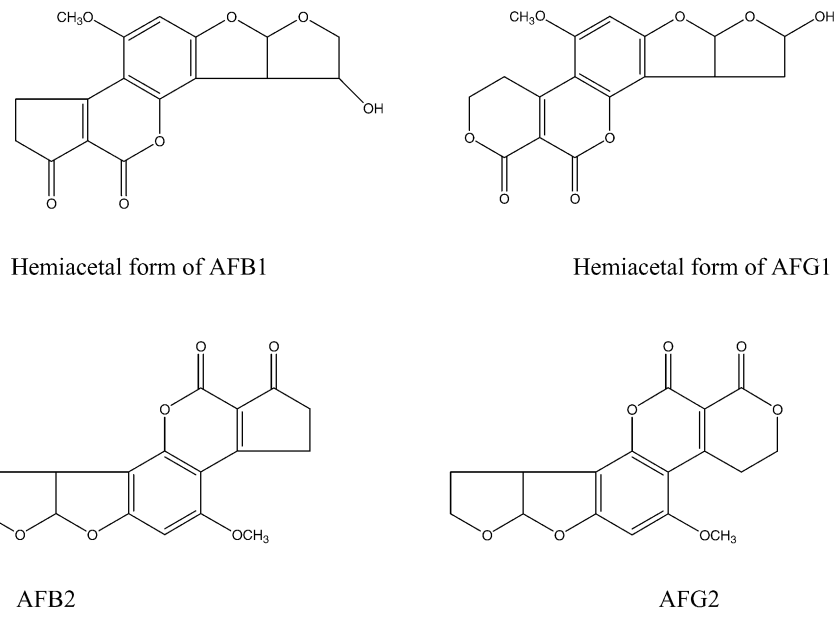


Fig. 3. Chemical structures of fluorescence active substances.

aflatoxins and 0.06 ng for ochratoxin A. These LODs were similar to those reported in the literature (0.0025 ng AFB1 and AFB2) [7].

As it used be expected, the greater sampled air volume the lower relative detection limit obtained. The results were the following: 1.2 ng/m^3 for OA, 0.4 ng/m^3 for AFB1, AFB2, AFG1, AFG2 for 50 L air samples and 0.04 ng/m^3 for OA, 0.013 ng/m^3 for AFB1, AFB2, AFG1, AFG2 for 150 L air samples.

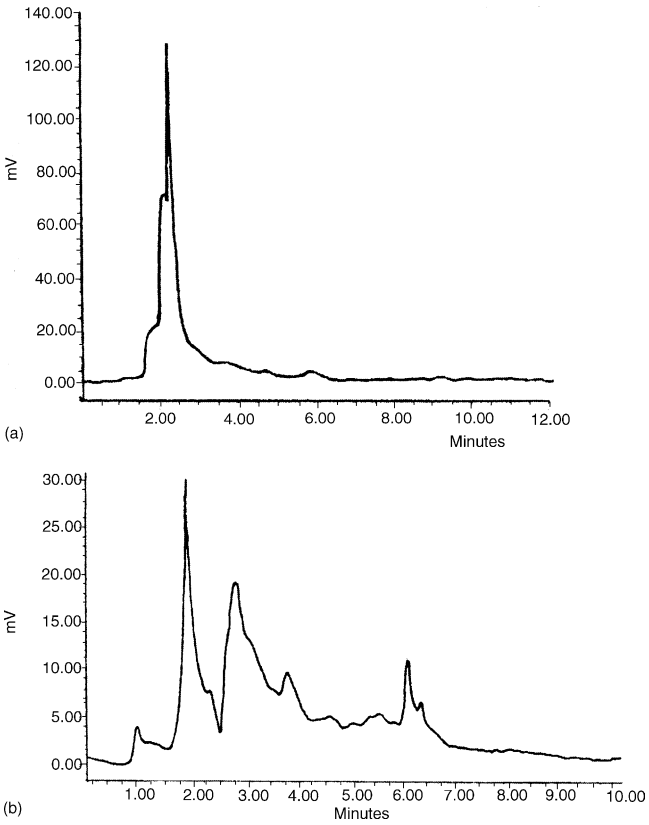


Fig. 4. HPLC-fluorescence chromatogram of (a) glass fibre filter blank extracted with methanol–acetonitrile (50:50, v/v), (b) glass fibre filter blank extracted with methano-deionised water (80:20, v/v).

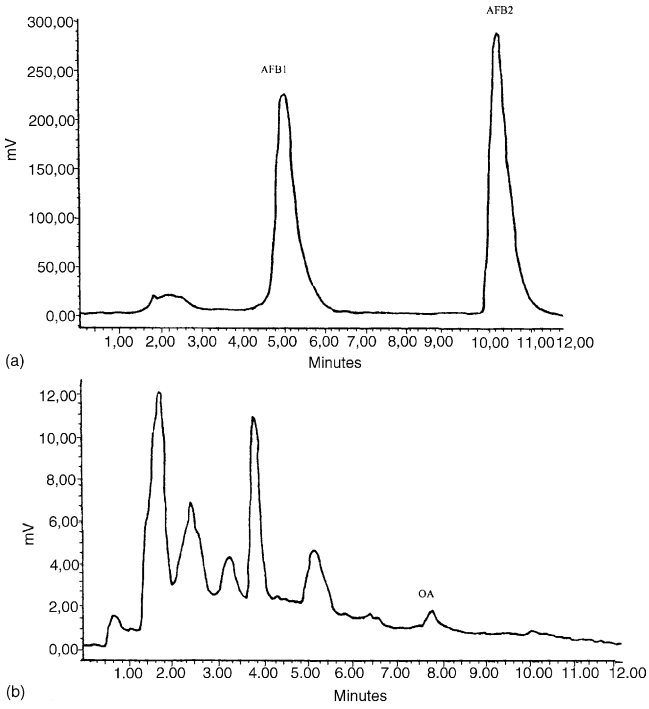


Fig. 5. HPLC-fluorescence chromatogram of (a) a glass fibre filter sample spiked with 2.5 ng/mL of AFB1 and AFB2, (b) a glass filter sample spiked with 0.04 ng/mL of OA.

