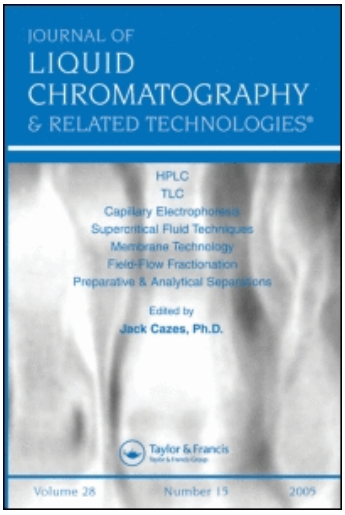

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W. Jeffrey Hurst[a]; Robert A. Martin Jr.[a]; C. H. Vestal[b]
[a] Hershey Foods Corporation Technical Center, Hershey, Pennsylvania [b] Vestec Corporation, Houston, Texas

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THE USE OF HPLC/THERMOSPRAY MS FOR THE CONFIRMATION OF AFLATOXINS IN PEANUTS

W. JEFFREY HURST[1], ROBERT A. MARTIN, JR.[1]
AND C. H. VESTAL[2]
[1]*Hershey Foods Corporation Technical Center*
1025 Reese Avenue
P.O. Box 805
Hershey, Pennsylvania 17033-0805
[2]*Vestec Corporation*
9299 Kirby Drive
Houston, Texas 77054

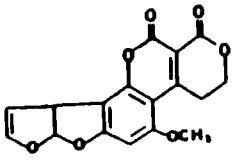
Abstract

An HPLC/Thermospray MS method is described for the determination of aflatoxins B_1, B_2, G_1 and G_2 in peanut extracts. Samples are extracted and prepared for analysis using an SPE method. The final determination utilizes reversed phase HPLC with a thermospray MS detector. The use of the MS allowed for unequivocal identification of aflatoxins in the extracts.

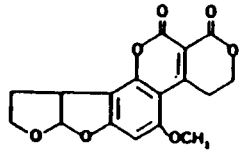
Introduction

Likely the most analyzed of the mycotoxins is aflatoxin in its various forms. Figure 1 provides structures of the aflatoxins of interest in this study. Since it's discovery in the 1960's as the Turkey X Virus (1) there has been extreme interest in developing methodology to allow it's accurate and precise analysis. Early methods centered on the use of TLC to separate the

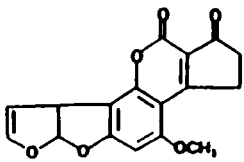
Aflatoxin G_1 (MW 328)



Aflatoxin G_2 (MW 330)



Aflatoxin B_1 (MW 312)



Aflatoxin B_2 (MW 314)

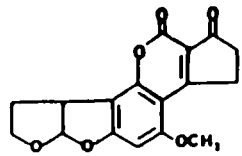


Figure 1. Aflatoxin Structures

various aflatoxins with the final quantitative step accomplished by a visual inspection or through the use of a scanning densitometer. Many of these TLC methods are presently being used and have official regulatory status (2-4). With the introduction of HPLC in the early 1970's, there was increased emphasis on this methodology since HPLC offered several distinct advantages over the TLC methodology such as better accuracy and precision. It also offered the analyst a lower level of detection. The early HPLC methodology offered detection limits of about 20 ng total aflatoxins while more recent developments permits the detection of less than 100 pg total aflatoxin (5-9).

Method developments were modernized with the emphasis on improvements in sample preparation and cleanup. These developments centered on the elimination of large packed columns to isolate the aflatoxins of interest, and the use of disposable solid phase extraction (SPE) columns (10-13). This manuscript reports the use of SPE columns to isolate the aflatoxins from a peanut sample and the use of Thermospray MS technique as the detection system.

Experimental

Sample Preparation

Aflatoxins were extracted from artificially contaminated peanut samples using a methanol/water mixture prior to cleanup on series-connected SPE columns. The method that was used has been previously published (11) and will not be repeated in this manuscript.

