Customer Application Note: 115

Clean-Up and Analysis of Aflatoxins and Ochratoxin A in Herbs and Spices

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Key Words

- Herbal Supplements
- Spices
- Aflatoxins
- Ochratoxin
- Fluorescence Detection

Introduction

The production of herbal supplements and spices is a fast growing industry. Unfortunately, the raw materials are often imported from countries that lack adequate quality control and whose weather conditions during the growing season, along with improper harvesting and storage practices, can cause toxic mold contamination. There are numerous reports on the presence of Mycotoxins in commercially available herbs and spices such as chamomile, black and white tea leaves, ginkgo leaves, paprika, and cumin. We developed a simple, sensitive, and robust HPLC method for determining Aflatoxins B1, B2, G1, G2, and Ochratoxin A in herbs and spices. Afla-OtaCLEAN™ (LCTech, Germany) Immunoaffinity columns contain antibodies specific for both classes of Mycotoxins and allow for fast and efficient sample clean-up. We used the AcceClean™ (LCTech, Germany) automated workstation, which processes three samples simultaneously. Post-column photochemical derivatization was used to increase the sensitivity of detection of Aflatoxins B1 and G1. The UVE™ (LCTech, Germany) photochemical reactor requires no additional reagents and is easy to install between the HPLC column and fluorescence detector. Ochratoxin A is a naturally fluorescent compound that does not require derivatization and can be analyzed together with all four Aflatoxins.

Method

Standards

Stock standards for the Aflatoxins B1, B2, G1, and G2 (Supelco Aflatoxin Mix, P/N 46300-U), and Ochratoxin A solution (P/N 46912) were obtained from Sigma-Aldrich Co.

Isolation of Aflatoxins B1, B2, G1, G2, and Ochratoxin A

Mix 5 g of finely ground sample with extraction solution (25 mL of methanol:water 80:20, 12.5 mL of hexane, 0.5 g of NaCl) and shake on a mechanical shaker for 1 h. Filter the extract through fluted paper. Dilute 14 mL of the aqueous layer with 86 mL of PBS buffer (pH 7.2), filter and apply 11 mL of solution to an Afla-OtaCLEAN™ Immunoaffinity column at a flow rate of 2 mL/min. Wash the column with 10 mL of water at a flow rate of 2 mL/min. Elute the toxins with two 1 mL portions of methanol at a flow rate of 0.3 mL/min. Allow 5 min before applying the second portion of the methanol to ensure complete disruption of the antibody-toxin bond.

Equipment

Thermo Scientific Dionex UltiMate 3000 system consisting of:

SR-3000 Solvent Rack

LPG-3400A Pump

WPS-3000SL Autosampler

RF 2000 Fluorescence Detector

Analytical Conditions

Analytical Column: Mycotox™ (Pickering Laboratories, Inc), C18, 4.6 × 250 mm

HPLC Eluent: Sodium Phosphate buffer (Pickering Laboratories Inc. Cat #1700-1108), Methanol, Acetonitrile

HPLC Gradient:

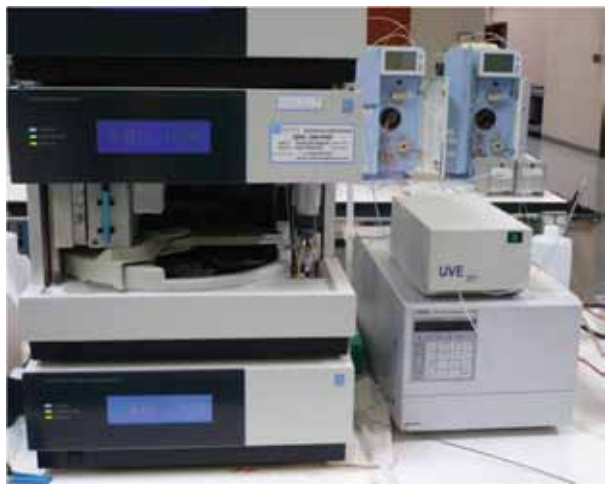
Time (min)	Sodium Phosphate (%)	Methanol (%)	Acetonitrile (%)
0	57	28	15
13	57	28	15
13.1	40	60	0
23	40	60	0
23.1	0	100	0
28	0	100	0
Equilibration:	12 min		

