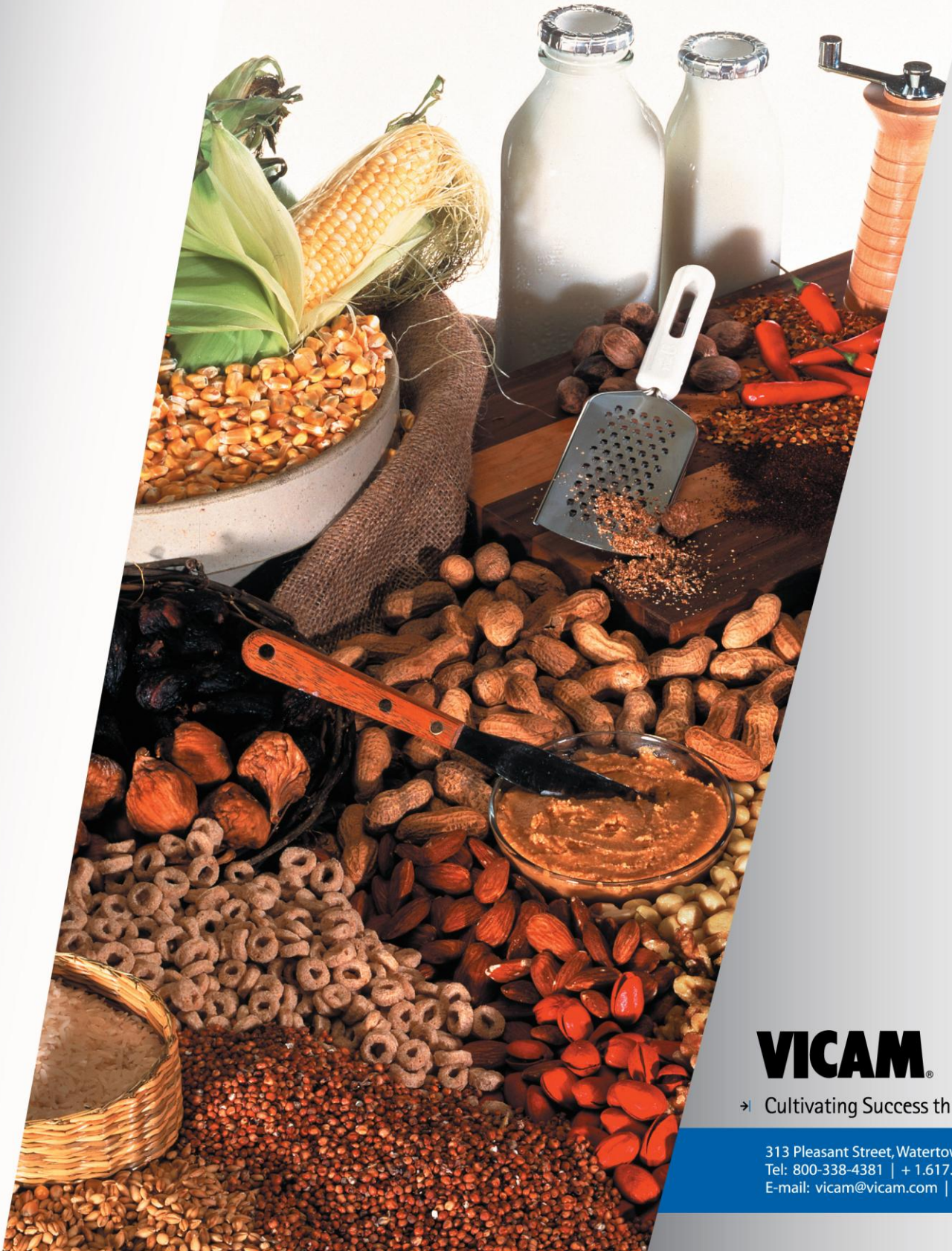
AflaTest®
Instruction Manual
(for HPLC use)



VICAM®

Cultivating Success through Science®

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Manual Number: 715001733 REV B

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1.1 INTENDED USER

AflaTest® with HPLC is a quantitative method for the detection of aflatoxin in many commodities. VICAM's advanced biotechnology permits the measurement of all the major aflatoxins (including AFB1, AFB2, AFG1, AFG2 and AFM1) without the use of toxic solvents like chloroform or methylene chloride. AflaTest® HPLC aflatoxin testing can be used in any laboratory with an HPLC system - from food processing Quality Control laboratories to food and feed company laboratories to commercial and government testing laboratories.

1.2 PRINCIPLE

Aflatoxin, a toxin from a naturally occurring mold, is a Group 1 carcinogen proven to cause cancer in humans. Aflatoxin can also cause economic losses in livestock due to disease or reduced efficiency of production. AflaTest® is a fast, simple, safe and highly accurate method for quantitatively measuring aflatoxin in many commodities.

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the AflaTest® column bound with specific antibodies to aflatoxin. At this stage, the aflatoxin binds to the antibody on the column. The column is then washed with water to rid the immunoaffinity column of impurities. By passing methanol through the column, the aflatoxin is removed from the antibody. This methanol solution can then be injected into an HPLC system. These steps are outlined in section 1.7, AflaTest® Overview.

1.3 APPLICABILITY AND APPROVALS

AflaTest® has been optimized for quantitative measurement of aflatoxins in many commodities. The Table of Contents lists the testing protocols developed for specific commodities as of the publication date of this manual. Assistance in measuring aflatoxin in commodities not listed in this manual can be obtained by contacting our Technical Services Department.

AflaTest® methods vary in the amount of sample passed through the affinity column. Greater amounts of sample passed through the column results in lower limits of detection. However, when lesser amounts of sample are passed over the column, the range of the assay is wider and the test can be completed quicker. In general, 0.2g methods have a wider testing range and are faster. 1.0g methods have a lower limit of detection. Both methods are accurate.

