

**Aflatoxins in Herbs and Spices
(Immunoaffinity Column Method)**

Purpose: To screen for the presence of aflatoxins in spices and herbs.

A. Apparatus:

1. Blender - High speed. With 500 mL blender jar and cover.
2. Filter paper - 24 cm, prefolded (Whatman 2V performed satisfactorily).
3. Glass microfiber filter paper - 11 cm (Whatman 934 AH performed satisfactorily).
4. Affinity column - Aflatest P Column (Vicam, 313 Pleasant St., Watertown MA, 02172) performed satisfactorily.
5. Syringe - 10 mL Luer tip for sample reservoir.
6. Hand pump - 20 mL syringe barrel and plunger with polyethylene stopper attached to outlet.
7. Fluorometer cuvet - 12 X 75 mm borosilicate test tube.
8. Fluorometer - 360 nm excitation filter and 450 nm emission filter (Torbex FX100 [Idetek, Sunnyville, CA or Source Scientific, Garden Grove, CA]).
9. Automatic dispenser - Amber 2 oz. bottle with 1.0 mL automatic dispenser (Tri-Continent Scientific, Inc., Grass Valley, CA 95945 performed satisfactorily).

B. Reagents:

1. Solvents - LC grade methanol, acetonitrile, and distilled water.
2. Extraction solvent - methanol/water (8:2).
3. Bromine Developer Solution - 0.002% bromine in water. Prepare fresh daily by mixing 5 mL 0.02% solution (Vicam Aflatest concentrated developer) with 45 mL H₂O. Keep solution in amber bottle.

C. Preparation of Sample:

1. Use Method 1.0.

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D. Procedure:

1. Weigh 25 g powdered sample into blender jar.
2. Add 5 g NaCl and 100 mL extraction solvent. Blend 1 minute at high speed.
3. Filter through fluted paper into a clean beaker.
4. Pipet or pour suggested volume of filtered extract (Table 1) into a clean vessel. Dilute extract with suggested volume of dilution solvent (Table 1).
5. Filter diluted extract through glass microfiber paper. Filtrate should be clear. If not, refilter. Proceed to column chromatography.
6. Attach column to pump assembly.
7. Pipet 4 mL of second filtrate (equivalent to 0.2 g test portion) into the Column and push it slowly through the column.
8. Pipet 10 mL distilled water into the column and push it through the column.
9. Repeat the column wash with another 10 mL portion of distilled water.
10. Elute aflatoxins from the column with 1.0 mL of HPLC grade methanol and collect eluate in a glass test tube which fits in the fluorometer.
11. Calibrate of fluorometer - Turn on fluorometer. Set red standard vial to 120 ppb and green standard vial to -2 ppb. Insert yellow check standard to verify the calibration. The yellow standard should give a reading of 60 ± 5 ppb. Recalibrate if the yellow standard does not give the correct readout.
12. Quantitation - Add 1.0 mL of the dilute bromine developer solution to the glass test tube with the methanol eluate. Vortex for 5 s. If bubbles adhere to the test tube, tap lightly to dislodge. Insert tube in fluorometer and wait 60 s. Record reading, which is equivalent to ppb total aflatoxins.

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E. Calculations:

Reading ppb directly from fluorometer.

F. Statistics:

See Ruggedness Table.

G. Notes:

Conduct affinity column chromatographic determination with duplicate aliquots of second filtrate and determine fluorometer reading of both.

H. References:

1. Official Methods of AOAC International (1996) 991.45 (49.2.10); for peanut butter.
2. Vicam, 313 Pleasant Street, Watertown, MA 02172.

**Aflatoxins in Herbs and Spices
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Product	Vol. of Filtered Extract	Dilution Solvent -V/V	Vol. of Dilution Solvent	Wash Solution
Nutmeg	5 mL	Tween 20 - water (2+8)	20 mL	water
Black Pepper	5 mL	Tween 20 - water (2+8)	20 mL	water
Onion	10 mL	water	40 mL	water
Parsley	10 mL	water	40 mL	water
Chili	10 mL	water	40 mL	methanol - water (2+8)
Oregano	5 mL	Tween 20 - water (2+8)	20 mL	water

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RUGGEDNESS STUDY RESULT

VARIABLES	NUTMEG	BLACK PEPPER	ONION	PARLSEY	CHILLI	OREGANO
A	0.62	0.78	1.26	1.89	10.03	0.17
DIFFERENCE	(-0.27)	(0.04)	(0.62)	(0.60)	(-0.96)	(0.00)
a*	0.35	0.82	1.88	2.49	9.07	0.17
B*	0.27	0.43	0.96	2.38	9.80	0.00
DIFFERENCE	(-2.52)	(-0.74)	(-1.23)	(0.38)	(0.50)	(-0.33)
b	2.79	1.17	2.19	2.00	9.30	0.33
C*	0.60	0.80	1.42	2.45	8.95	0.17
DIFFERENCE	(0.24)	(0.00)	(-0.30)	(0.52)	(-1.20)	(0.00)
c	0.36	0.80	1.72	1.93	10.15	0.17
D*	0.61	0.94	1.57	2.14	10.00	0.17
DIFFERENCE	(0.26)	(0.28)	(0.00)	(-0.10)	(0.90)	(0.00)
d	0.35	0.66	1.57	2.24	9.10	0.17
E	0.76	0.98	1.71	2.69	10.29	0.17
DIFFERENCE	(-0.37)	(-0.36)	(-0.03)	(-1.00)	(-1.48)	(0.00)
e*	0.39	0.62	1.68	1.69	8.81	0.17
eF*	0.57	1.13	1.17	2.33	9.48	0.17
DIFFERENCE	(0.17)	(0.66)	(-0.80)	(0.28)	(-0.14)	(0.00)
f	0.40	0.47	1.97	2.05	9.62	0.17
G*	0.63	0.64	1.65	2.44	9.76	0.17
DIFFERENCE	(-0.29)	(-0.32)	(0.16)	(0.50)	(0.42)	(0.00)
g	0.34	0.96	1.49	1.94	9.34	0.17

*Factor on which the difference is based.