

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

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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. Aflatoxins are analyzed using Aflatoxins Immunoaffinity columns contain antibodies specific for both classes of Mycotoxins and allow for fast and efficient sample clean-up. We used the AccuClean™ 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 filter 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 Aflatoxins 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



### Placement of UVE™

(Note: HPLC Model shown is to demonstrate placement of the UVE™ only. No endorsement expressed or implied.)

## 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.

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 & 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

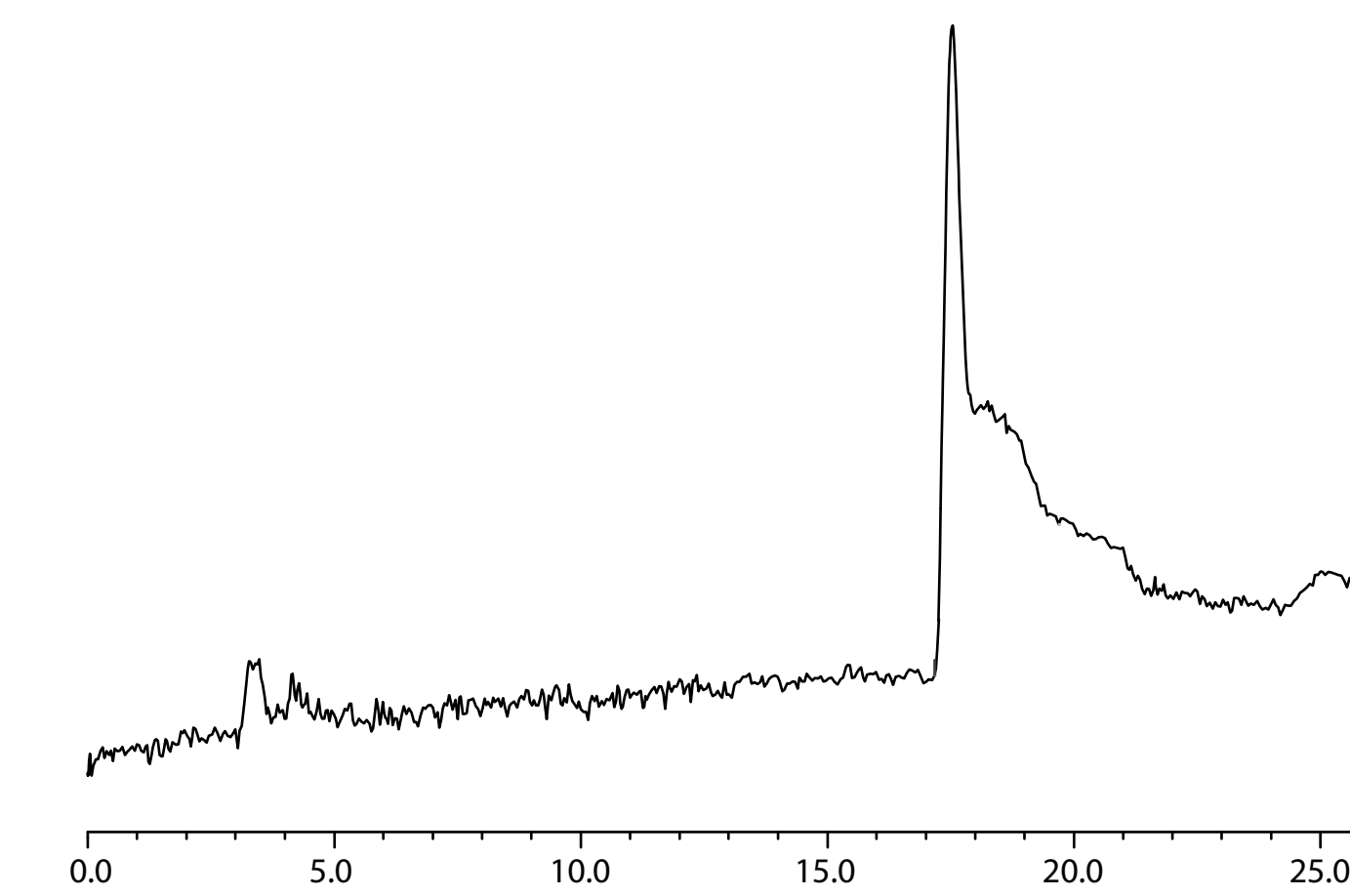
Mycotoxins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level, ng/g	5.06	1.45	4.33	1.45	10.1
Recoveries, %	96	97	84	62	72
Spike level, ng/g	2.53	0.72	2.16	0.72	5.05
Recoveries, %	77	80	78	63	80