This test kit is cited in the AOAC® Official Methods Program, as official method 991.31 applicable for the determination of aflatoxin B_1, B_2, G_1 and G_2 both by fluorometry and HPLC analysis in corn, peanuts and peanut butter. AOAC Offical Method 991.31 has final action status. AflaTest immunoaffinity columns can also be used in AOAC Official Methods 999.07 for aflatoxin determination in peanut butter, pistachio paste, fig paste and paprika powder as well as in AOAC Official method 2003.02 for the determination of aflatoxin in cattle feed.

1.4 LIMITATIONS

This test has been designed for use with the procedure and reagents described on the following pages. Do not use materials beyond the expiration date. Deviation from these instructions may not yield optimum results.

1.5 SAMPLING

Mycotoxins do not occur in every kernel in a lot and may only occur in a small percentage of the kernels in a lot. Because of the wide range in mycotoxin concentrations among individual kernels in a contaminated lot, variation from sample to sample can be large. It is important to obtain a representative sample from a lot. Product should be collected from different locations in a static lot based on a probing pattern. The probe should draw from the top to the bottom of the lot. The samples obtained from the probes should be ground and mixed well and a subsample taken for testing. For further information on grain sampling, refer to the following United States Federal Grain Inspection Service (FGIS) publications:

FGIS Aflatoxin Handbook
FGIS Grain Inspection Handbook, Book 1, Grain Sampling
FGIS Mechanical Sampling Systems Handbook

These can be viewed online at:

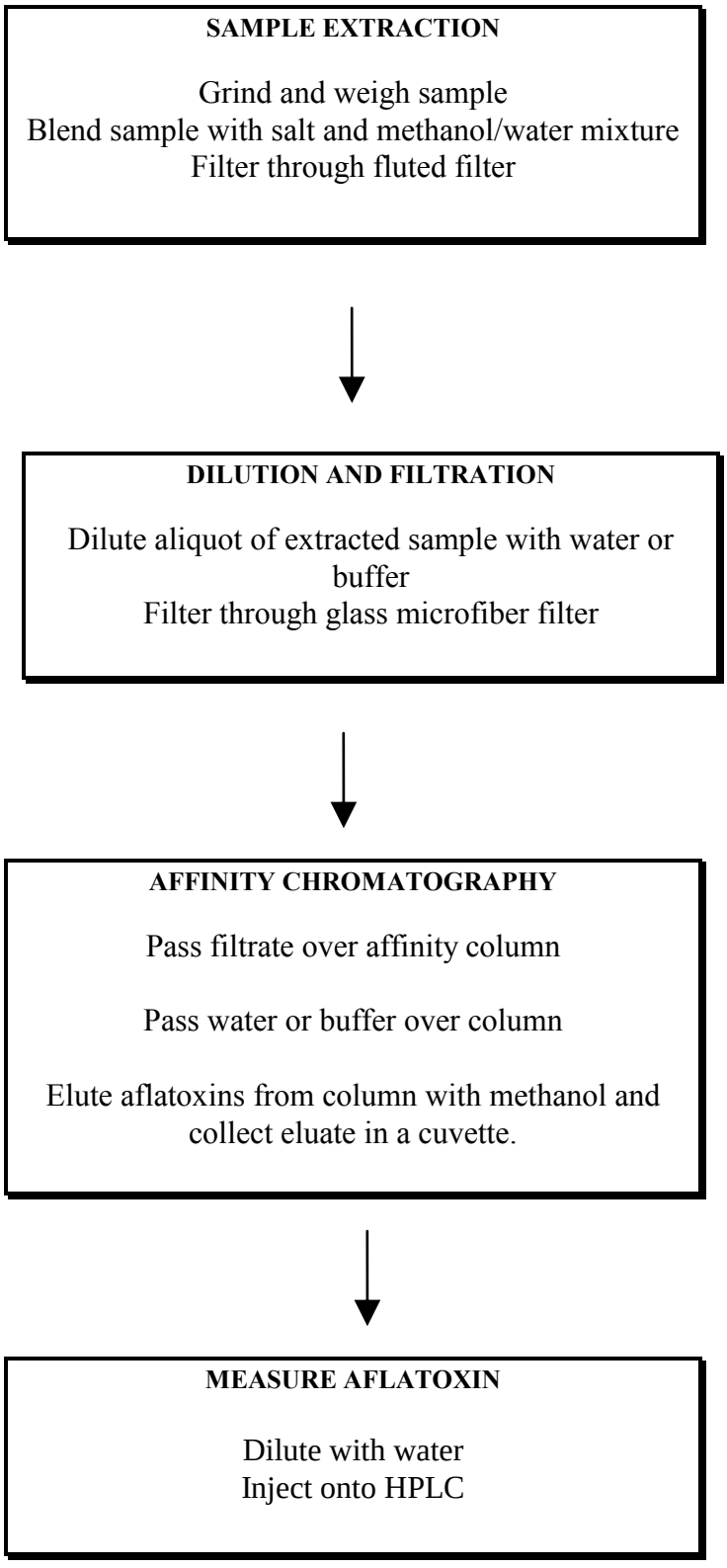
http://www.usda.gov/gipsa/reference-library/handbooks/handbooks.htm
http://www.usda.gov/gipsa/reference-library/brochures/sampling.pdf

European community sampling procedures can be found in Commission Regulation EC No 401/2006 of 23 February 2006.

1.6 SHELF LIFE AND STORAGE CONDITIONS

Store at room temperature. Storage at temperatures above 30°C for prolonged periods of time may reduce shelf life. If storage temperatures above 30°C are anticipated, all components may be stored refrigerated (2 - 8°C). It is recommended that reagents should be at room temperature (18 - 22°C) for usage.

1.7 AFLATEST® HPLC OVERVIEW



2.1 PUMP STAND SETUP

AflaTest® affinity chromatography is easily performed with the AflaTest® affinity column attached to a pump stand. The stand has a 10 mL glass syringe barrel that serves as a reservoir for the column. A large plastic syringe with tubing and coupling provides air pressure to manually push liquids through the column. An adjustable air pump (VICAM part #20650) can be attached to the pump tube instead of the large pump syringe barrel to operate without using hand pressure. Double position pump stands (part # 21030), four-position pump stands with aquarium pumps (VICAM part #21045), and twelve-position pump stands with aquarium pumps (VICAM part # G1104) are available for running multiple samples at one time.

