ApplicationNOTE

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THE ANALYSIS OF MILK AFLATOXINS BY HPLC USING FLUORESCENCE DETECTION

Mark E. Benvenuti, Waters Corporation

INTRODUCTION

Aflatoxins are secondary metabolites of molds which can be found in various food matrices. The more common aflatoxins, which include G2, G1, B2, and B1, have been identified as contaminants in cattle feed. On ingestion, aflatoxins B2 and B1 are metabolized to M2 and M1, potentially adulterating dairy products.

This note demonstrates the analysis of the three families of aflatoxins using HPLC and fluorescence detection. The fluorescence of G1 and B1 is enhanced using electro-chemically generated bromine.

The European Union has set a limit for aflatoxin M1 of 0.05 µg/kg for consumable milk and 0.025 µg/kg for infant formula. These limits are easily within detectable limits using a Waters® Alliance® HPLC System with a 2475 Scanning Fluorescence Detector.

EXPERMIMENTAL

Analytical Conditions for Aflatoxins (Electrolytic Bromine)

Eluent: 60:25:15 water:methanol:acetonitrile to which has been added 0.119 g KB and 87.5 µl HNO_3 per liter (filter and degas).
Note: electrolytic bromine is produced by use of a Kobra Cell (Rhone - Diagnostic Technologies Ltd.) using the 100 µa setting. A Waters RXN 1000 Reaction Coil is inserted between the outlet of the cell and the inlet of the fluorescence detector to allow time for the derivitization reaction.

Alliance 2695 HPLC System

Column:	Symmetry® C_{18}, 4.6 X 150 mm,3.5 µ
Flow rate:	1.0 ml /min (Isocratic)
Column Temp.:	30 °C
Injection Vol:	50 µl

2475 Fluorescence Detector

Excitation:	365 nm
Emission:	455 nm
Analog Out:	Sample Energy
Scaling Factor:	10
Filter:	Digital (Hamming) @ 20 sec.

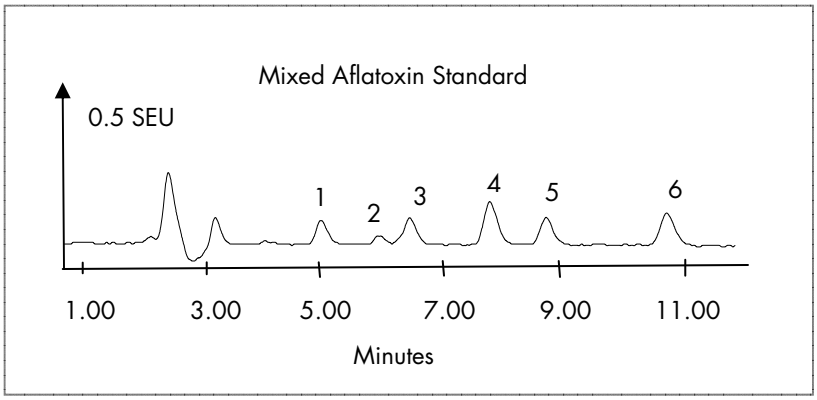

Figure 1. Mixed Aflatoxin standard (µg/L).1: M2- 0.020, 2: M1- 0.020 3: G2-0.006, 4: G1-0.020, 5: B2-0.006, 6: B1-0.020. * Regulated by the European Union.*

Milk Sample Prep

- Add 1 g NaCl to 40 ml fluid milk sample, mix well
- Carefully remove skim portion for analysis
- Pass 25 ml of sample through an Afla Test (Vicam) column at a rate of 1-2 drops/second
- Wash column with 10 ml of 90:10 water:methanol
- Elute aflatoxins by slowly passing 1 ml of methanol through the column
- Add 1 ml of water to eluate, mix well and inject

Spiking Procedure for Milk Sample

- Spike 50 ml milk with known amounts of aflatoxins
- Dilute 5 ml of spiked milk to 50 ml with water
- Add 1 g NaCl to 40 ml diluted spiked milk, mix well
- Pass 20 ml of diluted spiked milk through an Afla Test column at a rate of 1-2 drops/second
- Wash column by passing 10 ml of 90:10 water:methanol through column at a rate of 2 drops/second
- Elute aflatoxins by passing 1 ml of methanol through the column at a rate of 1-2 drops/second
- Add 1 ml of water to eluate, mix well and inject