HPLC System

The HPLC system consisted of a Model 600 Solvent Delivery System (Waters), a Model 712 WISP Autoinjector (Waters) and a Model 490 UV Detector (Waters). The interface used was a Model 201 Dedicated Thermospray Mass Spectrometer (Vestec). The interface conditions used were T aux = 318°C, T Block = 290°C , T tip = 185°C. The interface was optimized for the mobile phase of interest. The HPLC column used was an Altex Ultrasphere ODS (5 μm) (4.6 mm ID x 25 cm) with a mobile phase of 0.1M Ammonium Acetate/Methanol/ Acetonitrile 56/22/22 (v/v/v) at a flow rate of 1.0 ml/min.

Standards

The standards for aflatoxins B_1, B_2, G_1 and G_2 were obtained from Sigma Chemical Company and stored frozen until needed. Aliquots were withdrawn, evaporated under nitrogen and diluted with methanol. These working standards were refrigerated when not being used.

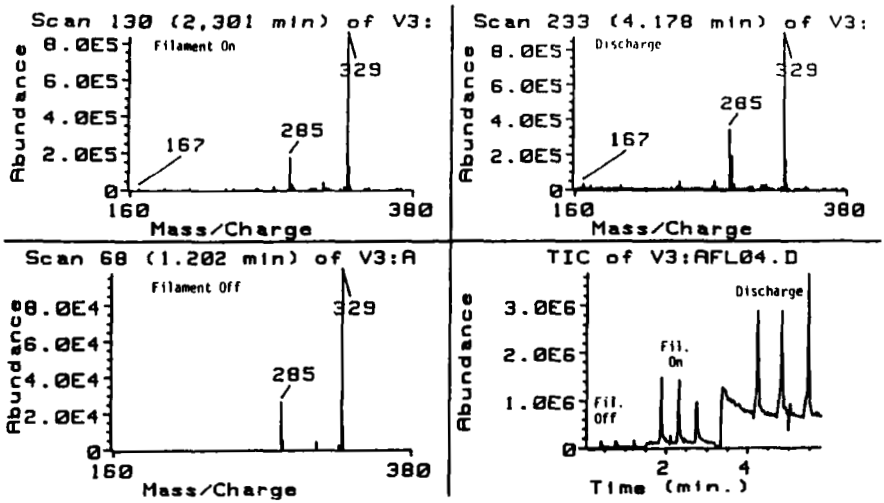


Figure 2. Mass Spectra of Aflatoxin G1 in Three Thermospray Ionization Modes

Analysis

Aliquots of both sample and standard were injected onto the HPLC column using the MS interface conditions specified previously. Additionally, standard compounds were injected under thermospray ionization, filament on and discharge ionization mode to determine the most appropriate conditions for these compounds.

Results

A series of studies were conducted using both the four standard aflatoxin compounds and also the peanut extract. After the establishment of the correct MS interface conditions that would allow for the determination of the aflatoxins, three modes of ionization were investigated. These modes were thermospray (filament off), filament on and discharge; each has its own distinct set of advantages which will not be discussed in this manuscript. Several references provide this information for interested parties (14-17).

Table 1. SIM and Full Scan Lower Limits of Detection

COMPOUND	FULL SCAN DETECTION LIMITS	SIM DETECTION LIMITS
Aflatoxin B_1	800 pg	60 pg
Aflatoxin B_2	500 pg	40 pg
Aflatoxin G_1	2 ng	100 pg
Aflatoxin G_2	2 ng	100 pg

Figure 2 illustrates the results of these studies for the injection of 1 µg of aflatoxin G1. The results indicated by monitoring at 329 (MH^+) and 285 ($MH^+ - 44$) show that both filament on and discharge provide a substantial increase in sensitivity. The remaining data in this manuscript were developed in the filament on mode. Additional studies were conducted for the determination of lower detection limits under limited scan conditions (260-380 AMU) and selected ion monitoring (SIM) conditions. This data is summarized in Table 1.