When using a pump stand:

1. Remove large top cap from column.

2. Cut bottom 1/8 inch off the end of the top cap with scissors or sharp blade. This provides a reusable coupling for attaching the column.

3. Attach column to coupling and place waste collection cup under column outlet. Keep bottom cap on column.

4. Pour extract into microfibre filter (see previous section) and collect desired amount of extract in glass syringe barrel using markings on the syringe barrel to measure extract.

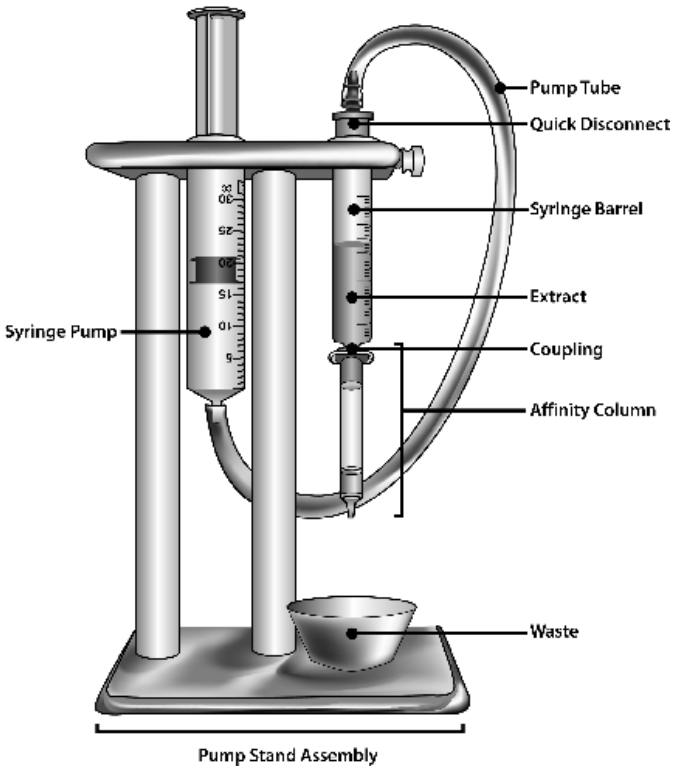
5. Pull up on the plastic syringe piston.

6. Inset coupling on end of tube into syringe barrel. Remove column bottom cap.

7. Apply pressure to piston of plastic syringe to push liquid through the column. Maintain a flow rate of 1-2 drops per second. Push all liquid through the column. Repeat for wash and elution steps (see procedures).

Note: Avoid pulling up on plastic syringe piston while coupling is attached to glass syringe barrel. This may displace the antibody coated support beads and affect test results.

Affinity Column Syringe Barrel Connection



2.2 CLEANING EQUIPMENT

Before Starting AflaTest® Testing

To eliminate background fluorescence make sure the equipment is clean and not contaminated with materials that might cause background fluorescence. This is particularly important when using brand new equipment or equipment that has not been used for a long period of time.

Before using the equipment, it should be washed with a mild detergent solution and then rinsed thoroughly with purified water. This includes the glass syringe barrels used for sample reservoirs. The syringe barrels are treated with a lubricant for use with a piston plunger. Wash new syringe barrel for pump stands using a brush with soap and water. Then rinse with purified water and methanol before using to remove lubricant. Other pieces of equipment that need to be cleaned with detergent before using are graduated cylinders, funnels and blender jars. Repipetters need only to be rinsed with methanol before use.

Between Assays:

After each assay, the blender jar assembly needs to be washed with a mild detergent solution and rinsed thoroughly with purified water. The same cleaning procedure must be performed for any equipment that will be reused to hold, collect or transfer sample extracts.*

Do not wash repipetter with soap. Methanol repipetter needs only to be refilled with methanol.

In between each assay, the syringe barrel reservoir can be rinsed with methanol followed by a rinse with purified water. This will be sufficient to prevent cross-contamination of samples. After a number of samples have been tested, the glass syringe barrel should be washed with a brush and detergent and rinsed well with water.

It is not recommended to wash and reuse the cuvettes. These cuvettes are designed for one-time use and should be discarded.

Other Important Precautions

Use only equipment specified by VICAM. Avoid contact of any test reagents or solutions (such as methanol, water, sample extract or column eluate) with rubber or soft flexible plastic. These materials may leach contaminating fluorescent materials into the sample and thereby affect results.

Note: Some blender jar lids are lined with waxed cardboard. These liners are not resistant to methanol and water solutions and will breakdown when used for sample extraction. The extract will then become contaminated with materials, which may cause background fluorescence. Lids with cardboard liner should not be used.

* More details on decontamination can be found in JAOAC **48**, 681 (1965); Am. Hyg. Assoc. J. **42**, 398 (1981); and IARC Sci. Publ. No. 37, IARC, Lyon, France, 1980.

3.1 PREPARATION OF EXTRACTION SOLUTIONS

The AflaTest® procedure uses a methanol or a methanol/water solution to extract aflatoxin out of the sample.

To prepare extraction solution: Use reagent grade (or better - i.e. HPLC grade) methanol when preparing extraction solutions.

Solution desired (methanol: water)	Methanol (mL)	Purified Water (mL)	Total Volume (mL)
80:20	800	200	1000 (1 liter)
70:30	700	300	1000 (1 liter)
60:40	600	400	1000 (1 liter)

CAUTION: Extraction solvent is flammable. Keep container tightly capped when not in use. Prepare extraction solution every week or as needed. The formulas above will prepare 1 liter of solution. Solution volume may be increased or decreased as needed provided the proportion of reagents is kept consistent.

