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Automated column-switching high-performance liquid chromatography for the determination of aflatoxin M1

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Abstract

An extractionless method for determining aflatoxin M1 (AFM1), a major metabolite of aflatoxin B1 (AFB1), in human urine was developed. The biological fluid is injected directly into the chromatographic system after simple dilution and centrifugation. A pre-column, packed with a cation-exchange phase and coupled on-line to a column-switching liquid chromatography (LC) system, is used for sample pre-treatment and concentration. The analytes are non-selectively desorbed with the LC eluent and cleaned by means of a column-switching procedure. Pre-treatment and analysis were performed within 40 min. Average AFM1 recovery reached 97% in the 10–100 ng/l range of urine. The detection limit of AFM1 in urine and milk was 2.5 ng/l for 1 ml of injected sample. A comparison with an immunoaffinity column clean-up and LC method was performed. The method was applied to determine AFM1 in the urine of AFB1 gavaged rats, and in the urine of both potentially exposed and supposedly unexposed workers. The method was also extended to milk. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Aflatoxins are mycotoxins produced by several species of *Aspergillus flavus* moulds. They have been found in various plant products including peanuts, copra and soja, or in cereals such as maize, rice and wheat. Aflatoxin B1 (AFB1) is the most commonly occurring variety and one of the most carcinogenic. Several epidemiological studies have found positive associations between AFB1 dietary exposure and an increased risk of human liver cancer [1–4]. In addition to dietary intake, occupational exposure can occur, and AFB1 has been determined in airborne dust from feed factories [5–7].

Dietary and occupational AFB1 (Fig. 1) exposure can be assessed by looking for and measuring metabolites in urine. Among the hydroxylated urinary metabolites, AMF1 (Fig. 1) was found to be an excellent marker with a linear relationship between its excretion and the AFB1 absorbed dose [2,8].

Several analytical methods have been described in

Fig. 1. Structural formulas of AFB1 and AFM1.

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the literature to determine AFM1 in urine or milk using various extraction and concentration methods [2–4,8–16]. They employ the simple but cumbersome and time consuming liquid–liquid extraction procedure [2,4,12] through to the automated or unautomated sample clean-up method with solid-phase extraction [10,11] or expensive immunoaffinity columns [3,8,13–16]. However, these methods require a treatment time of up to 30 min and a minimum analysis time of about 15 min. The column-switching technique, which has found many applications in clinical laboratories or in biological monitoring, offers numerous advantages to sample clean-up and routine analysis in liquid chromatography (LC). An interesting automated method was developed by Farjam et al. [13], but several drawbacks restrict its use.

The described method in this paper allows the fluorescence detection of AFM1 in urine or milk without liquid–liquid extraction step or purification by immunoaffinity columns. The samples are injected directly into the chromatographic system after simple dilution and centrifugation. A reusable precolumn was used as an injection loop and was on-line coupled to a column-switching cleaning system.

2. Experimental

2.1. Chemicals and reagents

All were of analytical reagent grade. Formic acid and ammonium acetate were obtained from Merck (Darmstadt, Germany), and water was purified by passing it through a Milli-Q treatment system (Millipore, Bedford, MA, USA). Chromatographic-grade acetonitrile and methanol were obtained from SDS (Peypin, France) and Merck, respectively. AFM1 and AFB1 were purchased from Supelco (Bellefonte, PA, USA). For sample immunoaffinity cleaning, Aflaprep M columns (Kit bx: FL 248) were provided by Rhône-Poulenc (Glasgow, UK).

2.2. Apparatus

The high-performance liquid chromatography (HPLC) system consisted of the following: two chromatographic pumps, Model 590 from Waters Associates (Milford, MA, USA) and Model 5000 from Varian (San Fernando, CA, USA); three automated switching valves (Model 7000, Rheodyne, Berkeley, CA, USA) combined with a Model 7125 sample injector (Rheodyne) equipped with a mini-column loop; a Model RF-551 fluorescence detector (Shimadzu, Kioto, Japan) set to 365 nm for excitation and 440 nm for emission. The entire system was controlled by a Waters chromatographic pump. The output signal was recorded with a Chromjet integrator (Thermo Separation Products, Fremont, CA, USA) or a recorder (Kipp and Zonen, Touzart et Matignon, Vitry-sur-Seine, France).