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

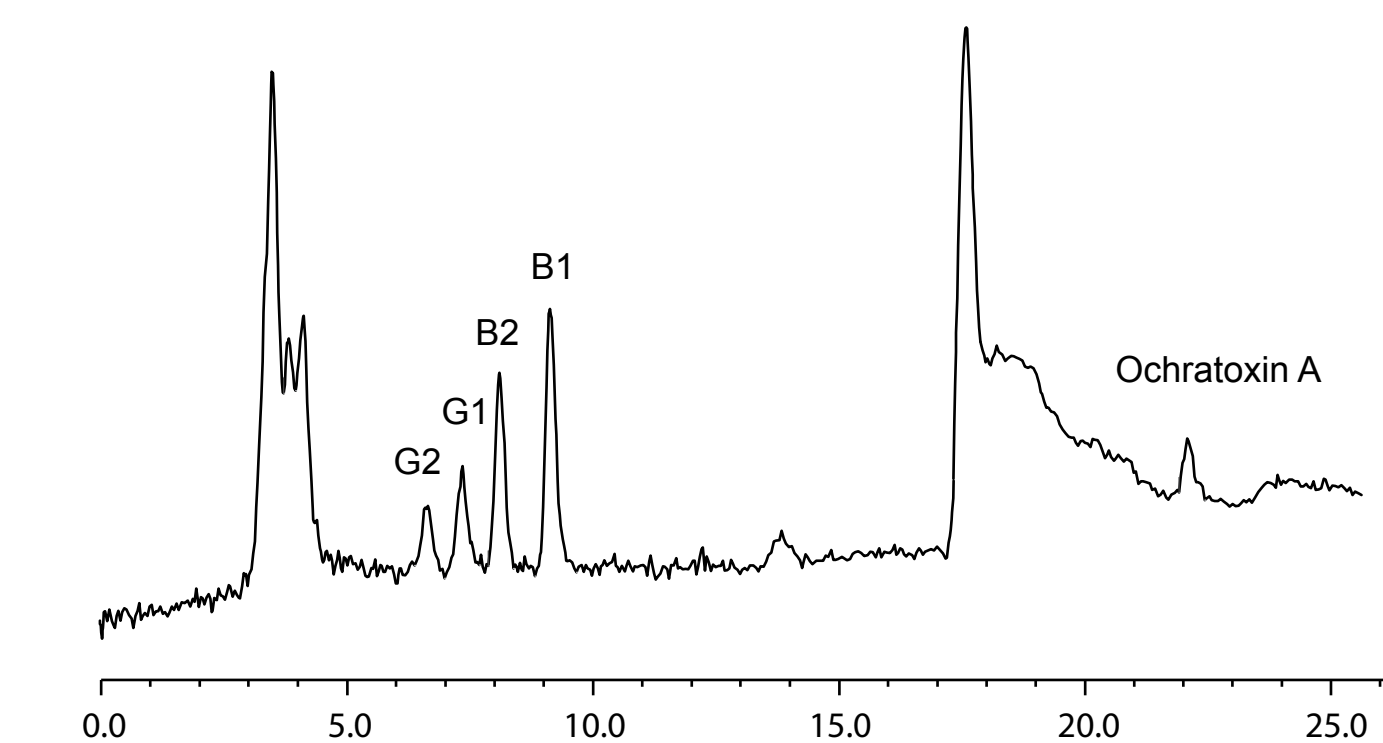
Table 3. Recovery Results for Ginseng Sample