3.2 PREPARATION OF DILUTION/WASH SOLUTIONS

The formulas below will prepare 1 liter of solution. Solution volume may be increased or decreased as needed provided the proportion of reagents is kept consistent.

1. Methanol:Water solutions; prepare every week or as needed

Solution desired (methanol: water)	HPLC Grade Methanol (mL)	Purified Water (mL)	Total Volume (mL)
10:90	100	900	1000 (1 liter)
20:80	200	800	1000 (1 liter)

2. Tween-20 solutions; prepare every month or as needed

Solution desired	Tween-20 (mL)	Purified Water (mL)	Total Volume (mL)
10% Tween-20	100	900	1000 (1 liter)
15% Tween-20	150	850	1000 (1 liter)

3.3 PREPARATION OF HPLC SOLUTIONS

1. HPLC Mobile Phases

Methanol:Water (45:55)

HPLC Grade Methanol (mL)	Purified Water (mL)	Total Volume (mL)
450	550	1000 (1 liter)

Water:Acetonitrile:Methanol (3:1:1)

Purified Water (mL)	Acetonitrile (mL)	HPLC Grade Methanol (mL)	Total Volume (mL)
600	200	200	1000 (1 liter)

Solutions should be filtered and degassed before use.

2. Iodine solution (0.05%)

0.5 g Iodine
100 mL Methanol
900 mL purified water

Dissolve iodine in methanol, stirring until completely dissolved. While stirring add purified water. Mix solution for at least 30 minutes. Filter solution through 0.45 micron nylon filter. This solution can be used for 2 weeks from preparation.

3. Kobra Cell Mobile Phase

450 mL MeOH
550 mL purified water
119 mg Potassium Bromide
87.5 µL Nitric Acid

Calculations are based on the following reagents:
Potassium Bromide: SIGMA catalogue # P-0838
Nitric Acid: 16M (70%) ALDRICH catalogue# 43,807-3

4.1 MATERIALS AND EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Materials Required

Description	Part #
AflaTest® Columns, for Fluorometer & HPLC (50/box)	12022
Disposable Plastic Pipets, 1 mL (50)	20652
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfiber Filters, 1.5µm, 11 cm (100)	31955
Tween-20 (50 mL) (for nutmeg, oregano, black pepper & turmeric)	33501
Disposable Cuvettes (250)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25)	36010
Acetonitrile, HPLC Grade (4 x 4 L) (for HPLC condition 3)	G1130
Noniodized sodium chloride (salt, NaCl)	G1124
Distilled, reverse osmosis or deionized water	

Equipment Required

Description	Part #
Graduated Cylinder, 50ml	20050
Digital Scale with AC Adapter	20100
Commercial Blender with Stainless Steel Container	20200
Graduated Cylinder, 250ml	20250
500 ml Bottle Dispenser for Methanol (0-3 ml range)	20501
Wash Bottle, 500 ml	20700
Cuvette Rack	21010
Single Position Pump Stand	21020
or 2-Position Pump Stand w/ Air Pump (10 mL)	21040
or 4-Position Pump Stand w/2 Air Pumps (10 mL)	21045
or 12-Position Pump Stand w/6 Air Pumps (10 mL)	G1104
Filter Funnel, 65 mm (10 per pack)	36020
Filter Funnel, 105 mm (4 per pack)	36022
Centrifuge capable of obtaining 2000 x g Relative Centrifugal Force (for milk only)	
HPLC System as specified in procedure	

Suggested but not required

Micro-pipettor, 1.0 mL	G4033
Micro-pipette Tips for 1 mL Micro-pipettor (100)	20656

4.2 AFLATEST® HPLC PROCEDURE FOR CORN, GRAINS & FEEDS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:
See HPLC condition #2

2.0 Sample Extraction:
- **2.1** Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 100 mL methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 10 mL filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 mL of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 10 mL filtered diluted extract (10 mL = 1g sample equivalent) completely through AflaTest ® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through the column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

5.0 Limit of Detection: (using post column photochemical reaction)

Commodity	Aflatoxin	Limit of Detection (ppb)
Corn	$7B_1:1B_2:3G_1:1G_2$	0.1
Corn	B_1	0.05
Corn	B_2	0.02
Corn	G_1	0.25
Corn	G_2	0.08

Note: Better limit of detection may be obtained using post column iodine

6.0 Recovery: On Certificate of Analysis supplied with columns

4.3 AFLATEST® HPLC PROCEDURE FOR CORN, RAW PEANUTS, PEANUT BUTTER (AOAC METHOD) (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:
See HPLC condition #3

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 125 ml methanol: water (70:30).
- **2.3** Cover blender jar and blend at high speed for 2 minutes.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 15 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 30 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 15 ml filtered diluted extract (15 ml = 1g sample equivalent) completely through AflaTest ® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through the column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 ml HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 ml) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC.

5.0 Limit of Detection: Note: The AOAC Collaborative study ran samples at a low level of 10 ppb. Other studies at VICAM showed quantitation possible at levels of 2 ppb.

6.0 Recovery: Greater than 70% of aflatoxin B_1, B_2, G_1, G_2.

4.4 AFLATEST® HPLC PROCEDURE FOR COTTONSEED MEAL & WHOLE COTTONSEED (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1

2.0 Sample Extraction:
- **2.1** Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 200 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 5 minutes.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 10 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest ® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 5 mL of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through the column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 50-200 µl onto HPLC.

Note: For greater sensitivity, more sample volume may be passed over column in step 4.1.

4.5 AFLATEST HPLC PROCEDURE FOR FLUID MILK SAMPLES USING AFLATEST® COLUMN (0 – 0.5 PPB)

1.0 HPLC Set up:
See HPLC condition #4 and #5.

2.0 Sample Extraction:

2.1 Add 1g NaCl to 40 ml fluid milk sample and mix well.

2.2 Centrifuge milk at 2000 g* for 10 minutes.
Note: The rpm value that corresponds to 2000 g Relative Centrifugal Force will vary depending on the centrifuge rotor. Use a nomogram to identify the rpm corresponding to 2000 g for your centrifuge rotor. Nomograms are usually supplied from the manufacturer with the rotor.

