AppNote 6/2007

Improving MS Detection of Aflatoxins using Automated Solid Phase Extraction and Derivatization coupled with an LC/MS System

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Introduction

Aflatoxins are metabolites from molds such as *Aspergillus flavus* and *Aspergillus Parasiticus*. Aflatoxins are classified as mycotoxins, they are among the most potent human carcinogens, found mainly in foods and feed of plant origin. High concentrations of aflatoxins have been found, for example, in pistachios, figs and cereals and aflatoxins have also been found in dairy products. Due to the high toxicity of aflatoxins, EU legislation specifies very low acceptable daily intakes and maximum residue limits (0.05-15 µg/kg).

Of 17 known Aflatoxins, four are mainly relevant: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 & Aflatoxin G2 (Figure 1). This application note describes the configuration and operation of an LC/MS system combined with automated solid phase extraction and analyte derivatization for determination of the four aflatoxins listed above. Using the combined system, lower detection limits and improved chromatographic separation were achieved.

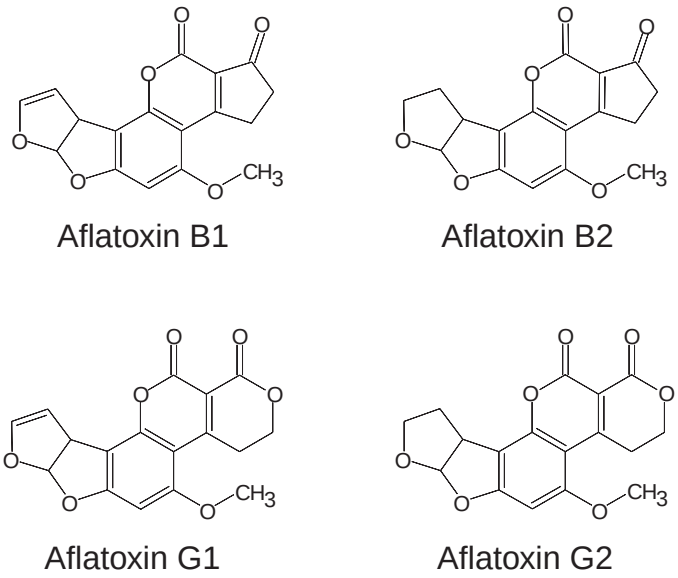

Figure 1. Chemical structures of aflatoxins B1, B2, G1 & G2.

EXPERIMENTAL

Sample preparation and derivatization. The clean-up and derivatization steps were performed using a GERSTEL SPE system, based on the GERSTEL MultiPurpose Sampler (MPS), a combined autosampler and sample preparation robot (Figure 2). The complete system was controlled using the GERSTEL MAESTRO software integrated with the Agilent Technologies ChemStation software.

Solid phase extraction (SPE) was performed using an SPE affinity column (LCTech AflaCLEAN™, 3mL) to separate aflatoxins from sample matrix. Following SPE clean-up, a derivatization step was performed in which aflatoxins B1 and G1 were brominated by stirring the extract with 3 % solution of bromine in chloroform.

Method for automated SPE clean-up and derivatization.

SPE steps:

MOVE	Transport sample vial into SPE vial position
MOVE	Transport cartridge to the SPE waste position
ADD 1	Add 4 mL sample to the cartridge; flow: 50 µL/s
ADD 2	Rinse cartridge with 20 mL H_2O; flow: 50 µL/s
SPE SHIFT	Slide SPE carriage with cartridge from SPE waste to SPE vial position
ADD 3	Elute aflatoxins with 0.5 mL MeOH; flow: 30 µL/s
WAIT	Wait 30 seconds for complete eluent transfer
MOVE	Discard SPE cartridge into the cartridge disposal container
SPE SHIFT	Slide SPE carriage with cartridge from SPE vial to SPE waste position

Derivatization

MOVE	Move eluate collection vial from SPE vial position to agitator tray
ADD	Add derivatization solution (bromine/ $CHCl_3$ 3%)
MIX	Mix for 2 minutes (derivatization)
MOVE	Move eluate collection vial to the tray for sample introdution
INJECT	Sample introduction to LC/MS system