Flow Rate: 1 mL/min

Injection Volume: 30 µL

FLD: Excitation 365 nm, Emission 430 nm for Aflatoxins (0–16 min)

Excitation 335 nm, Emission 455 nm for Ochratoxin A (16–28 min)



Placement of UVE next to the UltiMate™ 3000 system.

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Results and Discussion

The 6-point calibration curves were built in the range of 0.12–11.49 ppb for B1, 0.04–3.29 ppb for B2 and G2, 0.1–9.85 ppb for G1, 0.263–25.23 ppb for Ochratoxin A with R2 exceeding 0.999.

The samples of echinacea, ginger, and ginseng were purchased from a local herbal products store. These samples were not naturally contaminated with mycotoxins.

There were no matrix interferences present after immunoaffinity clean-up. The samples were spiked with five Mycotoxins at two levels and processed, along with sample blanks, as described above. The recovery data for Aflatoxins B1, B2, G1, G2, and Ochratoxin A are presented in Tables 1–3.

The removal of matrix interferences using the Immunoaffinity columns and the use of the UVE Photochemical Reactor greatly enhances the sensitivity and reproducibility of the analysis of Aflatoxins and Ochratoxin A in Herbs and Spices. The same procedure and columns can be applied to other natural products, including (but not limited to) coffee, tea juices, and essential oils.

Table 1. Recovery results for Echinacea sample.

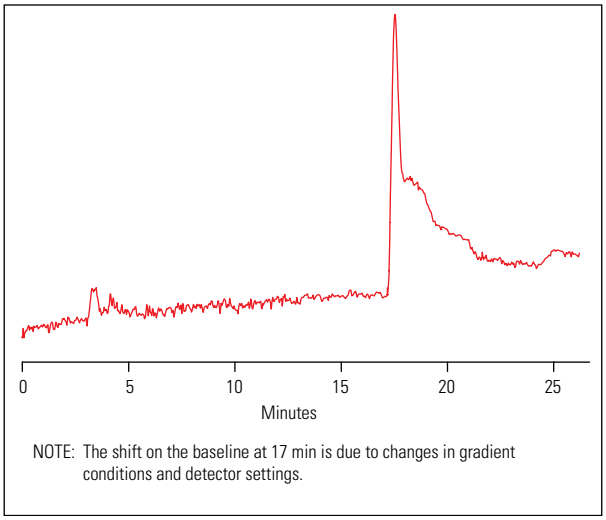
Mycotoxin	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level (ng/g)	5.06	1.45	4.33	1.45	10.1
Recoveries (%)	96	97	84	62	72
Spike level (ng/g)	2.53	0.72	2.16	0.72	5.05
Recoveries (%)	77	80	78	63	80

Table 2. Recovery results for the ginger sample.

Mycotoxin	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level (ng/g)	5.06	1.45	4.33	1.45	10.1
Recoveries (%)	72	78	86	75	62
Spike level (ng/g)	2.53	0.72	2.16	0.72	5.05
Recoveries (%)	66	89	74	59	60

Table 3. Recovery results for the ginseng sample.

Mycotoxin	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level (ng/g)	5.06	1.45	4.33	1.45	10.1
Recoveries (%)	87	89	86	97	68
Spike level (ng/g)	2.53	0.72	2.16	0.72	5.05
Recoveries (%)	75	69	64	58	68



NOTE: The shift on the baseline at 17 min is due to changes in gradient conditions and detector settings.

Figure 1. Ginseng sample blank.