2.3 Carefully remove the skim portion (bottom layer) of the milk for analysis without disturbing the top, fat layer (a syringe needle can be used to poke a hole into the bottom of a plastic centrifuge tube).

2.4 Immediately before affinity chromatography analysis, filter the skim sample through glass microfiber filter paper.

3.0 Column Chromatography:

3.1 Remove two end caps from AflaTest® column.

3.2 Cut off tip of column top cap to use as a coupling. Attach column to outlet of 10 ml reservoir on pump stand.

3.3 Pass 25 ml of filtered milk sample through the Aflatest column at a steady slow flow rate of about 1-2 drops per second.

3.4 After milk sample has completely passed through column, transfer Aflatest column to a clean syringe barrel and pass 10 ml 10% methanol: 90% water solution through Aflatest column twice at about 2 drops per second flow rate. Make sure all the liquid has passed through the column. A few seconds of air can come through the column.

3.5 Place glass cuvette (VICAM part # 34000) under AflaTest® column and add 1.0 mL **methanol: water (80:20)** into glass syringe barrel.

3.6 Elute AflaTest® column at a rate of 1 drop/second by passing the methanol/water through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.

3.7 Concentrate to 100 µl and inject entire eluate if using absorbance detector.

Note: Greater sensitivity is possible with fluorescence detection at excitation 360 nm, emission 440 nm. 1 ml eluate at step 3.5 can be diluted with 1 mL water and injected directly into HPLC without concentrating. Sample can also be eluted with 1 ml 100% methanol at step 3.5.

4.0 Limit of detection: 0.05 ppb

4.6 AFLATEST® HPLC PROCEDURE FOR NUTMEG (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 100 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 5.0 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 20 ml of 15% Tween-20 solution. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml of filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.3** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.4** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Recovery: 77% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

4.7 AFLATEST® HPLC PROCEDURE FOR DRIED ONIONS (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 100 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 10 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml of filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of 1 -2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Recovery: 87% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

4.8 AFLATEST® HPLC PROCEDURE FOR OREGANO (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 100 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 5.0 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 20 ml of 10% Tween-20 solution. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml of filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Recovery: 57% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

4.9 AFLATEST® HPLC PROCEDURE FOR PAPRIKA, CHILI PEPPER & RED PEPPER (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1.

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 100 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 10 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml of filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of methanol: water (20:80) through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Recovery: 76% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

4.10 AFLATEST® HPLC PROCEDURE FOR PARSLEY (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1.

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 200 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 10 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 8 ml of filtered diluted extract (8 ml = 0.2g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Recovery: 87% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

4.11 AFLATEST® HPLC PROCEDURE FOR PEANUTS, CASHEWS, APRICOT NUTS, ALMONDS, PISTACHIOS, WALNUTS & PECANS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:
See HPLC condition #2

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 125 ml methanol: water (60:40).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 20 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 20 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 10 ml of filtered diluted extract (10 ml = 1g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC.

6.0 Recovery: Average recovery of 70% total aflatoxins (7B1:1B2:3G1:1G2 ratio) over 2 - 20 ppb range

4.12 AFLATEST® HPLC PROCEDURE FOR BLACK PEPPER & TURMERIC (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1

2.0 Sample Extraction:
- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 100 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 5.0 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 20 ml of 10% Tween 20 solution. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml of filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest ® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Recovery: 97% from black pepper at 20 ppb, 83% from turmeric at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

4.13 AFLATEST® HPLC PROCEDURE FOR WHEAT MIDDS, OATS, CALF MIXING PELLETS, SAFFLOWER SEED, SAFFLOWER MEAL, CANOLA SEED, CANOLA MEAL, DRIED DISTILLERS GRAIN & HIGH FIBER SAMPLES (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:
See HPLC condition #1.

2.0 Sample Extraction:
- **2.1** Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 200 ml methanol: water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution
- **3.1** Pipet or pour 10 ml filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 ml of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography
- **4.1** Pass 4 ml filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest ® affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 5 ml of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Place glass cuvette (VICAM part # 34000) under AflaTest® column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest™ column at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette.
- **4.6** Add 1.0 ml of purified water to eluate. Inject 50-200 µl onto HPLC.

Note: For greater sensitivity, more sample volume may be passed over column in step 4.1.

6.0 Recovery:

Commodity	**Spike level** $7B_1:1B_2:3G_1:1G_2$ aflatoxin mix	**% recovery**
Wheat midds	30 ppb	70
Oats	30 ppb	73
Calf mixing pellets	30 ppb	65
Safflower seed	20 ppb	71
Safflower meal	20 ppb	51
Canola seed	20 ppb	75
Canola meal	20 ppb	66

4.14 OTHER PUBLISHED HPLC PROCEDURES

Beer

Scott PM, Lawrence GA. *Journal of AOAC International*. Determination of aflatoxins in beer. 1997 Nov-Dec; 80(6): 1229-34.

Cattle Feed

Stroka, J.; von Holst, C.; Anklam, E.; Reutter, M. *Journal of AOAC Int'l.* Immunoaffinity Column Cleanup with Liquid Chromatography Using Post-Column Bromination for Determination of Aflatoxin B1 in Cattle Feed: Collaborative Study **2003**, *86(6)*, 1179-1186.

Corn, Peanuts and Peanut butter (AOAC Method 991.31)

Trucksess MW, Stack ME, Nesheim S, Page SW, Albert RH, Hansen TJ, Donahue KF. *Journal of Association Official Analytical Chemist.* Immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization for determination of aflatoxins in corn, peanuts, and peanut butter: collaborative study. 1991 Jan-Feb; 74(1): 81-88.

Infant formula (AOAC 2000.16)

Stroka J, Anklam E, Joerissen U, Gilbert J. *Journal of AOAC International* Determination of aflatoxin B1 in baby food (infant formula) by immunoaffinity column cleanup liquid chromatography with postcolumn bromination: collaborative study. 2001 Jul-Aug; 84(4): 1116-23.