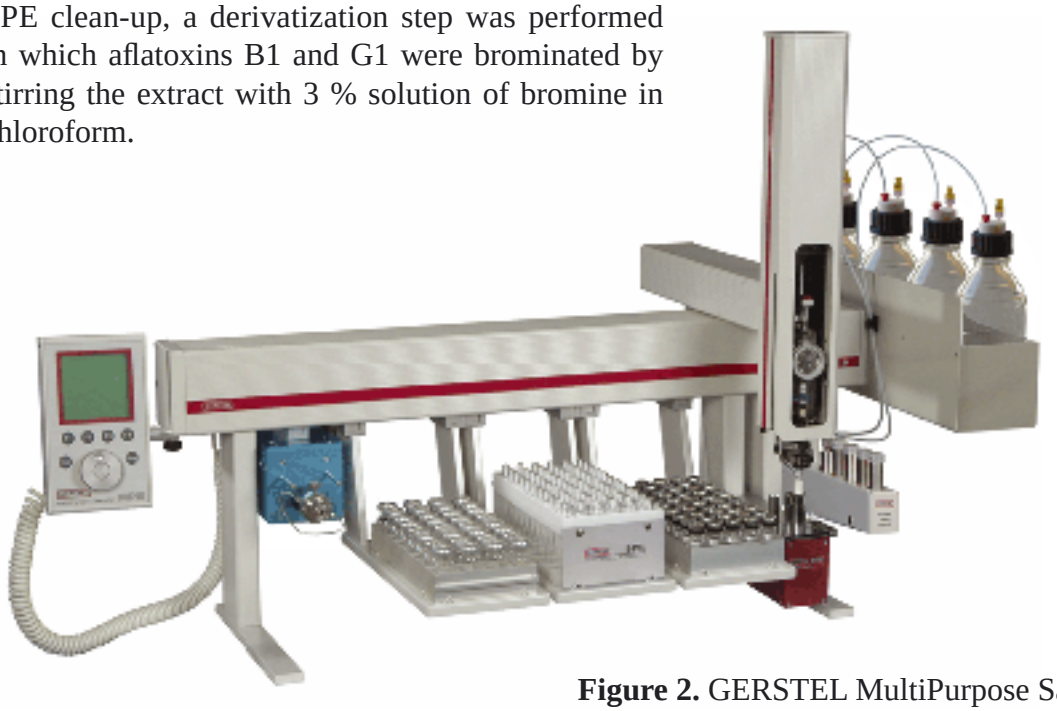

Figure 2. GERSTEL MultiPurpose Sampler MPS with SPE.

LC/MS Method. The GERSTEL SPE was integrated with an Agilent 1100 LC/MSD system, consisting of a binary pump, thermostatted column compartment, diode array detector and a single quadrupole Mass Selective Detector (MSD). The MSD was used with electrospray ionization in positive ion mode. Chromatographic separations were performed on a Phenomenex Max RP (250 x 2.1mm, 5µm), using a flow rate of 0.3 mL/min in gradient mode (Eluent A: 0.1% formic acid, Eluent B: Acetonitrile). Complete system control (including the autosampler) and data evaluation were performed using the Agilent ChemStation (Rev.A10.03) with integrated GERSTEL MAESTRO software.

Results and Discussion

The method was developed mainly for the determination of aflatoxins B1, B2, G1 and G2. After the clean-up step on an SPE affinity column, the aflatoxin compounds with an isolated, non-conjugated double bond (B1 and G1) were brominated (Figure 3) by stirring the extract with 3 % bromine in chloroform. MS detection (Figure 4) indicated that bromination resulted in the formation of 1-methoxy-2-bromo substituted compounds only as shown in figure 3. Under the chosen experimental conditions dibromo-aflatoxins were not detected.

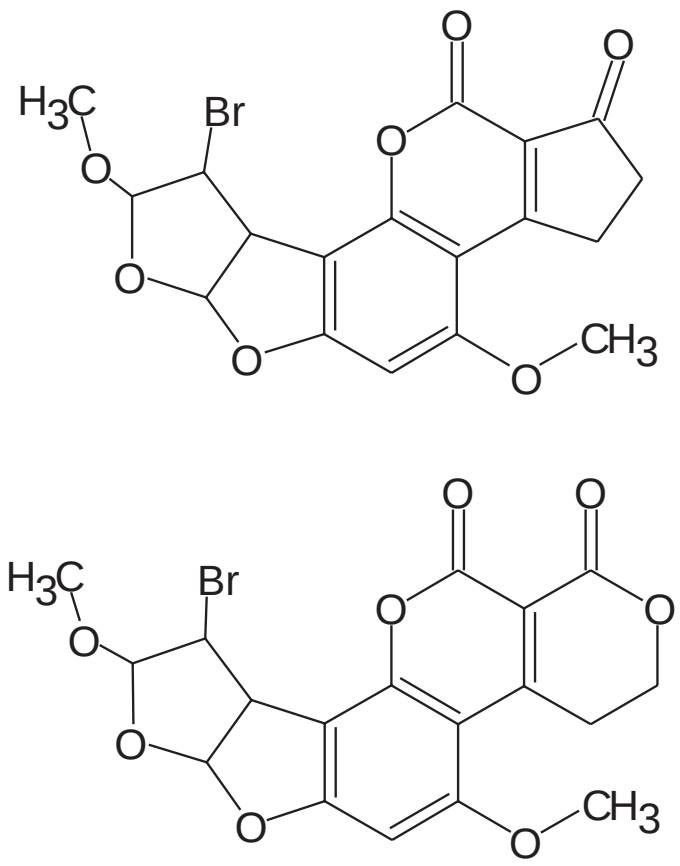

Figure 3. Chemical structures of brominated aflatoxins B1 and G1.

