Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1083 (2005) 127–132
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

# Determination of aflatoxin $B_1$ in sidestream cigarette smoke by immunoaffinity column extraction coupled with liquid chromatography/mass spectrometry

Leslie E. Edinboro, H. Thomas Karnes *

*Department of Pharmaceutics, Virginia Commonwealth University, School of Pharmacy, P.O. Box 980533, 410 N 12th Street, Richmond, VA 23298-0533, USA*

Received 6 April 2005; received in revised form 8 June 2005; accepted 9 June 2005

**Abstract**

Aflatoxins produced by food-borne molds are known carcinogenic toxins. Aflatoxin $B_1$ ($AFB_1$) is reported as the most toxic of this class of mycotoxins. We have coupled immunoaffinity column extraction with LC/MS to produce a sensitive and selective approach for the study of $AFB_1$. As $AFB_1$ can be potentially found in tobacco it is of interest to establish whether $AFB_1$ can be transferred from a cigarette fortified with $AFB_1$, to the sidestream smoke. Previous studies have found that $AFB_1$ does not transfer to the mainstream smoke. Since sidestream smoke may contain higher concentrations of some smoke components, a method was developed to analyze the sidestream smoke produced from machine-smoked cigarettes. Sidestream smoke condensates collected on Cambridge filter pads were extracted with isopropanol, then further purified using immunoaffinity extraction columns. The extracts were then analyzed by LC/MS and LC/MS/MS. An instrumental limit of detection (LOD) was established at 3.75 pg injected on column, with the limit of quantitation (LOQ) equal to 11.25 pg on column for both LC/MS and LC/MS/MS. The instrument was found to be linear from 11.25 pg to 150 pg ($r > 0.995$.) Precision ranged from 4.2% to 8.4% at the LOQ, while accuracy ranged from 0.53% to 1.33%. The immunoaffinity extraction method LOD was determined to be 100 pg fortified onto the Cambridge filter. The LOQ was 350 pg. The average recovery of the $AFB_1$ from the Cambridge pad was 82.9% over the range of 100–1000 pg fortified onto the pad. $AFB_1$ was not detected in unfortified cigarettes. A transfer experiment, fortifying cigarettes at 1 μg/cigarette determined that $AFB_1$ was transferred only slightly from the burning cigarette to the sidestream smoke. The mean percent transfer was 0.087%.


*Keywords:* Aflatoxin; Immunoaffinity; Sidestream smoke; LC/MS; *Aspergillus*

## 1. Introduction

Aflatoxins are produced by many species of microorganisms including the food-borne mold *Aspergillus flavus* [1]. Of the reported aflatoxins, aflatoxin $B_1$ ($AFB_1$) is the most toxic [2]. As aflatoxins have been found to be toxic, mutagenic and carcinogenic, much interest has been devoted to the analysis of $AFB_1$. The use of immunoaffinity columns for the extraction from biological and non-biological matrices has been reported [3–7]. Immunoaffinity columns utilize monoclonal antibodies covalently bonded to agarose beads to provide a selective extraction from interfering matrix components. Post immunoaffinity extraction detection has been reported for both chromatographic and non-chromatographic methods [3–7]. HPLC is by far the most reported chromatographic method using a variety of detection strategies. Post-column reaction with iodide or bromide coupled with fluorescence detection has yielded sensitive determinations of aflatoxins: these reactions and others have been extensively reviewed [8]. Liquid chromatography with mass spectrometric detection (LC/MS) has been reported as a sensitive and specific alternative to spectrophotometry methods without the need for post-column manipulations [6]. An LC/MS

* Corresponding author. Tel.: +1 804 828 3819; fax: +1 804 828 8359.
*E-mail address:* tom.karnes@vcu.edu (H.T. Karnes).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.06.032

method using an electrospray ionization (ESI) interface that was 10 times more sensitive than post-column derivatization with bromide and fluorescence detection for the analysis of $AFB_1$ in urine has been reported [6]. This report used immunoaffinity column extraction as well. Both single ion recording (SIR) using single quadrupole and multiple reaction monitoring (MRM) utilizing tandem quadrupoles have been reported. Atmospheric pressure photoionization (APPI) liquid chromatography/mass spectrometry has been reported as an alternative to ESI due to lower chemical noise and signal suppression caused by matrix effects [9].