Milk (AOAC Method 2000.08)

Dragacci S, Grosso F, Gilbert J. *Journal of AOAC International*, Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin M1 in liquid milk: collaborative study. 2001 Mar-Apr; 84(2): 437-43

Milk

Hansen T.J., *Journal of Food Protection*, Affinity column cleanup and direct fluorescence measurement of Aflatoxin M_1 in raw milk, **53** (1) (1990) 75-77.

Ioannou-Kakouri, E., Christodoulidou, M., Christou, E., Constantinidou, E., *Food and Agricultural Immunology,* Immunoaffinity column/HPLC determination of Aflatoxin M_1 in milk, **7** (1995): 131-137.

Peanut butter, Pistachio paste, Fig paste, and Paprika powder (AOAC Method 999.07)

Stroka J, Anklam E, Jorissen U, Gilbert J. J. *Journal of AOAC International* Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder: collaborative study. 2000 Mar-Apr; 83(2): 320-40.

5.1 SELECTING AN AFLATOXIN DERIVATIZATION METHOD

Aflatoxins B2 and G2 are naturally much more fluorescent than aflatoxins B1 and G1. Aflatoxin B1 and G1 fluorescence can be increased for HPLC with fluorescence detection by derivatization using one of the four methods:

1. Pre-column Trifluoroacetic acid (TFA) (See section 5.3)
2. Post-column iodine derivatization
3. Post-column electrochemically generated bromine (KOBRA or COBRA cell) (See section 5.4)
4. Post-column in-line photochemical derivatization (PHRED)

The following is a list of articles on derivatization methods for aflatoxins.

Jaimez J, Fente CA, Vazquez BI, Franco CM, Cepeda A, Mahuzier G, Prognon P. *Journal of Chromatography A.* Application of the assay of aflatoxins by liquid chromatography with fluorescence detection in food analysis. 2000 Jun 16; 882(1-2): 1-10. Review.

Kok WT. *Journal of Chromatography B Biomed Application.* Derivatization reactions for the determination of aflatoxins by liquid chromatography with fluorescence detection. 1994 Sep 23; 659(1-2): 127-37.

Joshua, H., *Journal of Chromatography,* Determination of aflatoxins by reversed-phase high-performance liquid chromatography with post-column in-line photochemical derivitization and fluorescence detection, 654 (1993) 247-254.

Waltking, Arthur, *Journal of AOAC International*, Liquid Chromatographic Analysis of Aflatoxin Using Post-Column Photochemical Derivatization: Collaborative Study, 2006, 89 (3), 678-692.

5.2 HPLC CONDITIONS

Condition 1

1.1 **Column**: reverse phase C18 (Waters Nova pak C18, 3.9mm X 150mm, 4µm column part #WAT086344, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5mm X 12.5cm, 5µm).
1.2 **Mobile phase**: methanol:water (45:55) isocratic degassed.
1.3 **Flow rate**: 0.8 mL/min.
1.4 **Fluorescence detector**: excitation 360 nm, emission 440 nm.
1.5 **Post column:**
Post column iodine: 0.05% iodine solution (see section 3.4, Preparation of HPLC Solutions).
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40[Eppendorf] heater & FIAtron [Eppendorf]TC-50 controller).
Reaction time: ~1 minute.

Condition 2

2.1 **Column**: reverse phase C18 (Waters Nova pak C18, 3.9 mm X 150mm, 4µm cartridge WAT086344, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5mm X 12.5cm, 5µm).
2.2 **Mobile phase**: methanol:water (45:55) isocratic degassed.
2.3 **Flow rate**: 1.0 ml/min.
2.4 **Fluorescence detector**: excitation 360 nm, emission 440 nm.
2.5 **Post column**:
Photochemical reactor: Aura Industries, Staten Island, NY, VICAM product #G8500

Condition 3

3.1 **Column**: 4.6mm x 25cm, 5µm, C18 (Rainin).
3.2 **Mobile phase**: water:acetonitrile:methanol (3:1:1) degassed.
3.3 **Flow rate**: 1.0 ml/min.
3.4 **Fluorescence detector**: Kratos 950 fluorescence detector, excitation 360 nm, emission >420 nm cut off emission filter.
3.5 **Post column**:
Post column iodine: 0.05% iodine solution (see section 3.4, Preparation of HPLC Solutions).
Flow rate: 0.3 ml/min.
Reaction temperature: 70°C (FIAtron FH-40[Eppendorf] heater & FIAtron [Eppendorf]TC-50 controller). Reaction time: ~1 minute.

Condition 4

4.1 **Column**: Whatman Partisphere RTF C_{18} 4.6 X 150mm or Merck 5mm X 12.5cm C_{18} column, 5 µm, Waters Novapak® C_{18}, 5 x 100mm, 4 µm.
4.2 **Mobile phase**: methanol:water (45:55) isocratic degassed.
4.3 **Flow rate**: 0.8 mL/minute.
4.4 **Fluorescence Detector**: excitation = 360 nm, emission = 440 nm.
4.5 **Peak retention time**: ~5 minutes.

Condition 5

5.1 **Column**: Rainin 4.6mm X 25cm Microsorb - C_{18}.
5.2 **Mobile phase**: methanol:water (50:50) isocratic degassed.

5.3 **Flow rate**: 0.7 ml/minute.
5.4 **Absorbance Detector**: Beckman 160 detector 365 nm.
5.5 **Peak retention time**: ~8 minutes.

*DISCLAIMER
Although specific equipment and HPLC columns are listed in this document, there are a number of equally suitable components that can also be used.

5.3 TFA DERIVATIZATION

- Evaporate aflatoxin-containing eluate to dryness in a silanized glass vial (on steam plate, under nitrogen or using an evaporator/concentrator).
- Redissolve aflatoxins in 200 µL Hexane and add 200 µL TFA. Mix Well. Cap vial tightly.
- Incubate at 40^{o}C for 10 minutes.
- Evaporate derivatized sample to dryness.
- Redissolve derivatized aflatoxins in 200 µL of mobile phase.
- Inject appropriate amount onto HPLC.