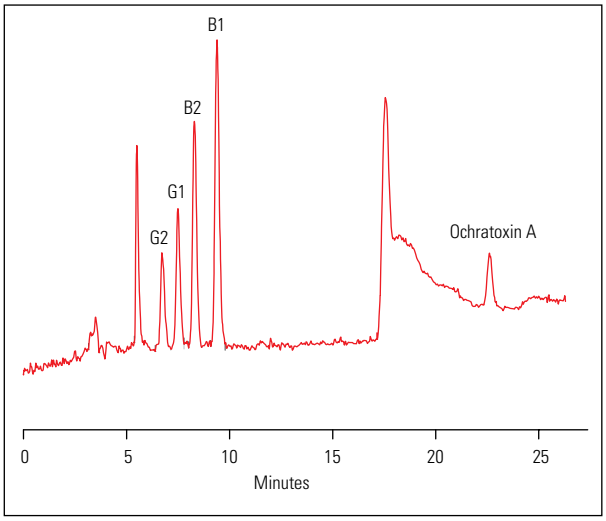


Figure 3. Ginger sample spiked with Mycotoxins. Aflatoxin B1 – 5.06 ng/g, Aflatoxin B2 – 1.45 ng/g, Aflatoxin G1 – 4.33 ng/g, Aflatoxin G2 – 1.45 ng/g, Ochratoxin A – 10.1 ng/g.

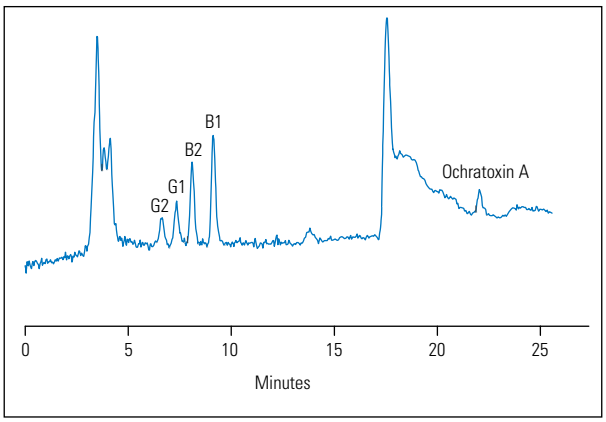


Figure 2. Ginseng sample spiked with Mycotoxins. Aflatoxin B1 – 2.53 ng/g, Aflatoxin B2 – 0.72 ng/g, Aflatoxin G1 – 2.16 ng/g, Aflatoxin G2 – 0.72 ng/g, Ochratoxin A – 5.05 ng/g.

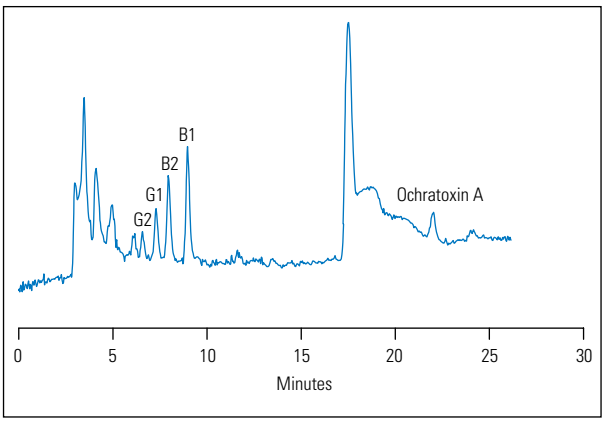


Figure 4. Echinacea sample spiked with Mycotoxins. Aflatoxin B1 – 2.53 ng/g, Aflatoxin B2 – 0.72 ng/g, Aflatoxin G1 – 2.16 ng/g, Aflatoxin G2 – 0.72 ng/g, Ochratoxin A – 5.05 ng/g.

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CAN115_E 07/16SV LPN3044