Tobacco has been reported to contain aflatoxins if it is not properly cured to prevent the growth of the *Aspergillus* mold [10,11]. Aflatoxin $B_1$ concentrations of 43.53 ppb have been reported for chewing tobacco products in India [11]. Previous studies found that aflatoxins fortified into cigarette tobacco did not transfer into the mainstream smoke [12,13]. The high temperatures of the burning cigarette core are thought to degrade the aflatoxins prior to collection. It should be noted, however, that the limit of detection for the analytical methodologies used was 0.01 μg. Current detection levels for LC/MS/MS have been reported in the pg range (6.9). The sidestream smoke, which comes from the burning end of the cigarette, has not previously been tested. We therefore developed a method utilizing immunoaffinity column extraction of collected sidestream smoke samples followed by LC/ESI-MS detection for the analysis of $AFB_1$. The method was developed to provide the lowest limit of detection possible using both SIR and MRM modes of LC/MS/MS.

The use of an isotopically labelled internal standard was desired for this evaluation although none were commercially available. A procedure was developed to re-methylate aflatoxin P (AFP; the demethylated metabolite of $AFB_1$), using iodomethane-$^{13}$C-$d_3$ to produce $AFB_1$-$^{13}$C-$d_3$.

## 2. Methods

### 2.1. Chemicals

Aflatoxin $B_1$—1 μg/mL in methanol (Supelco, Bellefonte, PA, USA). Aflatoxin $B_1$—1 mg, aflatoxin P—10.0 μg, formic acid, ACS, 97.0% (Sigma, St. Louis, MO, USA). Iodomethane-$^{13}$C-$d_3$;99% (Aldrich, Milwauke, WI, USA). Acetonitrile (HPLC grade; Burdick and Jackson, Muskegon, MI, USA). Glacial acetic acid, isopropanol, acetone, diethyl ether, ammonium formate, sodium sulfate (anhydrous), potassium carbonate (anhydrous), and benzene were all ACS grade or better. Deionized water (di$H_2O$) was prepared by a Barnstead NANOpure Diamond water purification system.

### 2.2. Equipment

Aflatest P aflatoxin immunoaffinity columns (VICAM, Watertown, MA, USA). The smoking machine consisted of a controller, syringe pump and software: KC Automatic Smoker, V1.6.0 (KC Automation, Richmond, VA, USA). Fishtail chimneys, filter holders and Cambridge filters were supplied by Philip Morris (Richmond, VA, USA). Cigarettes used throughout the study were Kentucky Reference cigarette 2R4F, University of Kentucky, supplied by Philip Morris. A vacuum manifold, 24 position (Varian, Walnut Creek, CA, USA). Evaporator, Turbo-Vap LV (Zymark, Hopkinton, MA, USA).

### 2.3. LC system (LC)

Two Shimadzu LC10ADvp high-pressure pumps connected via a high-pressure, low-dead-volume mixing tee, system controller: SCL10Avp, solvent degasser: DGU-14A (Shimadzu, Columbia, MD, USA). Autosampler: LEAP CTC PAL-HTS (LEAP Technologies, Carrboro, NC, USA). Analytical column: 150 mm × 3.9 mm Nova-Pak Phenyl, 4 μm (Waters, Milford, MA, USA). Guard column: Phenomenex SecurityGuard ODS, 4.0 mm × 2.0 mm (Phenomenex, Torrance, CA, USA). Mobile phase composition: solvent A—67% and solvent B—33%. Solvent A: 0.05% formic acid (v/v) with 10 mM ammonium formate in di$H_2O$. Solvent B: 0.05% formic acid (v/v) in ACN. Flow rate was 0.75 mL/min utilizing a 1:10 split post column.

### 2.4. MS system (MS)

A Micromass Quattro LC triple quadrupole mass spectrometer with an ESI source was interfaced to the LC system. MassLynx 3.5 software was used to integrate the LC system, control data acquisition and data processing. The MS system was tuned using a 100 ng/mL $AFB_1$ solution in mobile phase directly infused into the ESI source at 10 μL/min. Conditions were established for the LC/MS selected ion recording (SIR) mode at 312.80 *m/z* for AFB1 and 317.00 for the internal standard. Multiple reaction monitoring (MRM, MS/MS) conditions (LC/MS/MS) for $AFB_1$ were optimized for the transition 312.80 → 240.90. As MRM was used for identification of $AFB_1$, no MRM conditions for the internal standard were developed.