5.4 KOBRA CELL INSTALLATION

Add to one liter of the existing mobile phase 119 mg of potassium bromide and 350ul of 4M Nitric acid or see section 3.3, Preparation of HPLC Solutions. Connect the reservoir to the HPLC.

The Kobra Cell is supplied filled with water in order to keep the ion-exchange membrane wet. Disconnect all capillary tubing from the Kobra Cell and connect the HPLC column discharge to the Kobra Cell inlet (top left side, i.e. closest port to the red terminal) and the Kobra Cell outlet (same side of housing) to the detector inlet-this length of tubing is critical. The length is dependant on the flow rate and the internal diameter of the tubing. Cut an appropriate length of tubing according to the flow rate to be used. See table, "Determining the Reaction Coil Length" in "Kobra Cell Instructions For Use" manual.

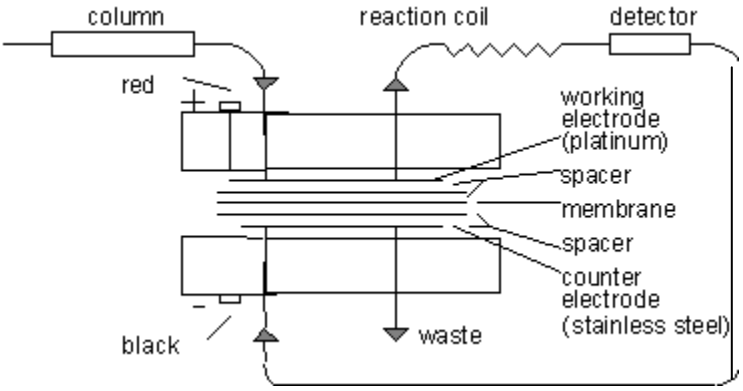
Connect the detector outlet to the Kobra Cell inlet (reverse bottom side closest port to the black terminal) and the Kobra Cell outlet (same side of housing) to waste. All connections are made to hand tightness and should be plastic, not metal.

Switch on the HPLC pump and flush mobile phase through the system for approximately 5 minutes.

Connect the Kobra Cell current source to the cell, red lead to red terminal and black lead to black terminal. Switch "on" current source with the setting at 100uA. Do not switch on the current source without the mobile phase flowing through the Kobra Cell, otherwise damage to the ion exchange membrane will occur.

After the HPLC has been allowed to stabilize (approx. 15 minutes) the Kobra Cell is ready to be used.

Kobra Cell assembly:



5.5 HPLC STANDARD PREPARATION AND SAMPLE SPIKING

A Hamilton Syringe is preferred for spiking samples and preparing standards, but an adjustable micropipettor with disposable plastic tips can also be used. The Supelco aflatoxin standard product # 46304-U comes in sealed ampules. The concentration of this aflatoxin standard stock solution is about 2.6ng/µL in methanol. This standard is prepared according to AOAC Official methods. The certificate of analysis will show the exact concentration of each of the 4 different aflatoxins. An opened ampule should be able to be used for up to two weeks when stored at 2 – 8 °C. Use only HPLC grade methanol when preparing aflatoxin solutions.

5.5.1. Aflatoxin solutions

Prepare a 0.26 ng/µL aflatoxin standard by adding 100µL of the 2.6ng/µL aflatoxin standard stock solution to 900µL methanol.

Prepare a 0.026 ng/µL aflatoxin standard by adding 100µL of the 0.26ng/µL aflatoxin standard to 900µL methanol.

5.5.2. Spiking corn with aflatoxin at 26 ppb level

26 ppb (ng/g) X 50g corn = 1300ng
1300ng ÷ 2.6 ng/µL = 500µL
Add 500µL of the 2.6 ng/µL aflatoxin standard to 50g of aflatoxin-free corn

Allow the spiked sample to dry in a hood for at least 30 minutes before assaying.

5.5.3. Preparing HPLC standards for 1 gram equivalent procedures

1.3 ppb (0.5 B_1:0.15 B_2:0.5 G_1:0.15 G_2 ng/g) X 1g = 1.3 ng
1.3 ng ÷ 0.026ng/µL standard = 50µL
50µL 0.026ng/µL standard added to 950µl methanol

2.6 ppb (1.0 B_1:0.3 B_2:1.0 G_1:0.3 G_2 ng/g) X 1g = 2.6 ng
2.6 ng ÷ 0.026ng/µL standard = 100µL
100µL 0.026ng/µL standard added to 900µl methanol

26 ppb (10 B_1:3.0 B_2:10 G_1:3.0 G_2 ng/g) X 1g = 26 ng
26 ng ÷ 0.26ng/µL standard = 100µL
100µL 0.26ng/µL standard added to 900µl methanol

52 ppb (20 B_1:6.0 B_2:20 G_1:6.0 G_2 ng/g) X 1g = 52 ng
52 ng ÷ 0.26ng/µL standard = 200µL
200µL 0.26ng/µL standard added to 800µl methanol