2.3. Columns

The pre-column loop was a 2 cm×0.32 cm I.D. stainless steel guard column cartridge (Upchurch Scientific, Oak Harbor, WA, USA) filled with approximately 0.1 g of 20–40 μm Chromabond SA (Macherey-Nagel, Düren, Germany). The purification columns C_1, C_2 and C_3 were 7.5 cm×0.46 cm I.D. stainless steel tubes packed with: 10 μm Vydac 401 TPB (The Separation Group, Hesperia, CA, USA), cation-exchange phase; 5 μm Nucleosil SA (Macherey-Nagel), second cation-exchange phase; and 5 μm Nucleosil SB (Macherey-Nagel), anion-exchange phase. The analytical column C_4 was a 20 cm×0.32 cm I.D. stainless steel tube packed with 5 μm Spherisorb ODS 1 (Phase Separations, Deeside, Clwyd, UK), an octadecyl-bonded phase.

About 20 other manufactured ion-exchange and alkyl-bonded HPLC phases for purification and analysis were tested in this study, as well as 20 solid-phase bonded silicas (or membranes) to carry out a crude clean-up and concentration of the samples. With the exception of the pre-column loop, which is dry filled, all the columns were made in the laboratory and slurry-packed at $4 \cdot 10^7$ Pa using a mixture of 95% ethanol–2-propanol–toluene (1:1:1, v/v/v) as slurry solvent, and methanol and then water as displacement liquid.

The mobile phases used, E1 and E2, were a mixture of water–methanol–acetonitrile–formic acid (71:22.5:5.5:1, v/v) for the ion-exchange purification columns, and a mixture of the same solvents in a ratio of 61.9:28.5:9.5:0.1 (v/v) for the analytical
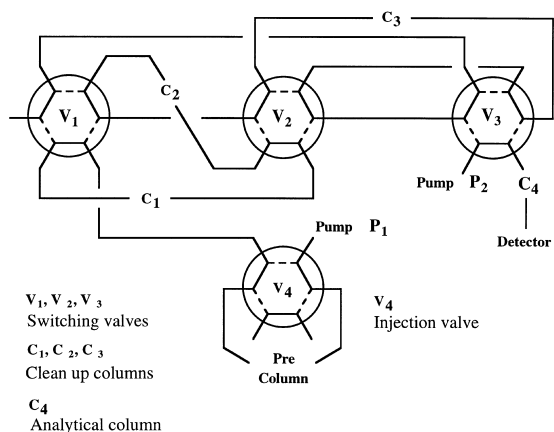
Fig. 2. Layout of the automated sample pre-treatment system for determining AFM1: valves V1–V4 are all shown in position A (cf. Table 1). The system is described in Section 2.

column. The flow-rate was set to 0.5 ml/min for both columns.

2.4. Analytical procedure

A schematic diagram of the switching system is shown in Fig. 2, and the time schedule of the analytical procedure is given in Table 1. Initially, the ion-exchange pre-column loop was manually pre-conditioned with 0.5 ml of water. The sample was then loaded with a HPLC glass syringe. The pre-column was flushed with 0.5 ml of water, then with 0.5 ml NH_4OH $5 \cdot 10^{-1}$ M (in water–MeOH, 9:1, v/v), and finally with 0.5 ml of $2 \cdot 10^{-1}$ M HCOOH (in water–MeOH, 9:1, v/v) at about 2 ml/min to remove the impurities liable to interfere with the analysis. Following this, the pre-column and the

Table 1
Column switching analytical procedure using the scheme according to Fig. 1

Step event	Valve positions[a]			
	Valve 1	Valve 2	Valve 3	Valve 4
1. Manual pre-column flushing[b] with sample, water, NH_4OH and HCOOH solutions (about 2 min)	B	A	A	B
2. Start of automated analytical sequence. Flushing pre-column with eluent pump P1 (1 min, 0.5 ml), analyte transfer on C_1 (injection)	A	B	A	A
3. Switch injection valve; manual pre-column wash. Purification on C_1	A	B	A	B
4. Analyte transfer from C_1 to C_2	A	A	A	B
5. Purification on C_2. Clean-up of C_1 in backflush mode	B	B	A	B
6. Analyte transfer from C_2 to C_3	A	A	A	B
7. Purification on C_3. Clean-up of C_1 and C_2 in backflush mode	B	A	A	B
8. Analyte transfer from C_3 to C_{18}. Flushing C_1 and C_2 with eluent pump P2	B	A	B	B
9. Analysis on C_{18}. Flushing C_1, C_2, C_3 with eluent pump P1 (reconditioning)	B	A	A	B

[a] Position A corresponds to the valve positions shown in Fig. 1.
[b] The flushing pre-column procedure could be automated with the appropriate material.