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

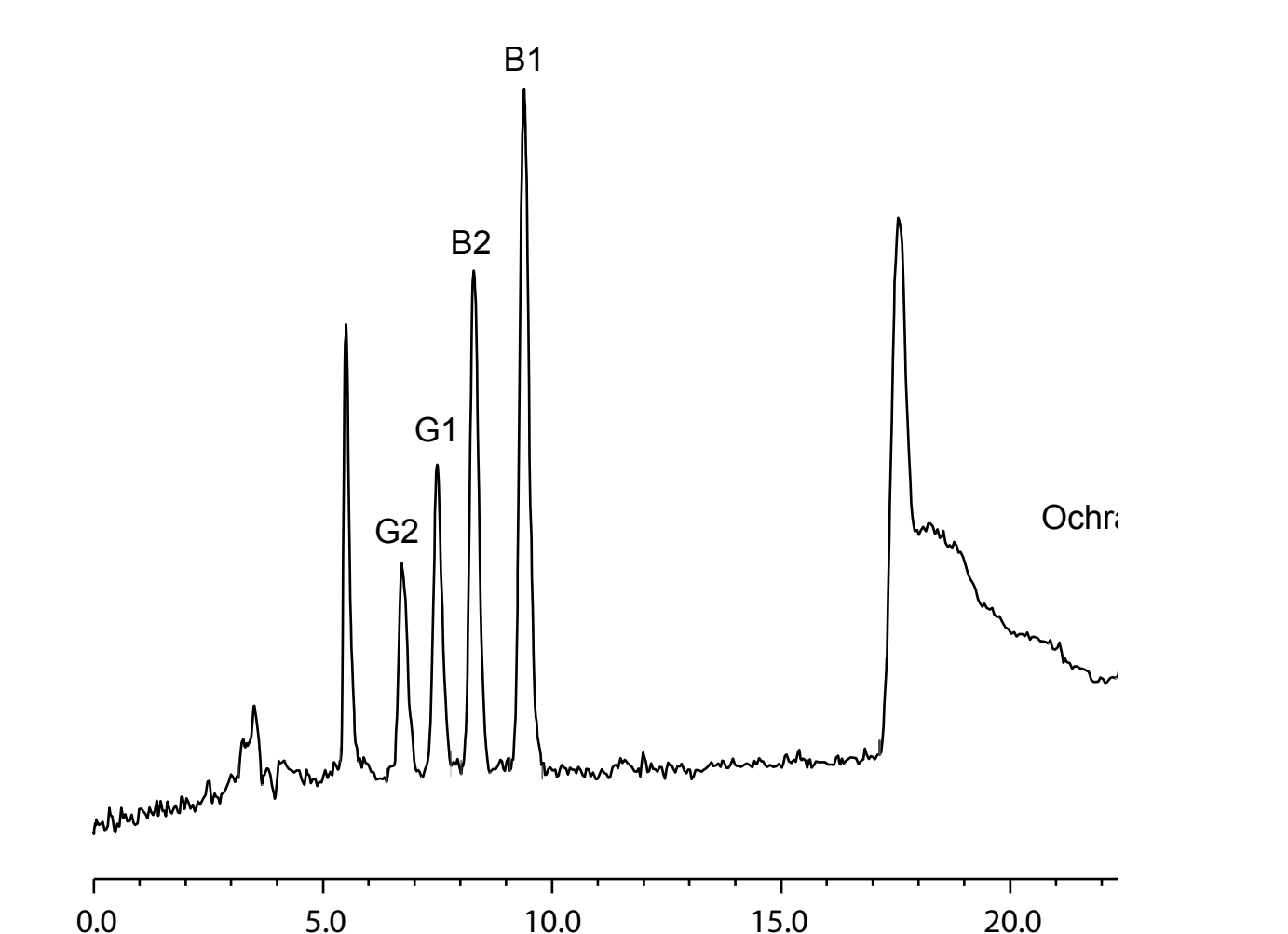


Pic 1. Ginseng sample blank.