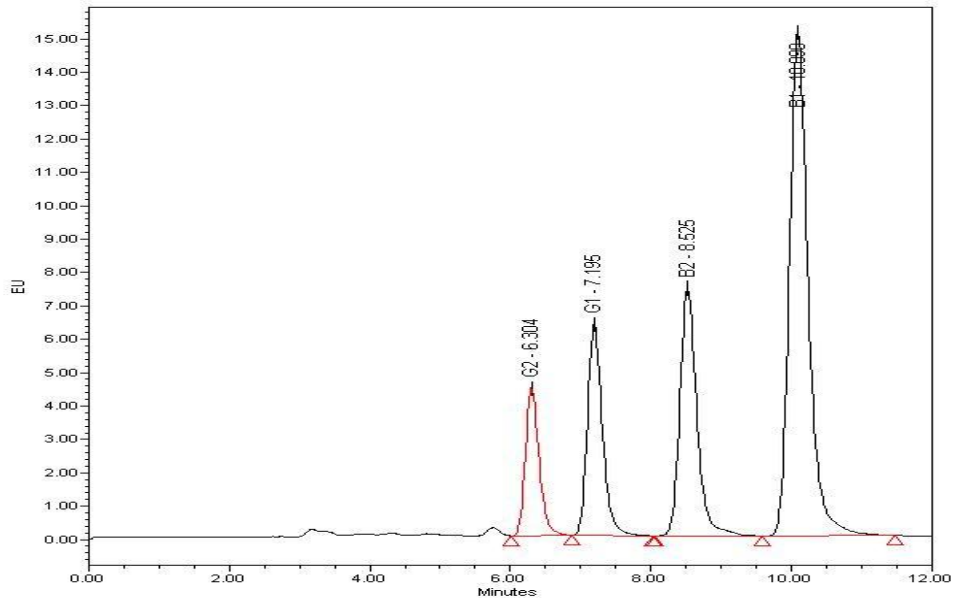
Add 1 mL water to the 1 mL of methanol eluate for all standards and samples before injecting onto the HPLC. Adding water to the standards and samples makes them more similar to the HPLC mobile phase.

Make a graph of ppb level of the standards vs peak area. The peak area of the unknown samples are then plugged into the equation of this line to calculate the ppb value of the samples. This calculation can be done with the software provided by an HPLC manufacturer. In addition, this calculation can be done using Microsoft Excel software.

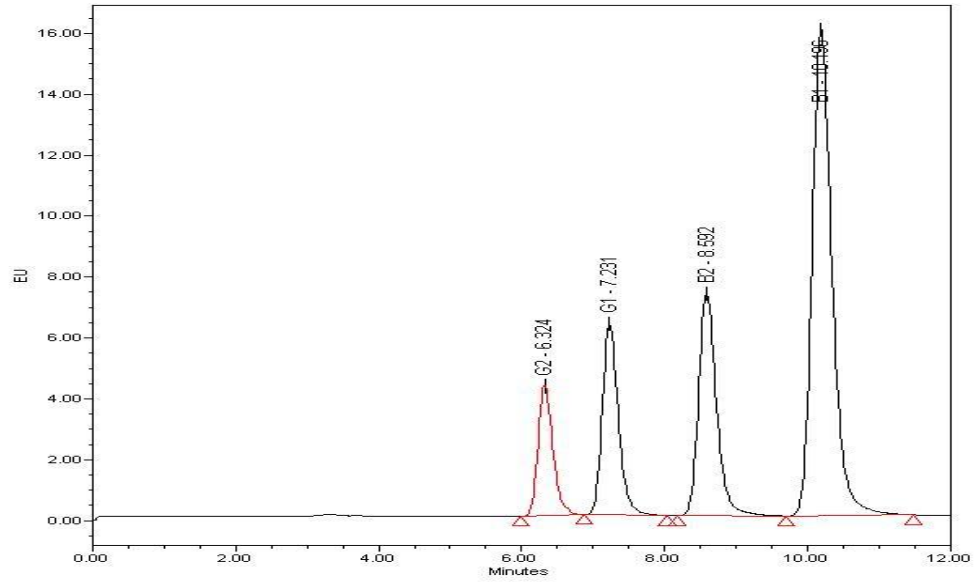
5.6 REPRESENTATIVE CHROMATOGRAMS

AflaTest® Representative Chromatogram for Corn Using HPLC Condition 2

4.3 ppb aflatoxin contaminated corn



5 ppb total aflatoxin standard



6.0 GENERAL PRECAUTIONS FOR HPLC PROCEDURES

Absorbance detection without post column derivatization is less sensitive. For greater sensitivity, add 100µl purified water to elute and concentrate the volume of the eluate to about 100 - 200 µL on a steam plate, under nitrogen or on an evaporator. Inject entire sample quantitatively.

If drying is performed, use siliconized vials to avoid irreversible binding of aflatoxins to the tube walls.

7.0 PROCEDURE FOR SILANIZING GLASSWARE

7.1 Make a 2% solution of Dimethyldichlorosilane (Sigma Chemical Co. # 3879) in toluene.
7.2 Fill glassware with DMDCS/Toluene solution.
7.3 Heat at 40°C for about 30 minutes.
7.4 Rinse three times with toluene.
7.5 Rinse three times with methanol.
7.6 Bake in oven at 180°C for three hours.

Alternatively, you can use Pierce SurfaSil (product # 42800). Dilute Surfasil 1:10 in hexane, dip cuvettes into the solution, rinse 3 times with hexane, rinse 3 times with methanol and let air dry without heating.

8.0 TECHNICAL ASSISTANCE

For assistance please contact your local distributor or VICAM Technical Services:

Phone:	800-338-4381	Canada, Mexico and the United States
	617-926-7045	all International and United States customers
Fax:	617-923-8055	
e-mail:	techservice@VICAM.com	

9.0 LIABILITY

The analytical methods described above have been developed by VICAM to be used exclusively with the reagents in this test. The user assumes all risk in using AflaTest® analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that AflaTest® products conform to VICAM's printed specifications and quality control standards. VICAM will at its option repair or replace any product or part thereof which proves to be defective in workmanship or material. VICAM's undertaking to repair or replace such products is exclusive and is in lieu of all warranties whether written, oral expressed, or implied, including any implied warranty of merchantability or fitness for a particular purpose. VICAM shall have no liability for anticipated or lost profits or any loss, inconvenience or damage whether

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All VICAM products are protected by worldwide patents and trademarks.

10.0 ORDERING INFORMATION

To place an order contact your local VICAM distributor or VICAM at:

In the United States:

Phone:	877-228-4244	Canada and the United States
	800-338-4381	Mexico
	617-926-7045	all International and United States customers
Fax:	617-923-8055	
e-mail:	vicam@vicam.com	

Effective Date: September 28, 2007