### 2.5. Preparation of $AFB_1$-$^{13}$C-$d_3$ internal standard (IS)

One gram of sodium sulfate (anhydrous) was placed in a beaker and placed into a 150 °C vacuum oven for 24 h. Upon removal, it was cooled in a vacuum dessicator. The sodium sulfate was transferred to a funnel with filter paper. Ten milliliters of dry acetone (0.28% water) was passed through the sodium sulfate, immediately capped and placed in the dessicator. Fifty milligrams of potassium carbonate (anhydrous) was weighed into a reaction vial and placed into the vacuum oven for 1 h prior to use. After removal, it was placed in the dessicator. One milliliter of the dry acetone was placed into the vial containing the 10.0 μg of aflatoxin P. The vial was swirled for 1 min and the contents transferred to the reaction vial. 200 μL of iodomethane-$^{13}$C-$d_3$

was immediately added, a Teflon stirring bar inserted and the vial capped. The reaction vial was stirred for one minute at room temperature, 5 μL removed and directly injected on the LC/MS (operating in scan mode) to check progress of the reaction. This continued for 15 min until the product *m/z* 317.00 started to decrease. The reaction was immediately stopped at this point with 400 μL of acetic acid. The reaction vial was placed under a stream of nitrogen in a 40 °C water bath to remove the remaining acetone. One milliliter of diethyl ether was added to extract the IS. Five microliters of the ether extract was transferred to a glass tube, evaporated and reconstituted in mobile phase for analysis of purity. No contamination of the internal standard with 313 *m/z* (native $AFB_1$) was noted. Based upon response of injected $AFB_1$ standards, the internal standard was determined to have an approximate concentration of 2.7 μg/mL. As this compound was to be used as an internal standard, no further purification or quantitation was necessary. The remaining ether solution was stored at −18 °C.

### 2.6. Preparation of working analytical solutions

#### 2.6.1. Preparation of working internal standard solution, 20 ng/mL

To a 12 mm × 75 mm glass tube 7.4 μL of IS standard was transfered and then evaporated to dryness with nitrogen, but no heat. A 1.0 mL volume of 25:75 mobile phase A:ACN was added to the tube. The tube was capped, vortex mixed and then stored at −18 °C. The working IS solution was prepared fresh weekly.

#### 2.6.2. Preparation of calibrators for LC/MS/MS calibration

A seven point calibration curve was prepared in mobile phase A. The calibrators were prepared as pg on-column/injection (pg o/c). The range utilized was 3.75–150.0 pg o/c.

#### 2.6.3. Preparation of $AFB_1$ 100 μg/mL solution for transfer experiment

A solution of 97:3 benzene:acetonitrile was prepared. This solution was used to quantitatively transfer the contents of the solid $AFB_1$ (1.0 mg) to a 10.0 volumetric flask. The flask was then brought to the mark with 97:3. The solution was stored at −18 °C.

### 2.7. Collection of sidestream smoke

A computerized smoking machine was used to automatically smoke the cigarettes to ensure the reproducibility of the smoke pattern between cigarettes. A 2R4F cigarette was inserted 9 mm into a Cambridge filter holder, which was in turn connected to the syringe pump of the smoking machine. The smoke collected in this manner is known as the mainstream smoke. After ignition of the cigarette, the cigarette was inserted into a fishtail chimney apparatus for collection of the sidestream smoke. The top outlet of the fishtail chimney was connected, via two serially connected Cambridge filter holders, to a vacuum pump. A mass flow controller was used to maintain a constant 2.5 L/min flow from the top of the chimney through the Cambridge filters. Preweighed Cambridge filters were inserted into the holders prior to each smoking session. Three cigarettes were smoked in a single session and constituted a single smoke collection sample. The puff volume was set at $35.0 \pm 0.3\,cm^3$. The puff duration and interval were set at 2.0 s and 60 s, respectively. Each cigarette was smoked for nine puffs or until the burning edge reached approximately 3 mm from the filter.

After each session, the mainstream Cambridge filter was removed and weighed. The weight increase was required to be 12.0 ± 1 mg/cigarette. This ensured that the cigarettes used maintained the proper conditioning prior to use and the smoking conditions were consistent. Filters from sessions that did not meet this criterion were discarded. No further analysis was performed on the mainstream Cambridge filter and it was discarded. The sidestream Cambridge filters were removed and weighed. The primary (closest to the chimney) sidestream Cambridge filter was removed, weighed and transferred to a 50.0 mL polypropylene centrifuge tube and capped. The secondary sidestream Cambridge filter was weighed and discarded. The smoking apparatus was disassembled and the fishtail chimney was rinsed with successive aliquots of isopropanol (IPA). Each rinse aliquot was transferred to the centrifuge tube containing the primary sidestream filter. A total rinse volume of 25 mL was used for each chimney.