cation-exchange column (C_1) were switched in series, and the pre-column was desorbed with 0.5 ml of eluent E1 in the backflush mode using pump P1. The sample was purified with E1 onto C_1 by dispatching the bulk of the undesired matrix to waste. Just before eluting the AFM1 from C_1, the six-port valve V2 was rotated and the fraction containing the eluting AFM1 was introduced into C_2. After AFM1 elution from C_1, valves V1 and V2 were switched in order to continue the purification on C_2 while C_1 was simultaneously cleaned in backflush mode with E1. After purification on C_2 with E1, the analyte fraction of interest was transferred from C_2 to C_3 with E1 and, finally, again with E1 for analysis. During the transfer of AFM1 into C_4, C_1 and C_2 were flushed in backflush mode with E2, and during the elution of AFM1 on C_4 with E2, columns C_1, C_2 and C_3 were cleaned and reconditioned with E1 for the next injection. The pre-column loop was cleaned with 1 ml of CH_3CN–HCOOH (99:1, v/v) and reconditioned with water. Every thirty injections the purification columns and analytical column were cleaned with a mixture of water–CH_3CN–MeOH–HCOOH (10:70:20:1, v/v).

2.5. AFM1 analysis

For a given urine or milk sample, the retention time was compared with that of an external standard, and the peak-height measurement method was used for quantitative assessment. The crude clean-up on the pre-column loop lasted about 3 min and the analysis of each sample was completed within 40 min.

2.6. Preparation of standards

The commercial AFM1 standard solution was dissolved in acetonitrile (10 mg/l) and stored at $-20°C$. It was further diluted in water, artificial urine [17], and different blank urines or milk to obtain spiked AFM1 solutions of 100, 50 and 10 ng/l, then stored in a refrigerator at $8°C$ for up to one week. Urine (0.1 ml to 1 ml) was diluted in half with ammonium acetate buffer (10^{-1} M), briefly vortex-mixed (0.5 min) and centrifuged at 2000 g for 0.5 min. The variable volume from the mixture could then be directly injected into the pre-column loop.

When the urine samples were not being analysed, they were kept frozen at $-20°C$. As for urine samples, the calibration solutions of standard AFM1 are passed regularly on the whole column system to test the reliability of the method and to detect also small leaks in the switching system, or changes of retention times on the purification columns.

2.7. Human urine collection and treatment

"Spot" urine samples from sixty volunteers from within our Institute were collected in polyethylene bottles and refrigerated. If urine samples were not analysed within the week after collection, they were fractionated and kept frozen at $-20°C$. Urine (0.5 ml to 1 ml) was diluted in half with ammonium acetate buffer (10^{-1} M), briefly vortex-mixed for 0.5 min, and then centrifuged at 2000 g for 0.5 min. A variable volume (0.1 ml to 2 ml) of the mixture was then injected directly into the pre-column loop. To check the specificity of the method and to detect an eventual influence of the diet, urine samples were not pooled.

The urine sample preparation described by Kussak et al. [16] was employed for the immunoaffinity method. This can be summarised as follows: the immunoaffinity column was conditioned with water, and the 10-ml urine samples were diluted with 10 ml of 0.1 M sodium acetate buffer (pH 5.0), then loaded on the column, washed with 10 ml water, and dried using nitrogen for 1 min. AFM1 was then eluted with 2 ml of acetonitrile into silanized test tube. The solvent containing the AFM1 was evaporated (but not to dryness) under a flow of nitrogen and redissolved in eluent E2 for analysis, which was performed on the analytical column only.

2.8. Milk treatment

Liquid milk (0.25 ml to 1 ml) was diluted in half with water, and briefly vortex-mixed (0.5 min). A variable volume (0.1 to 2 ml) of the mixture could then be injected directly into the pre-column loop. When milk powders were analysed, water–milk solutions were sonicated for about 2 min as injection samples. After loading, the pre-column loop was flushed in accordance with the protocol described in Section 2.4.

When the immunoaffinity method was used for clean-up, the milk sample was prepared in accordance with a previously described method [15].

2.9. Rats experimental protocol

Six-week old male Sprague-Dawley rats were purchased from Charles River Breeding Labs. (Lyon, France) and housed individually in glass metabolic cages throughout the study period in a temperature and humidity controlled environment with a 12 h light, 12 h dark photoperiod. Food and water (free from AFB1) were available ad libitum. Twelve male rats were gavaged with a single dose of 10 ng AFB1 (2 ml of water solution of 5 mg/l AFB1); the urines were then collected on ice at time 0 h and 8, 16, 24, 32, 40 and 48 h after exposure, and stored at −20°C until analysis. The urine volume ranged from 0.38 to 14 ml.