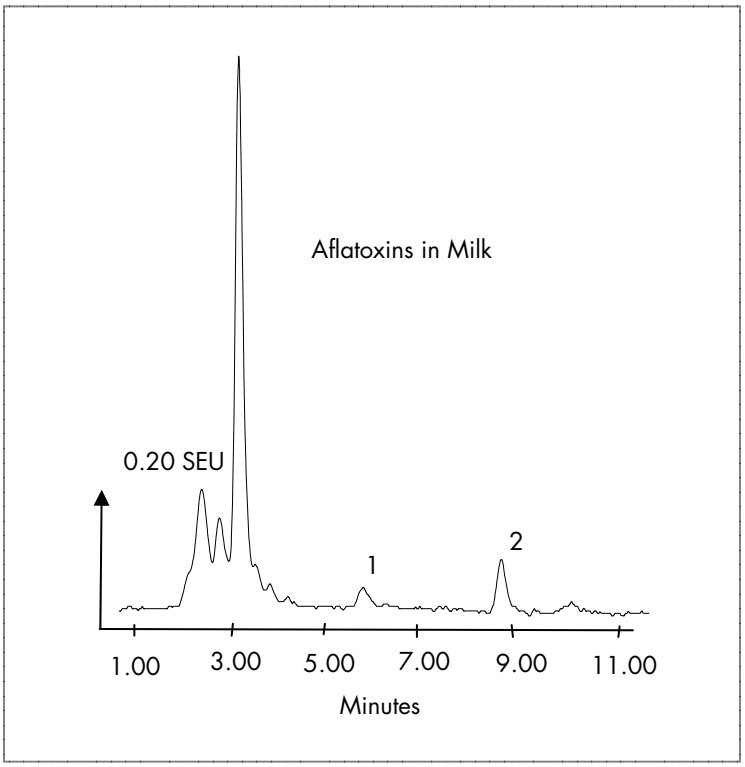

Figure 2. Aflatoxins in milk (µg/L) 1: M1- 0.0039, 2: B2- 0.0006.

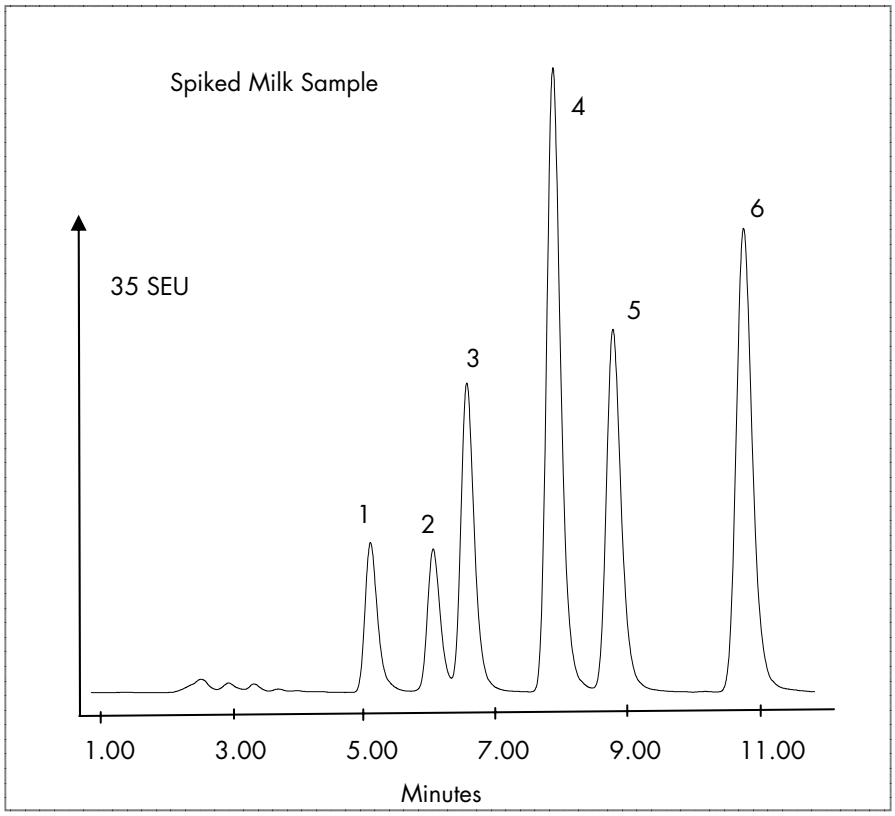

Figure 3. Spiked aflatoxins in milk (µg/L). 1: M2- 2.0, 2: M1- 0.6, 3: G2- 0.6, 4:G1- 2.0, 5: B2- 0.6, B1- 0.6.

Aflatoxin	Spike Amount	Recovery Amount	% Recovery
M2	2.0	0.986	49.4
M1	2.0	2.593	129.5
G2	0.6	0.622	103.4
G1	2.0	2.347	117.4
B2	0.6	0.691	115.2
B1	2.0	2.330	116.5

Table 1. Recovery data for spiked milk sample.

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Note: The low recovery of M2 could be due to a spiking error. The recoveries of the other aflatoxins are within expected ranges.

Note: Dairy samples with high fat content may require more extensive sample preparation. For further information on this and the AflaTest cartridges, please refer to Vicam's AflaTest Instruction Manual available from:

Vicam
313 Pleasant St.
Watertown,MA 02472
800-338-4381
www.vicam.com

Software

Data was acquired and processed with Waters Empower™ Software.

CONCLUSION

The Waters Alliance HPLC System and 2475 Fluorescence Detector can easily meet the desired detection limits for aflatoxins in dairy products. While electrochemical derivitization is described here, photochemical UV and post column addition of aqueous saturated Iodine are acceptable alternative methods for aflatoxin derivitization.

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

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