### 2.8. $AFB_1$ transfer experiment

Ten microliters of the 100 μg/mL AFB1 solution (1 μg $AFB_1$) was brought up in a 10 μL glass GC injection syringe. The syringe was inserted into the center of the non-filter end of the cigarette. The insertion continued length-wise into the cigarette until the tip of the syringe was approximately 5 mm from the filter. As the syringe was withdrawn from the cigarette, the AFB1 solution was dispensed in a uniform manner the length of the cigarette. The cigarette was stored at room temperature for 24 h prior to smoking to ensure the residual solvents had evaporated. The cigarettes were smoked as previously described.

### 2.9. Sidestream smoke extract analysis

The conical centrifuge tube containing the primary sidestream Cambridge filter pad with the 25 mL fishtail chimney wash was shaken for 1 h. The tube was then centrifuged at 2000 × *g* for 10 min. A 5.0 mL aliquot of the IPA was transferred to a second conical tube, 15.0 mL of deionized water added and the tube vortex mixed. An Alfatest immunoaffinity (IA) column was placed in the vacuum manifold and the storage buffer drained by gravity. Fifty milliliters plas-

tic reservoirs columns were attached to the IA column. All 20.0 mL of the IPA:water mix was applied to the IA column and a flowrate of 1–2 drops/s established. After the 20.0 mL had passed through the IA, it was washed with 10.0 mL of di$H_20$. The IA was then briefly dried to remove as much water as possible without drying out the column. The IA column was then eluted with 1.0 mL of ACN with 0.125 mL of mobile phase A added into a 12 mm × 75 mm glass tubes. Twenty-five microliters of working IS solution was then added to each collection tube, mixed and dried under nitrogen at 40 °C to approximately 200 μL. The 200 μL was then transferred to an autosampler vial and capped for LC/MS analysis. Seventy-five microliters of the extract was injected for LC/MS analysis.

### *2.10. Determination of the immunoaffinity extraction method LOD and LOQ*

After a smoke matrix sample has been collected as described in Section 2.7, the primary sidestream filter pad would be fortified with $AFB_1$ by transfer of the required amount of $AFB_1$ standard by pipet. The pad would then be equilibrated for 30 min prior to the extraction procedure described in Section 2.9.

## 3. Results

### *3.1. Specificity*

Three blank samples were prepared and analyzed for $AFB_1$ and IS (Fig. 1). None of the samples contained peaks that would interfere with the analysis. An infusion of $AFB_1$ (100 ng/mL stock solution) at 10 μL/min was established

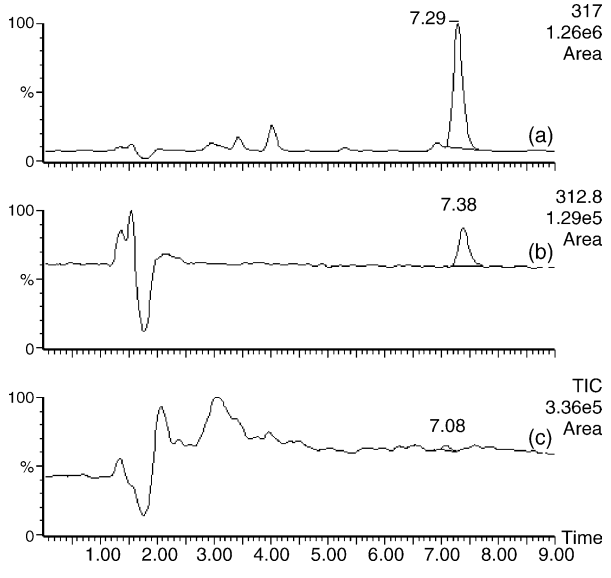


Fig. 1. Chromatograms of blank smoke matrix and injected $AFB_1$ standard: (a) internal standard, (b) $AFB_1$ (11.25 pg on column) and (c) blank smoke matrix.