2.10. Atmospheric sampling and analysis

The airborne dust was collected during the unloading of a cargo vessel and the analysis carried out in accordance with the method previously described by Lafontaine et al. [6]. Briefly, the aerosols were collected on GF/C filters which were extracted with a mixture of $CHCl_3$–water (9:1); the extracts were then purified on Sep-Pak or immunoaffinity minicolumns. The analyses were performed by HPLC with fluorimetric detection.

3. Results and discussion

Liquid–liquid extraction of AFM1 from urine or milk is a cumbersome and time-consuming technique; solid-phase extraction was used as a more specific clean-up method with immunoaffinity columns, but the steps of extraction, solvent evaporation and the redissolution of the AFM1 in HPLC eluent have not been eliminated. Farjam et al. [13] have developed an interesting automated method which employs short columns packed with immobilised antibodies for selective sample pre-treatment, on-line coupled to column-switching HPLC. However, a number of drawbacks – such as no quantitative desorption, rather poor repeatability, and the need to use a fresh immuno pre-column for each analysis – restrict its general application.

As a result, we followed up the use of an automated switching-column, which had previously been exploited in our laboratory [18] as a technique for sample clean-up and routine analysis. However, as it is essential to obtain a low detection limit to determine traces of AFM1 in urine, the switching system was improved. A short pre-column filled with preparative bonded HPLC phase was introduced instead of the injection loop to obtain both a concentration and a crude purification of the urine sample, and the number of purification columns was increased. The direct transfer technique or "heart cut" technique was retained; this consists in discarding the uninteresting parts of a sample initially eluted on a primary column, and in transferring the fraction of interest onto a secondary column with a minimum of overlapping interference. By increasing the "heart cut" number and, consequently, the column number through changing the nature of phases, sample purification was considerably improved. In addition to looking for the best eluents (constitution, ionic strength, pH, nature of organic modifiers, etc.), combinations of various anion-exchange, cation-exchange and alkyl-bonded phases together with chromatographic parameters such as particle diameter, column dimension and diameter, column number, etc.) were investigated. The results of our investigations are shown in Figs. 3 and 4 where the chromatograms of five different blank urines, selected as being among the most representative, demonstrate the high selectivity of the developed method.

3.1. Determining AFM1 in urine

Standards of AFM1 were initially established in artificial urines in order to check the efficiency of the purification and concentration procedure on the pre-column by comparing it to loop injection. No loss of AFM1 occurred during the pre-treatment of standard samples; the recovery of AFM1 from spiked blank urines was then calculated by comparison with the extraction of these standards. The results of the extractions are shown in Table 2; the recoveries are quasi complete and independent of the loaded AFM1 amount, contrary to the immunocolumn method described by Farjam et al. [13].
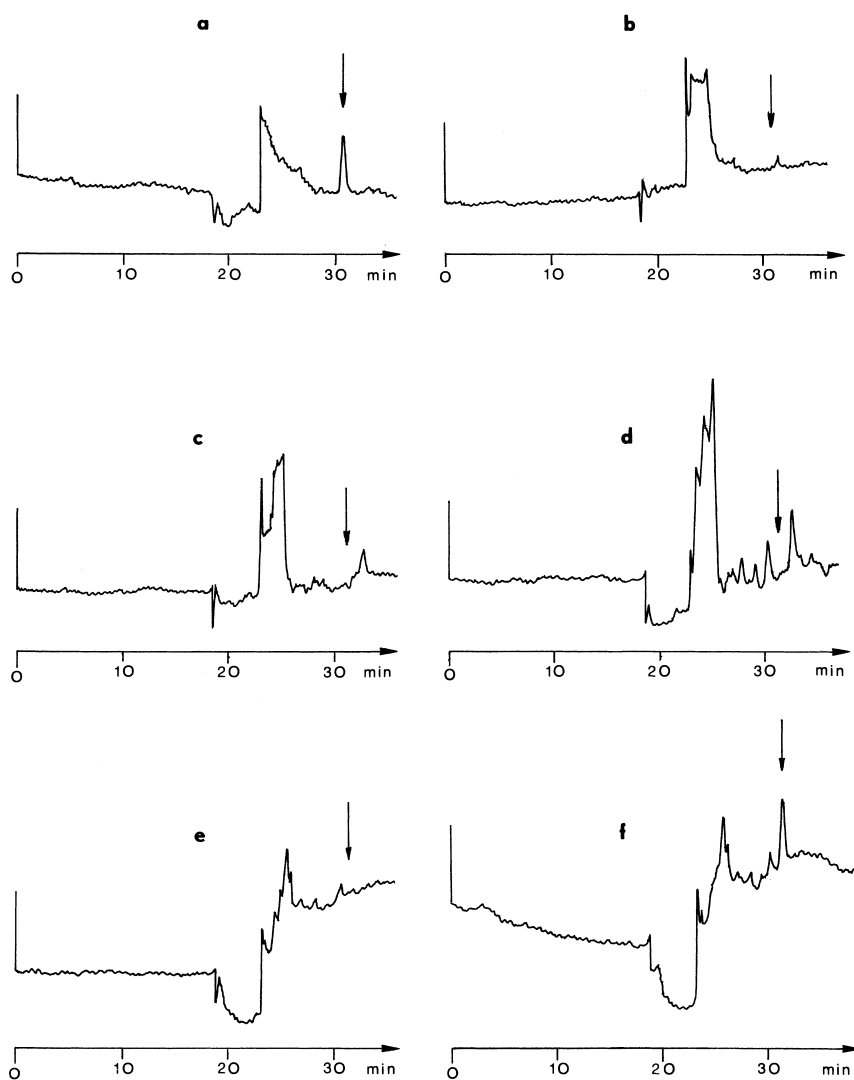
Fig. 3. Chromatograms of various urine samples obtained after clean-up with the switching system: (a) Standard of AFM1 10 ng/l, 0.5 ml injected; (b) urine sample 1, AFM1<2.5 ng/l, 1 ml injected; (c) urine sample 2, AFM1<5 ng/l, 0.5 ml injected; (d) urine sample 3, AFM1<5 ng/l, 0.5 ml injected; (e) urine sample 4, AFM1<5 ng/l, 0.5 ml injected; (f) urine sample 4 spiked with AFM1 10 ng/l, 0.5 ml injected. The system peak at time 19–24 min corresponds to the transfer and injection of analyte from C_3 into the analytical column. The chromatographic conditions are detailed in Section 2.

