

► CLEAN-UP AND ANALYSIS OF AFLATOXINS AND OCHRATOXIN A IN HERBS AND SPICES

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 analyzing Aflatoxins B1, B2, G1, G2 and Ochratoxin A in herbs and spices. AflaOTAClean™ Immunoaffinity columns contain antibodies specific for both classes of Mycotoxins and allow for fast and efficient sample clean-up. We used the AcceClean™ 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

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 hour. 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 AflaOTAClean™ 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 breaking of the antibody-toxin bond.

Analytical Conditions

Analytical Column: Mycotox™ (Pickering Laboratories, Inc), C18, 4.6x250 mm

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

Flow Rate: 1 mL/min

Injection Volume: 30 uL

FLD: Excitation 365 nm, Emission 430 nm for Aflatoxins

Excitation 335 nm, Emission 455 nm for Ochratoxin A

<i>HPLC Gradient:</i>	Time, min	1700-1108, %	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

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.

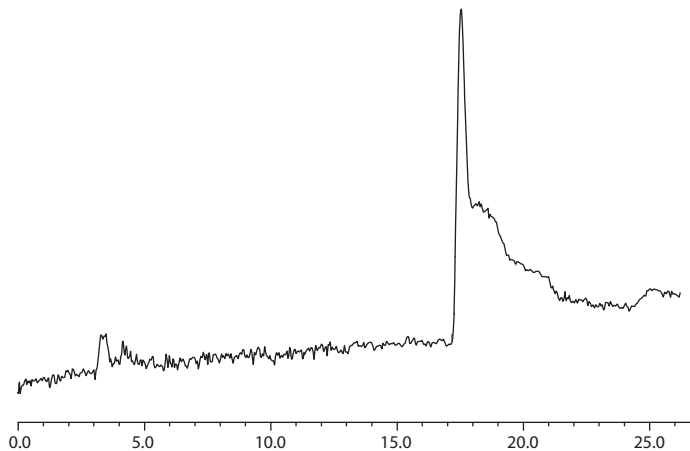
The samples of echinacea, ginger and ginseng were purchased from a local herbal store. These samples were not naturally contaminated with Mycotoxins.

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.

Table 1. Recovery Results for Echinacea Sample					
Mycotoxins	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

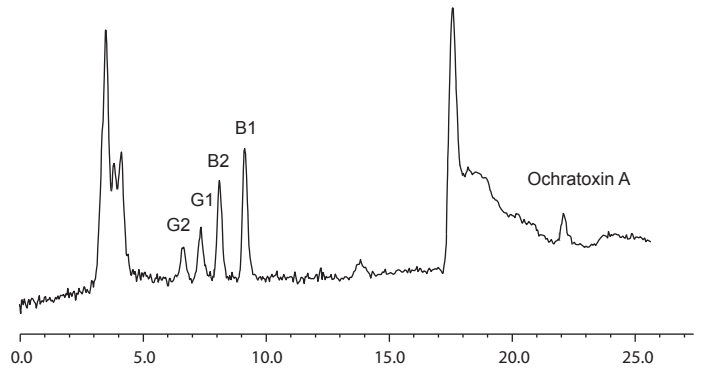
Table 2. Recovery Results for Ginger Sample					
Mycotoxins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level, ng/g	5.06	1.45	4.33	1.45	10.1
Recoveries, %	72	78	86	75	62
Spike level, ng/g	2.53	0.72	2.16	0.72	5.05
Recoveries, %	66	89	74	59	60

Table 3. Recovery Results for Ginseng Sample					
Mycotoxins	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

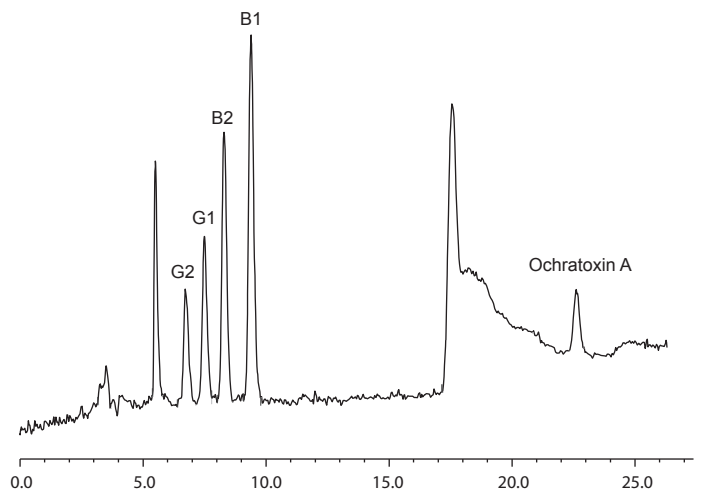


Pic 1. Ginseng sample blank.

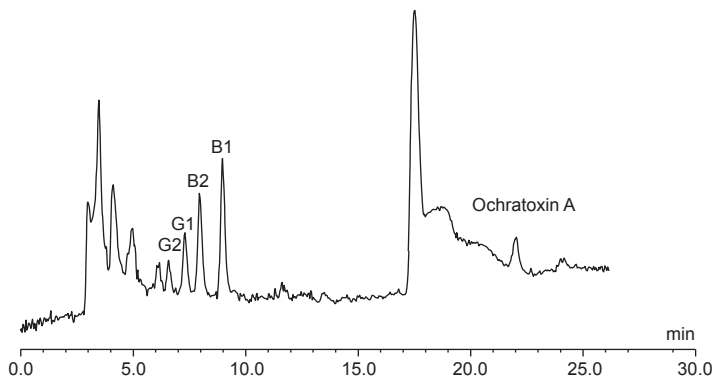
NOTE: The shift on the baseline at 17 min is due to changes in gradient conditions and detector settings.



Pic 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.



Pic 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.



Pic 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.