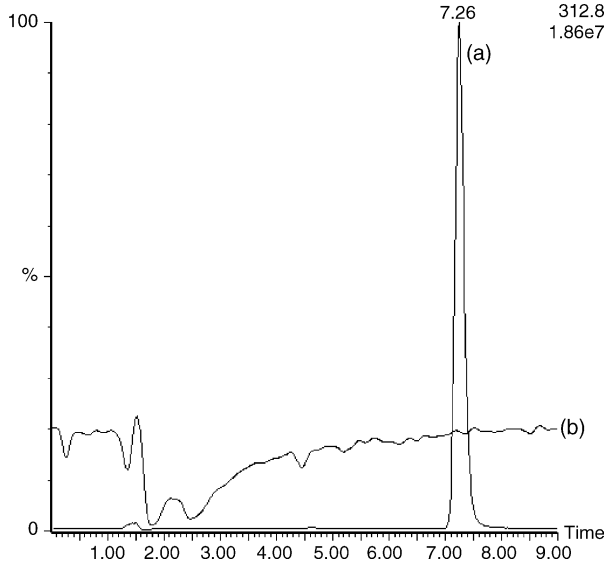


Fig. 2. (a) $AFB_1$ (37.5 ng) injected on column. (b) Chromatogram demonstrating ion suppression after injecting blank smoke matrix into a 1.0 μg/mL $AFB_1$ post-column infusion.

using a Harvard syringe pump. A blank sample was injected to establish the location of any ion suppression due to the matrix. Fig. 2 shows a chromatogram from the ion suppression/matrix effect experiment that is representative of a blank (non-fortified) smoke matrix. The chromatogram established that no significant ion suppression occurred at the retention time (RT) of $AFB_1$ or the IS (RT = 7.4 min).

### *3.2. Instrument linearity*

The calibration curves ($n = 9$) were evaluated over the range of 3.75 pg $AFB_1$ on column (o/c) to 150.00 pg o/c. Both external and internal standard calibrations were examined. Correlation coefficients ($r$) were greater than 0.99 for all calibration models examined (Table 1). The precision and accuracy of the back-calculated calibrators were all within acceptable limits of less than 20% (Table 1).

Table 1
LC/MS calibration parameters

| | SIR (IS) | SIR (external) | MRM (external) |
|---|---|---|---|
| $r$ | 0.9948 | 0.9953 | 0.9976 |
| LOD | 3.75 | 3.75 | 3.75 |
| Precision (%RSD) | 36.1 | 60.5 | 51.0 |
| Accuracy (%DFN) | 0.13 | 10.64 | 22.72 |
| LOQ | 11.25 | 11.25 | 11.25 |
| Precision (%RSD) | 4.2 | 8.4 | 4.4 |
| Accuracy (%DFN) | 1.33 | 0.89 | 0.53 |
| Upper limit of linearity (pg) | 150 | 150 | 150 |
| Precision of calibrators (%RSD) | 3.8–4.2 | 3.4–10.3 | 1.7–5.5 |
| Accuracy of calibrators (%DFN) | −1.29–1.45 | −3.95–0.99 | −2.67–1.71 |

### 3.3. Instrument limit of detection

An initial instrumental LOD was calculated based upon integration of noise in the elution window of the $AFB_1$ from the specificity blanks. The calculation based on three standard deviations of the blank noise yielded a LOD of 0.3 pg on o/c. The experimental injected concentration of $AFB_1$ that produced a peak that was approximately 3:1 was 3.75 pg o/c, so this value was established as the experimental instrumental LOD. While the accuracy was less than 20% difference from nominal (DFN), the precision was greater than 20% relative standard deviation (RSD), for both internal and external standard calibrations (Table 1).

### 3.4. Instrument limit of quantification

The limit of quantification (LOQ) was estimated as three times the LOD or 11.25 pg o/c $AFB_1$. Precision at the LOQ ranged between 4.2% and 8.4% RSD, respectively for the internal and external calibration. Accuracy ranged between 0.53% and 1.33% DFN for the same calibration models.

### 3.5. Immunoaffinity extraction limit of detection

Preliminary experiments to determine the LOD with the extraction procedure incorporated were started at an $AFB_1$ amount that yielded an injected amount of $AFB_1$ equivalent to the experimental LOD of 3.75 pg o/c. The amount of $AFB_1$ was incrementally raised until the signal-to-noise was just greater than 3:1. The final extraction method experimental LOD was therefore established to be 100 pg fortified onto the pad (o/p), Fig. 3. The precision ($n = 3$) at the extraction LOD was 7.6% for SIR and 1.6% for MRM. This is a practical limit involving real matrix background and the improvement in precision was likely due to less background noise with the MRM resulting in better integration of peaks The ratio of SIR/MRM was $1.024 \pm 0.0835$, RSD = 8.1%. This would indicate that the SIR mode was measuring $AFB_1$ without significant interference from other compounds extracted from the sidestream smoke matrix.