The calibration curve of AFM1 using peak-height measurement was linear over the range investigated. The regression equation was $y = -2.033 + 1.913x$, with a correlation coefficient greater than 0.999. The accuracy of the method was established on a sample of pooled human urine (1.45 g/l creatinine) to which AFM1 was added in concentrations of 10, 50 and 100 ng/l; for the 10 ng/l concentration, the co-

efficients of variation (C.V.s, $n=6$) were less than 6% and 10% for within-day and between-day accuracy, respectively, and less than 2% and 5% for the 50 and 100 ng/l concentrations.

The detection limit varied with the injected human urine sample volume or standard volume; for a 1-ml injection, the detection limit was estimated at 2.5 ng/l ($S/N=5$), and 5 ng/l for 0.5 ml. To check the
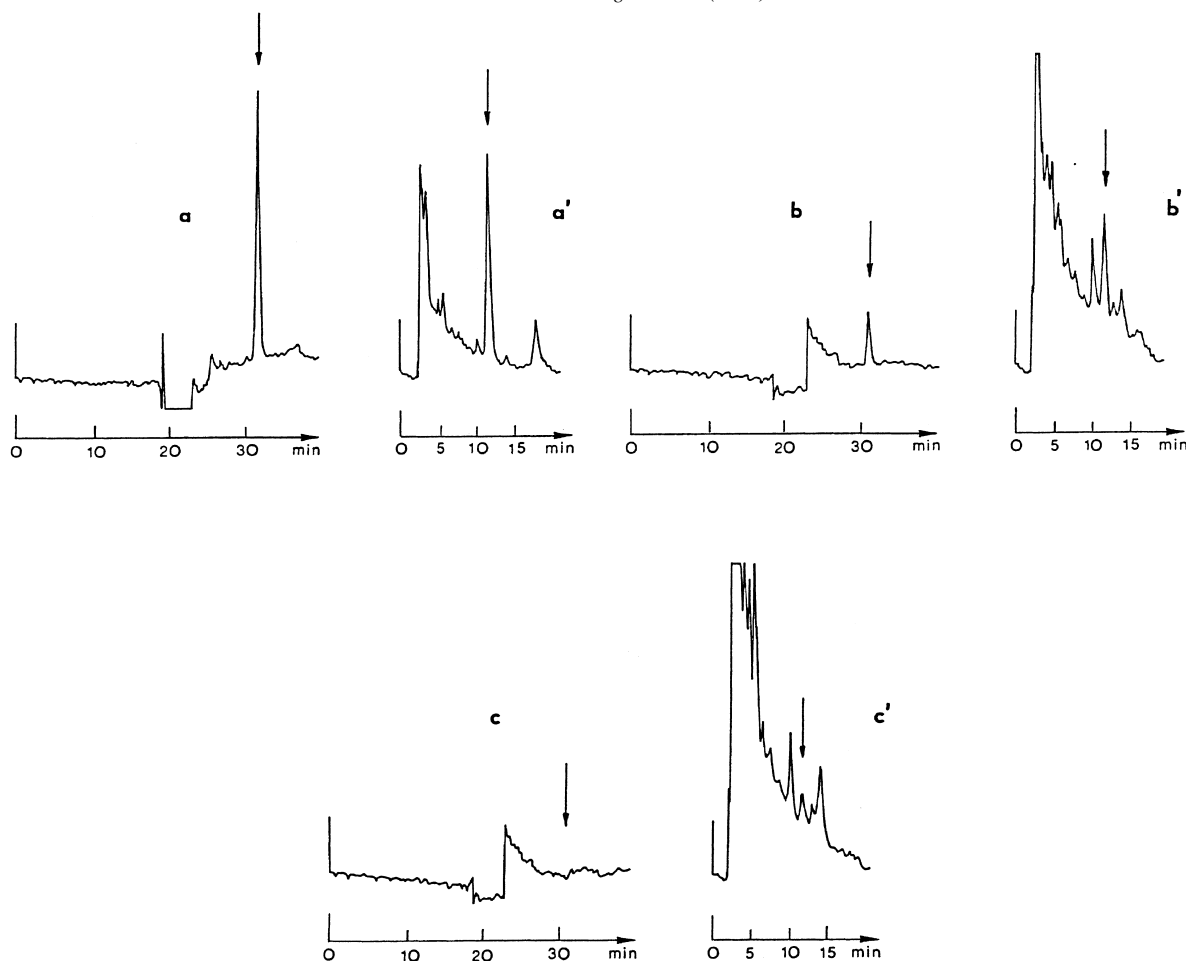
Fig. 4. Chromatograms of the urine samples obtained after purification with the described method (a, b, c) and with immunoaffinity micro-columns (a′, b′, c′): (a) urine sample 5 spiked with AFM1, 100 ng/l, 0.5 ml injected; (a′) same urine sample concentrated 10×, 0.05 ml injected; (b) urine sample 5 spiked with AFM1, 10 ng/l, 0.5 ml injected; (b′) same urine sample concentrated 10×, 0.05 ml injected; (c) urine sample 5, AFM1<5 ng/l, 0.5 ml injected; (c′) same urine sample concentrated 10×, AFM1 about 3.5 ng/l, 0.05 ml injected. The system peak at time 19–24 min in the chromatograms a, b and c corresponds to the transfer and injection of analyte from C_3 into the analytical column. The chromatographic conditions are detailed in Section 2.