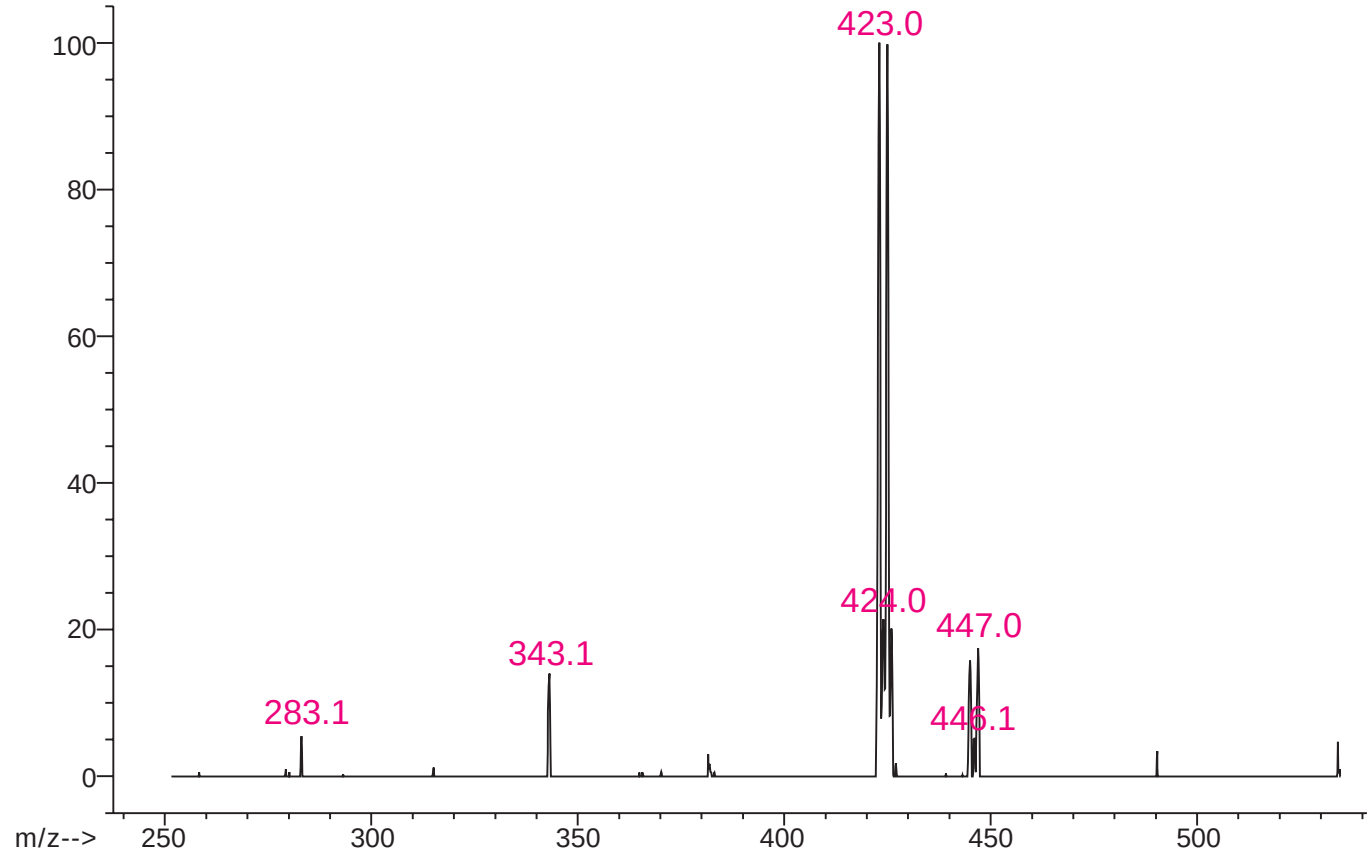

Figure 4. Mass spectrum of monobrominated aflatoxin B1.

The mono-brominated compounds (Figure 3) exhibited longer retention times in reversed phase chromatography than the non-brominated species, enabling baseline separation for the four aflatoxins and minimizing interference from residual matrix (Figure 5). More importantly, the derivatization resulted in a significantly improved MS response, combined with a characteristic bromine pattern in the mass spectra (Figure 4) for improved selectivity. The combined system delivered detection limits below 0.01 µg/kg for aflatoxins B1, B2, G1 and G2 using a single quadrupole system. Reproducibility data are shown in figure 6. The relative standard deviations were 3.4 and 5.3 % respectively for the brominated species of aflatoxins G1 and B1 based on twelve replicates. The extraction recoveries for the two monobrominated aflatoxins were 89.5 and 90.3% respectively.