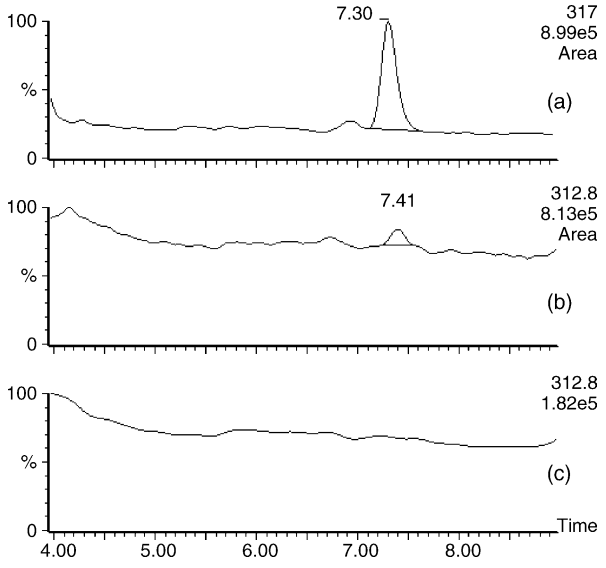


Fig. 3. Chromatogram of extracted smoke matrix with 100 pg $AFB_1$ fortified onto a Cambridge filter pad: (a) internal standard, (b) $AFB_1$ and (c) extracted blank smoke matrix.

### 3.6. Immunoaffinity extraction limit of quantitation in matrix

The extraction method LOQ was evaluated at 3.5 times the extraction method LOD, 350 pg $AFB_1$ o/p. At this amount, the signal-to-noise was found to be greater than 10:1. Precision at the extraction LOQ was evaluated by analyzing six replicates of sidestream smoke samples in three separate analytical runs ($n = 18$). The intra-run precision ranged between 6.5% RSD and 11.3% RSD within each of the three analytical runs. The inter-run precision was 19.1% RSD. The ratio SIR/MRM was $1.097 \pm 0.0836$, RSD = 7.6%.

### 3.7. Immunoaffinity extraction method recovery

Comparing the actual response from sidestream smoke samples to unextracted standards provided a means to assess recovery of the immunoaffinity extraction method. Recovery was assessed at 100 pg o/p, 350 pg o/p and 1000 pg o/p. The average recovery was found to be $82.93 \pm 3.569\%$, RSD = 4.3% across all amounts (Table 2). The 1000 pg o/p demonstrated a significantly greater recovery than the 100 or 350 pg o/p samples.

### 3.8. $AFB_1$ transfer experiment

The transfer experiment was conducted in triplicate. The initial results exceeded the upper limit of linearity, so the samples were repeated using a dilution, of 1:10 versus 1:5 after the initial extraction with IPA. The average percent transfer of $AFB_1$ from the fortified cigarettes to the sidestream smoke was $0.087 \pm 0.0107\%$, RSD = 12.3%. Fig. 4 is a representative chromatogram for the transfer experiment. No $AFB_1$ was detected in the sidestream smoke of cigarettes that were not fortified with $AFB_1$.

### 3.9. Carryover

Injection of blank solvent following injection of a 5 ng of $AFB_1$ was used to assess carryover. No $AFB_1$ was detected in the solvent following injection indicating a lack of carryover.

Table 2
Immunoaffinity extraction recovery

| o/p (pg) | Mean | SD | RSD (%) |
|---|---|---|---|
| 100 | 83.82 | 4.46 | 5.32 |
| 350 | 82.03 | 3.09 | 3.77 |
| 1000 | 96.27 | 2.24 | 2.33 |