specificity of the method, AFM1 measurements were carried out on the urine of workers who had not intentionally been exposed to AFB1 or AFM1. Sixty-

Table 2
AMF1 recovery from spiked blank human urine and milk samples

Concentration (ng/l)	Recovery ($n=6$)		S.D. (%) ($n=6$)	
	Urine	Milk	Urine	Milk
10	98.5	94.8	5.5	2.1
50	97	95.8	1.1	0.9
100	98.7	97.2	1.9	2.1

two urine samples were analysed, and the AFM1 levels were lower than the estimated detection limit. The described method was compared with a method that uses immunoaffinity columns [15] to purify and concentrate the urine sample. For the human urine sample, which was spiked with 10 and 100 ng/l of AFM1, the arithmetic means ($n=6$) of recuperated quantity were 7.8±1.6 ng/l and 90.0±4.3 ng/l for the immunoaffinity method versus 9.6±0.8 ng/l and 99.0±2.8 ng/l for the switching method. The results of the comparison on gavaged rat urine are summarised in Table 3, whereas Fig. 4 shows the chromato-

Table 3
Comparison of AFM1 measurements of different urine samples by immunoaffinity column and switching-column purification methods

Samples (rat urine)	Immunoaffinity (ng/l)	Switching (ng/l)
U1	15	18
U2	41	40
U3	36	39
U4	12	12
U5	22	25
U6	30	34

grams of the same urine samples for both treatment methods. For the studied urine samples, the chromatograms show that the obtained selectivity was generally better with the switching method than with the immunoaffinity method. However, the chromatogram profiles can vary with the urine samples and poor selectivity has been occasionally observed with the switching method as shown in Fig. 3d.