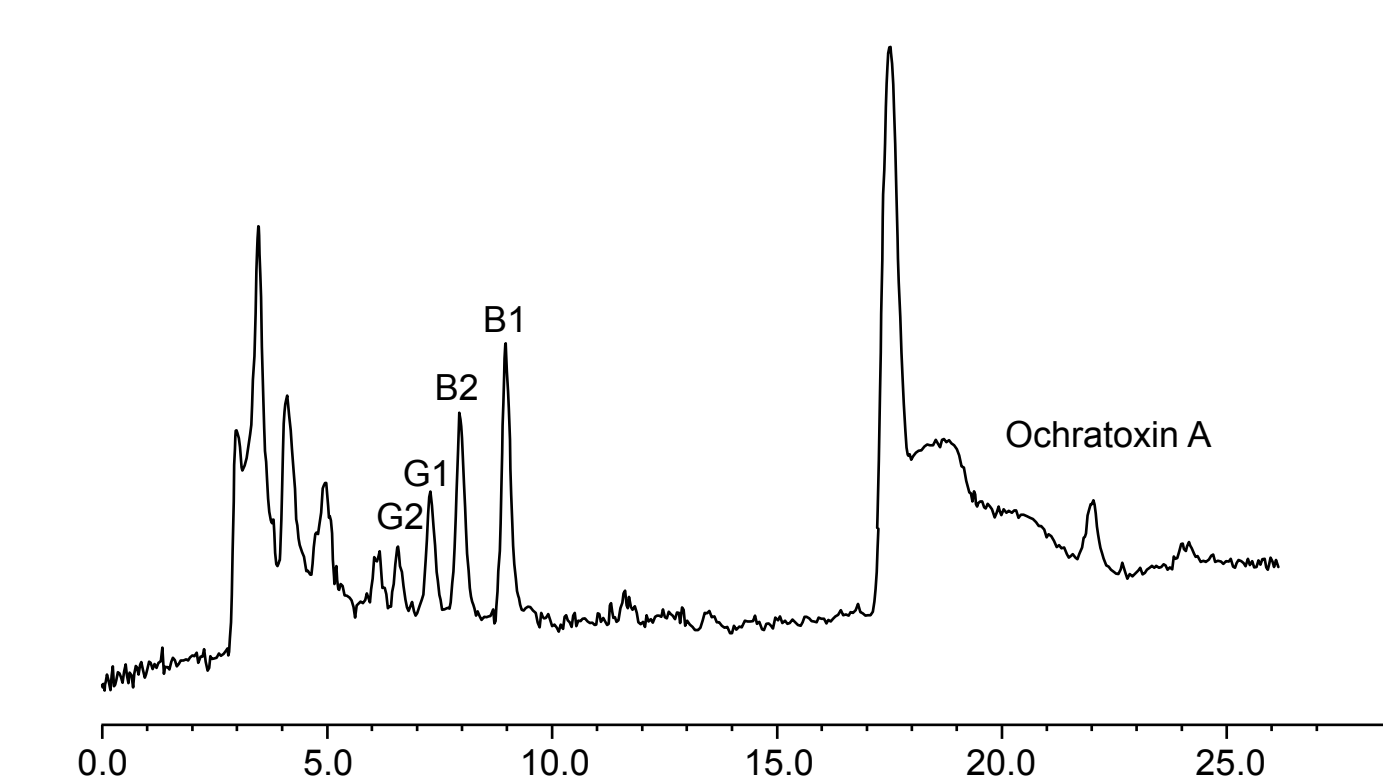
NOTE: The shift on the baseline at 17 min is due to changes in gradient conditions and detector settings.



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.