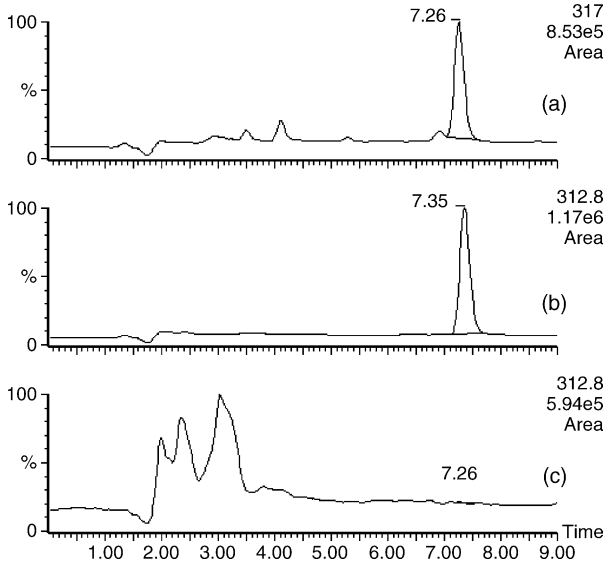


Fig. 4. Chromatogram from $AFB_1$ transfer experiment: (a) internal standard, (b) $AFB_1$ and (c) blank extracted smoke matrix.

## 4. Conclusion

A bioanalytical method for the determination of $AFB_1$ in cigarette sidestream smoke was developed. Immunoaffinity solid phase extraction has been effectively used in this work to remove the numerous compounds present in sidestream cigarette smoke that are known to cause matrix ion suppression in LC/MS analysis. While the method demonstrated good selectivity with an apparent lack of ion suppression, some loss of response was noted. This loss could not be accounted for by adjusting for recovery. Previous reports had shown $AFB_1$ not to be transferred to mainstream smoke so the method was developed with a focus on achieving the lowest limit of detection possible in anticipation of potentially low levels. The final method provided for detection of 33 pg of $AFB_1$ in an individual cigarette. This level of detection would provide for the evaluation of $AFB_1$ approximately three orders of magnitude lower than the amount of $AFB_1$ reported to be present in one gram of contaminated chewing tobacco. We fortified control cigarettes at a level of 1 μg of $AFB_1$ per cigarette to represent what might be considered a "worst case" scenario of *Aspergillus* mold contamination. The current method was therefore capable of detecting five orders of magnitude less than this fortification amount. Approximately 0.09% was transferred into the sidestream smoke from these fortified cigarettes but no $AFB_1$ was detected in unfortified cigarettes. The transfer precision was found to be 12.9%, indicating some stability during the smoking and transfer process. Further investigation is needed to establish the amounts of $AFB_1$ expected in *Aspergillus* contaminated cigarettes and normal cigarettes maintained under commercial cigarette storage conditions. The minimum amount of $AFB_1$ per cigarette that would result in detectable levels of $AFB_1$ in side stream smoke ("breakthrough") also needs to be determined.

## Acknowledgements

This study was funded by a grant from Philip Morris USA, Richmond, VA, USA. Philip Morris USA also provided the smoking instrumentation necessary to perform this study.

## References

[1] C.W. Hessletine, O.L. Shotwell, J.J. Ellis, R.D. Stubblefield, Bacteriol. Rev. 30 (1966) 795.
[2] R. Schoental, Ann. Rev. Pharmacol. 7 (1967) 343.
[3] J.D. Groopman, K.F. Donahue, Aflatoxin J. Assoc. Off. Anal. Chem. 71 (1988) 861.
[4] A.A. Candlish, J.E. Smith, W.H. Stimson, Biotechnol. Adv. 7 (1989) 401.
[5] M.W. Trucksess, M.E. Stack, S. Nesheim, S.W. Page, R.H. Albert, J. Assoc. Off. Anal. Chem. 74 (1991) 81.
[6] A. Kussak, C-A. Nilsson, B. Andersson, Rapid Commun. Mass. Spectrom. 9 (1995) 1234.
[7] A. Kussak, B. Andersson, K. Andersson, J. Chromatogr. B 672 (1995) 253.
[8] W.T. Kok, J. Chromatogr. B 659 (1994) 127.
[9] M. Takino, T. Tanaka, K. Yamaguchi, T. Nakahara, Food Addit. Contamin. 21 (2004) 76.
[10] O.M.O. El-Maghraby, M.A. Abdel-Sater, Zentrabl. Mikrobiol. 148 (1993) 253.
[11] R.J. Verma, A.S. Kolhe, H.C. Dube, Proc. Natl. Acad. Sci. India 65 (1995) 167.
[12] T. Tso, T. Sorokin, Beitr. Tabakforsch. 4 (1967) 18.
[13] E.J. Kaminski, J.C. Lazanas, L.L. Wolfson, O.E. Fancher, J.C. Calandra, Beitr. Tabakforsch. 5 (1970) 189.