The results obtained for both methods are in agreement. Nevertheless, with the immunoaffinity method the recovery and accuracy were weaker, with greater background interference levels ranging from 4 to 12 ng/l for blank urines. These variations were attributed to the weak ruggedness of the immunoaffinity method which is sensitive to various experimental factors such as elution flow-rate, pH, temperature, contact time of the solute with the antibody, etc. These drawbacks are well known and have already been described in the literature [13,19,20]. Thus, during the repeatability assays, different results and chromatograms were obtained for the same sample with a slight change in the dryness of the immunoaffinity support before desorption of the solute.

The feasibility of the method was verified on the urine of rats gavaged with very small doses of AFB1. After administration of a single dose of 10 ng of AFB1/day (25 to 50 ng/kg), sequential 8-h urine samples were collected and analysed; the total AFM1 was excreted within 24 to 32 h of the gavage, and represented 1–3% of the daily AFB1 dose. This finding is consistent with data from the literature [8], where between 2 and 6% of AFB1 dose was found to be excreted into the urine as AFM1 within 24 h. In this study, the main experimental differences

concerned the AFB1 doses which were 500-fold to 15 000-fold greater than those used in our study, and the vehicle was DMSO instead of water.

Aflatoxins have been detected in the airborne dust of agricultural products used in feed production [5–7]. A research project was therefore undertaken to detect AFM1 in the urine of exposed dockers during the unloading of a cargo vessel and to assess potential AFB1 exposure to contaminated dust. However the AFB1 airborne concentrations found, <10 ng/m^3, were too low in comparison to the previously reported values [6] to detect urinary AFM1 with respect to its detection limit. No AFM1 was detected around the limit of detection of the method, as confirmed by the analyses of the urine samples.

3.2. Determining AFM1 in milk

The method was tested on milk samples, Fig. 5 showing the chromatograms obtained. The recoveries from spiked milk samples are given in Table 2. As in the case of the urine samples, the performance and the repeatability of the method were tested regularly by injecting a standard solution every five samples. AFM1 was detected in the 2.5–6.5 ng/l range in six blank milk samples. Naturally contaminated reference powdered milks containing 0.037, 0.049 and 0.669 mg/kg, respectively (in other words 0.0037, 0.0049 and 0.0669 mg/l after dissolution 1:10 in water) were analysed; the divergence between the values found and the theoretical values did not exceed 10%.

The ability to adapt the method to determining AFM1 in milk was expected as the clean-up procedure initially developed to purify the urine samples had itself been adapted from a method described by Takeda [10], who used an ammonia solution and an acetic acid solution to clean-up milk samples on Sep-Pak C$_{18}$ cartridges. With the milk samples, the clean-up procedure on the pre-column was modified slightly for three reasons: firstly, to avoid aggregation, the milk was diluted in water instead of ammonium acetate buffer; secondly, after sample loading on the pre-column, it was flushed with 1 ml of water instead of 0.5 ml; thirdly, after activation of the injection valve, AFM1 was desorbed from the pre-column for 1.5 min instead of 1 min. If no filtration or centrifugation had been performed before
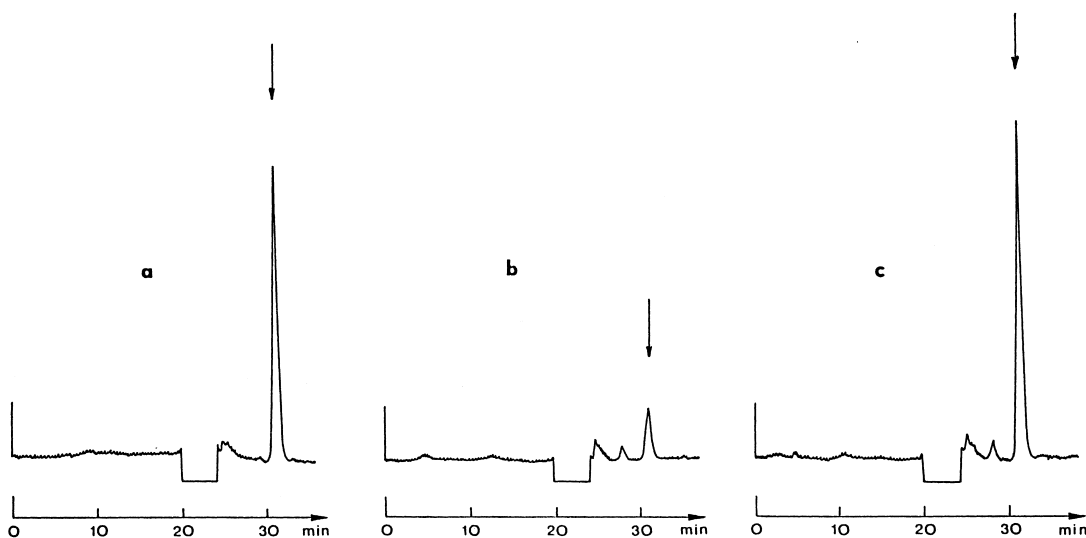
Fig. 5. Chromatograms of milk samples obtained after clean-up with the switching system: (a) standard of AFM1 50 ng/l, 0.5 ml injected; (b) milk sample 1, AFM1 4.4 ng/l, 1 ml injected; (c) milk sample 2, AFM1 60.2 ng/l, 0.5 ml injected. The system peak at time 19.5–24.5 min corresponds to the transfer and injection of analyte from C_3 into the analytical column. The chromatographic conditions are detailed in Section 2.