Figure 3 illustrates the separation of these four compounds at a concentration of 300 pg for each toxin using SIM. The total analysis time is less than 12 minutes. Figures 4-6 provide the thermospray mass spectrum necessary to allow for the confirmation of the peaks for B1, B2 and G2. Data on G1 can be seen in Figure 2. The mass spectra of the aflatoxins B1 and B2 indicate very strong base peaks a 313 and 315 respectively which correspond to the MH^+ peak. The spectra for aflatoxins G1 and G2 have much stronger peaks at 285 and 287 respectively which correspond to $MH^+ - 44$ peaks in addition to

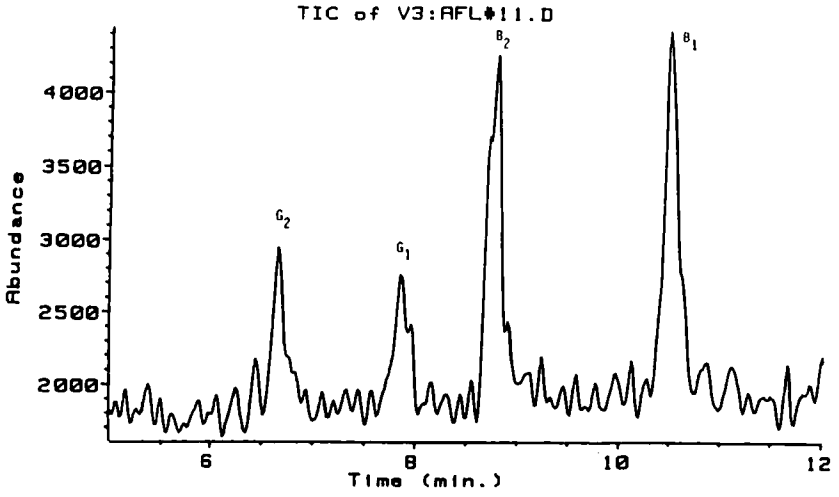


Figure 3. 300 pg of Aflatoxins B_1, B_2, G_1 and G_2 Using SIM (Selected Ion Monitoring)

