

Pretreatment

1. Weigh 50 g ground sample with 5 g sodium chloride and place in blender jar.
2. Add 100 mL 80:20 methanol: water (v/v) to jar.
3. Blend at high speed for 1 minute.
4. Filter extract with fluted filter paper. Collect filtrate in a clean vessel.
5. Pipet or pour 65 mL filtered extract into a clean vessel.
6. Dilute extract with 60 mL of phosphate buffer saline. Mix well.
7. Filter extract through glass microfiber filter into a clean vessel.

SPE Procedure

AflaTest® affinity column

Pass 4 mL of filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest affinity column at a rate of about 1-2 drops/seconds until air comes through column

Pass 10 mL of 20:80 methanol:water through the column at a rate of about 2 drops/second

Repeat previous step once more until air comes through column

Place glass cuvette under AflaTest column and add 1 mL HPLC grade methanol into glass syringe barrel

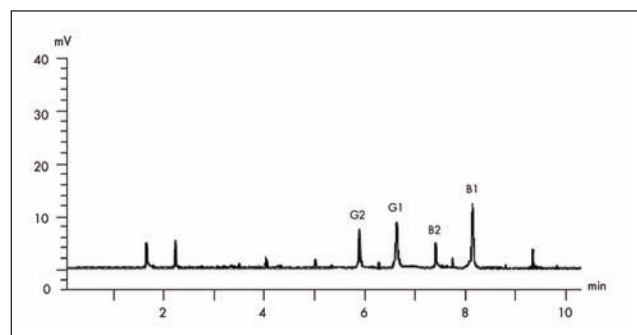
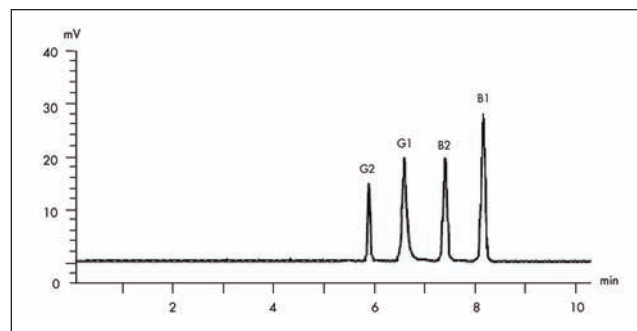
Elute AflaTest column at a rate of 1 drop/second by passing methanol through the column and collecting all the sample eluate (1 mL) in a glass cuvette

Add 1 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

LC Conditions

Instrument:	Waters Alliance® HPLC System
Column:	XBridge™ C ₁₈ , 4.6 x 250 mm, 5 µm
Flow rate:	1 mL/min
Mobile phase:	acetonitrile/water/methanol (17:54:29, v/v/v)
Injection:	100 µL
Detector:	2475 Multi Wavelength Fluorescence
Detection:	excitation wavelength: 333 nm emission wavelength: 460 nm

Results



Chromatogram of aflatoxins in red pepper extract. Recovery: 76% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix).

Action Levels for Aflatoxins

United States (FDA) action levels (B1, B2, G2, G2, M1)

Food Stuff	Level	Regulation
All products - except milk - designated for humans	20 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Milk	0.5 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for immature animals and dairy cattle	20 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for breeding beef cattle, swine and mature poultry	100 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for finishing swine	200 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for finishing beef cattle	300 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Cottonseed meal (as feed ingredient)	300 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
All feedstuff other than corn	200 ng/g	Policy Guides 7120.26, 7106.10, 7126.33

Aflatoxin regulatory action limits.

Ordering Information

Description	Part Number
VICAM aflaOchra HPLC™ Columns, 25/box	G1017
VICAM Glass Cuvette	34000
XBridge C ₁₈ , 4.6 x 250 mm, 5 µm	186003117
LCMS Certified Combination Packs	600000751CV