injection, the pre-column would only have been able to clean-up about 25 0.5-ml milk samples without damaging its purification or retention characteristics.

3.3. Technical remarks

Regarding the HPLC switching system, the pumps, valves, columns and connections were laid out in such a way as to minimise the number of devices (pump, valves and columns) and so that two out of the three purification columns could be cleaned and reconditioned in backflush mode during AFM1 elution on the analytical column. The characteristics of the purification column were chosen by determining the capacity factor of AFM1 for different packing materials.

As successive purifications on alkyl bonded phases were insufficient, ion exchangers were tested as stationary phases. The ionic bonded phases selected were the best compromise, offering a minimum number of interfering components transferred with a minimum retention time. The better results obtained with ion-exchange phases can be explained by the fact that more interactions take place between analytes and adsorbent than with reversed-phase supports, which are primarily partition effects.

The dimensions of the purification columns were also optimised so that the elution strength of the mobile phase was lower than that of the eluent of the analytical column in order to reconcentrate the AFM1 on top of the analytical column. Up to 500 injections, using about 400 urine samples, were performed with the same purification and analytical columns without loss of resolution and damage to the purification characteristics. The reliability of the method was tested regularly by injecting a standard solution every five samples. The AFM1 standard solutions are passed on the whole column system as for urine samples, in order to subject the standards to the same purification treatment. This procedure is necessary to detect chromatographic troubleshootings occurring upstream the analytical column, for example, small leaks of eluent, or changes of retention times of AFM1 on the purification columns. These troubleshootings cause a change of the standard chromatogram, especially a decreasing of peak area. By passing the standard solutions only on the analytical column, these troubleshootings can not be detected.

Concerning the injection pre-column, about 20 different chromatographic supports were tested; Chromabond SA was finally selected, but other

cation exchangers such as Bakerbond SAX can also be used. If this adsorbent is used, the proportion of MeOH must be increased in the clean-up solvent of the pre-column. The pre-column is easy to change and to fill with cheap bonded phase. It is able to purify up to 100 0.5-ml urine samples without damaging its clean-up or retention characteristics. The manual flushing pre-column procedure could be automated with the appropriate material.

When the laboratory is not air-conditioned, excessive retention time variations (C.V.>10%) can occur on the purification ion-exchange columns. As a result, the purification columns should, as far as possible, be thermostatically controlled.

4. Conclusions

An automated column-switching LC system including on-line sample injection, and concentration and purification with a bonded-silica pre-column was developed to determine AFM1 in urine and milk. The main advantage of the proposed method is the reduction in sample handling by omitting the tedious liquid–liquid extraction step. Moreover, the useful urine or milk sample volume is low at less than 1.5 ml, and the sample pre-treatment time is short, not exceeding 5 min. In addition, the AFM1 detection limit is low, about 2.5 ng/l, and indeed less for 1 ml of injected biological sample. The possibility of reusing the silica support of the pre-column to concentrate the AFM1 with good extraction repeatability is another interesting aspect of the method, in contrast to the method of Farjam et al. [13] which uses a new immuno pre-column for every analysis, particularly with milk samples; milk components probably deteriorate the immunosorbent. Moreover, the use of silica support is also more practical than using the immunoaffinity support.

The method will be extended to looking for traces of AFM1 in other matrices than urine or milk such as agricultural produces (coffee, corn, pistachio nuts, etc.), and by modifying switch times, will be applied to determining AFB1 in urine.

Acknowledgements

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