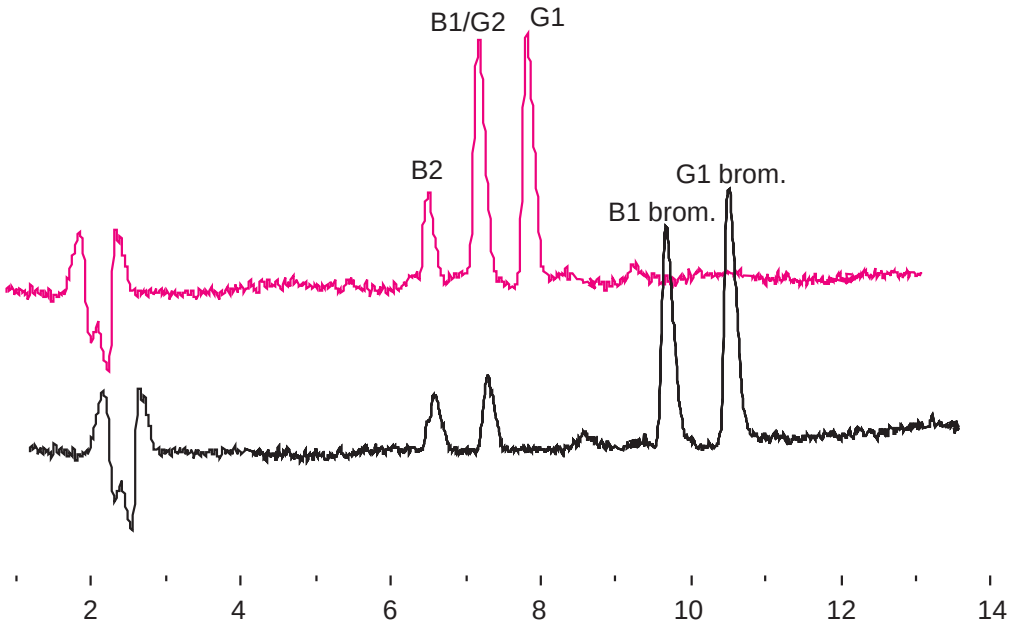

Figure 5. TICs of aflatoxins before (red) and after derivatization (black).

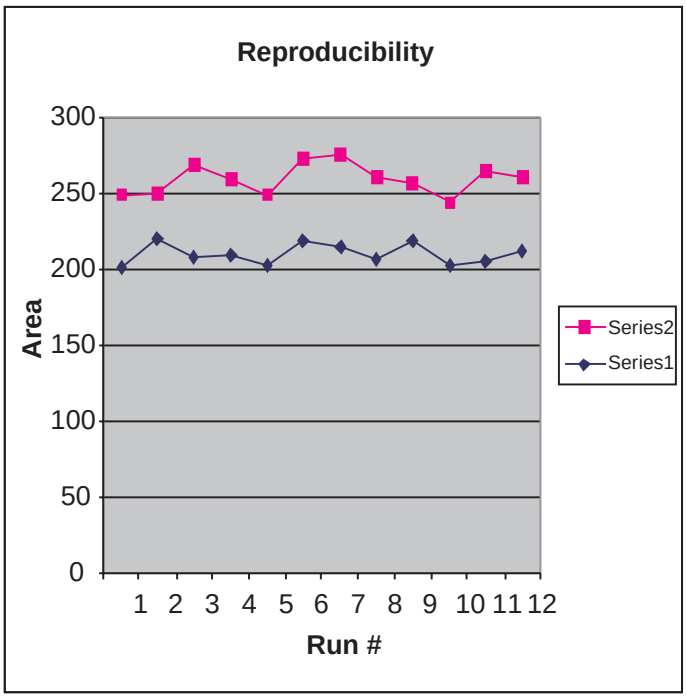

Figure 6. Reproducibility for the derivatited compounds.

Conclusions

The described LC/MS system with the integrated GERSTEL SPE system enables automated clean-up, derivatization and sample introduction for fully automated determination of aflatoxins B1, B2, G1 and G2. Bromination of aflatoxins B1 and G1 results in improved chromatographic resolution, less interference from residual matrix and significantly better MS response.

The method is robust and stable (RSDs 3.4-5.3 %) and provides good extraction recoveries (89.5-90.3%). Automation of the SPE and sample preparation process provides time savings in excess of 50 % while limiting exposure of lab personnel to solvents and reagents and thus helping to ensure a safer work environment.

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