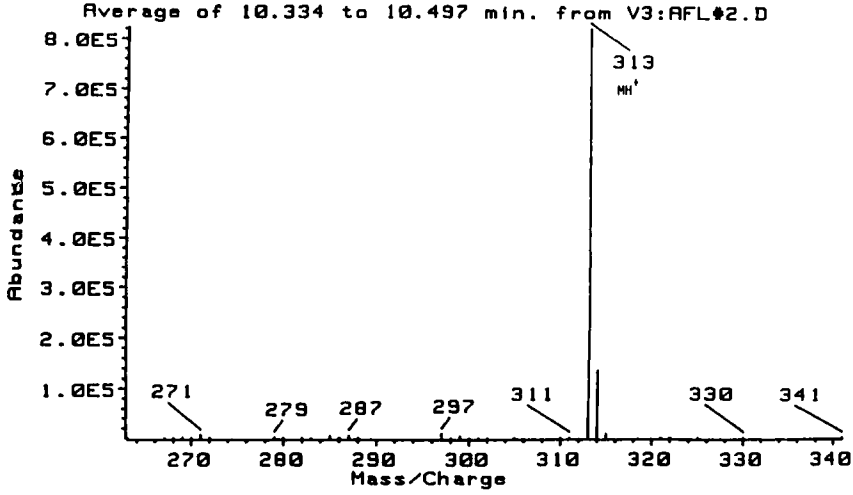


Figure 4. Thermospray Mass Spectrum of Aflatoxin B_1

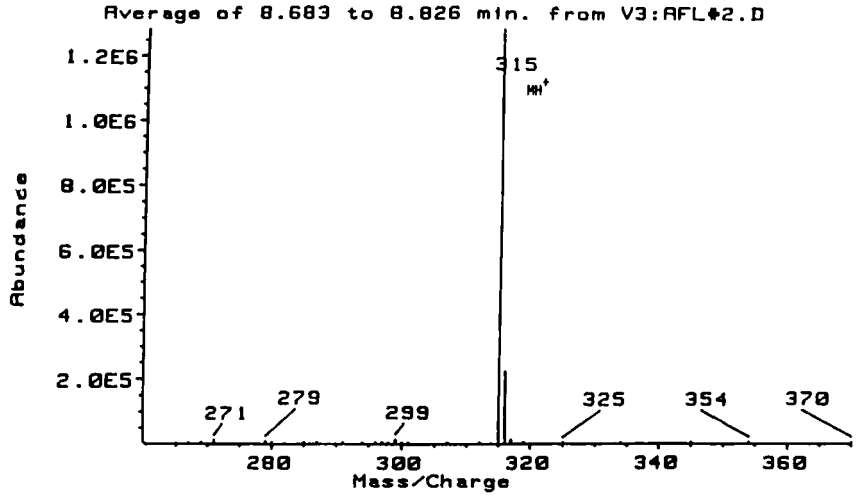


Figure 5. Thermospray Mass Spectrum of Aflatoxin B_2

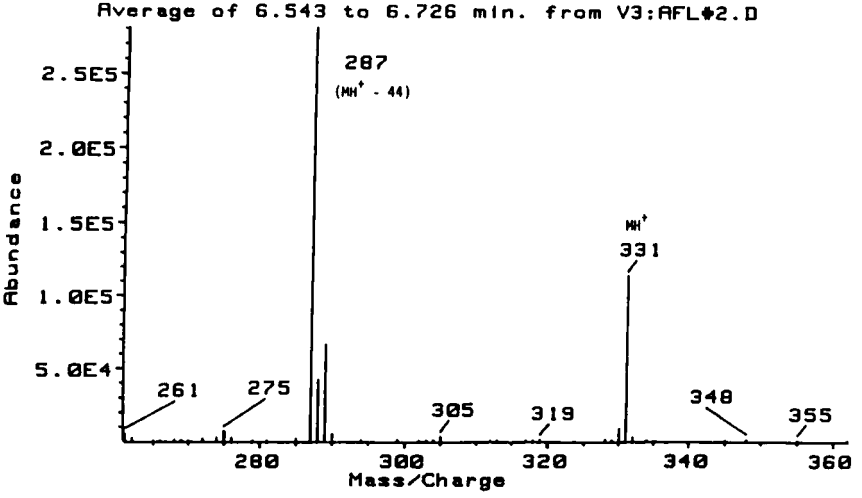


Figure 6. Thermospray Mass Spectrum of Aflatoxin G_2

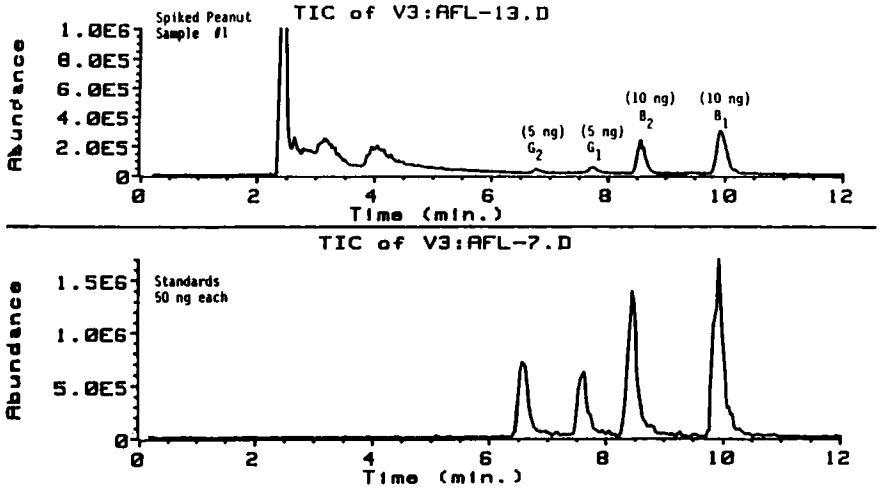


Figure 7. Analysis of Aflatoxins B_1, B_2, G_1 and G_2 in Spiked Peanut Extract (Top) Compared with Standard Injection (Bottom)

providing the characteristic MH^+ peaks at 329 and 331. Finally the technique that was developed was evaluated on a spiked peanut extract. The result of this experiment can be seen in Figure 7.

Conclusions

This study indicates that thermospray MS coupled with SPE techniques will allow for the positive identification of aflatoxins. While these experiments did not evaluate all of the possible variables such as concentration of buffer or the use of a postcolumn pumping system as has been done by others (18,19), it does provide the necessary information to allow it to be used on a routine basis. It also illustrates that LC/MS is a valuable confirmatory technique and shows continued promise for the determination of aflatoxins in various.

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