Table 1
Recovery percentages and precision (R.S.D.) of aflatoxins

Aflatoxins	Concentration spiked (ng/mL)		
	0.1	0.5	2.5
AFB1	85.0 (2.7)	98.0 (2.8)	88.8 (1.2)
AFB2	98.3 (5.8)	82.0 (3.4)	86.4 (2.0)

R.S.D. values (%) in parentheses.

Table 2
Recovery percentages and precision (R.S.D.) of ochratoxin A (OA)

Ochratoxin A (OA)	Concentration spiked (ng/mL)		
	0.04	0.2	1
	80.3 (5.5)	96.8 (1.7)	103 (5.0)

R.S.D. values (%) in parentheses.

The limit of quantitation (LOQ) is the lowest concentration of ochratoxin A and aflatoxins that can be measured with an approved precision (relative standard derivation, R.S.D. <7%). Thus, the LOQ for ochratoxin A and aflatoxins using 150 L air samples was estimated as 0.8 ng/m^3 for ochratoxin A, 0.13 ng/m^3 for AFB1, AFB2 and 0.33 ng/m^3 for AFG1 and AFG2.

3.4. Extraction recoveries and intra assay-precision

After 18 fibre filters were spiked with working standard solutions, extraction recoveries and intra assay-precision of ochratoxin A, aflatoxin B1, and aflatoxin B2 were calculated by comparison of peak areas of the compounds from fibre filters with those from working standards solutions at the intended final concentrations. The intended final concentration of the 18 glass fibre filters were 0.04, 0.2 and 1 ng/mL of ochratoxin A, and 0.1, 0.5 and 2.5 ng/mL of AFB1 and AFB2 (Fig. 5), respectively, on three separate runs.

The recovery percentages obtained for aflatoxins AFB1, AFB2, were 85, 98 and 88.8% for AFB1 and 98, 82.5 and 86.4% for AFB2 for glass fibre filter spiked with 0.1, 0.5 and 2.5 ng/mL aflatoxins B1, B2. The percentages for ochratoxin A (OA) were 80.30, 96.80 and 103% for a glass fibre filter spiked with 0.04, 0.2 and 1 ng/mL of ochratoxin A. Results are represented in Tables 1 and 2.

To evaluate the precision of the method, glass fibre filter samples spiked with 0.1, 0.5 and 2.5 ng/mL of AFB1 and AFB2 were analysed three times at each concentration on three separates runs. Similarly, samples spiked with 0.04, 0.2 and 1 ng/mL of OA were analysed six times at each concentration on three separates runs. Precision of the developed method was expressed as relative standard derivation, R.S.D. (Tables 1 and 2).

3.5. Analysis of the airborne particulate from a coffee factory

The optimised method was applied to assess the likely occurrence of airborne aflatoxins (AFB1, AFB2, AFG1, AFG2) and ochratoxin A (OA) in a coffee factory during the performance of different work activities. Over 2 days, 11 samples of airborne dust were collected. As it has already been mentioned, the volume of air sampled was in accordance with the operations carried out at the specific workplace. All the results show that the concentration of aflatoxins and ochratoxin A (OA) during the performance of the activity in the coffee factory were less than the LOD of the here proposed method (Table 3).

4. Conclusions

A new method for the determinatione of ochratoxin A and aflatoxins B1, B2, G1 and G2 in air has been described. Since

Table 3
Concentration of OA and AFB1, AFB2, AFG1, AFG2 of air samples collected during the usual production process in a coffee factory

Sampling site	Volume sampled (L)	Ochratoxin A (ng/m^3)	Aflatoxins (ng/m^3)
Unloading			
Big-bags of Brazilian coffee[a]	98	<0.6	<0.02
Bulk container of green coffee[a]	93	<0.6	<0.02
Coffee big-bags from the lorry[a]	102	<0.6	<0.02
Coffee big-bags in a conveyor belt[a]	144	<0.4	<0.01
Bulk container of green coffee[a]	58	<1.0	<0.03
Bulk container of green coffee[b]	57	<1.0	<0.03
Green coffee from big-bags[b]	30	<2.0	<0.06
To fill in			
Decaffeinated green coffee in big-bags[b]	49	<1.2	<0.04
Decaffeinated green coffee in big-bags[a]	187	<0.32	<0.01
Ambient samples			
From a cabin control room[a]	58	<1.0	<0.03
From a reception office[b]	56	<1.0	<0.03

[a] Temperature and humidity during the sampling: 25.5 °C, 56%.
[b] Temperature and humidity during the sampling: 16 °C, 25%.

the results concerning linearity, recovery and precision are similar to those described in the literature for other mycotoxins and, consequently they can be considered as acceptable.

The proposed method has been used to assess the occurrence of the above mentioned substances in the air of a coffee factory during the production process. Results show a mycotoxin (aflatoxins or ochratoxin A) content lower than the limit of detection of the method. Specifically, the detected concentration of AFB1 was lower than the recommended limit (<1 ng/m^3) [15]. Occupational exposure limits for AFB1, AFB2, AFG1, AFG2 and OA have not been set.

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