




AflaTest WB SR

Instruction Manual

VICAM®

A Waters Business

34 MAPLE STREET, MILFORD MA 01757 USA
TEL: 800.338.4381, 508.482.4935
FAX: 508.482.4972 EMAIL: VICAM@VICAM.COM

AflaTest® WB SR

Instruction Manual

Manual Number: 715001720 REV A

VICAM®

313 Pleasant Street
Watertown, MA 02472 USA

Tel: 800-338-438
+ 1.617.926.7045

Fax: +1.617.923.8055

E-mail: vicam@vicam.com
www.vicam.com

TABLE OF CONTENTS

1.1 INTENDED USER

AflaTest® WB SR with HPLC detection 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® WB SR 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® WB SR 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® WB SR 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® WB SR Overview.

1.3 APPLICABILITY AND APPROVALS

AflaTest® WB SR has been optimized for quantitative measurement of aflatoxins in corn and almonds. Assistance in measuring aflatoxin in commodities not listed in this manual can be obtained by contacting our Technical Services Department.

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

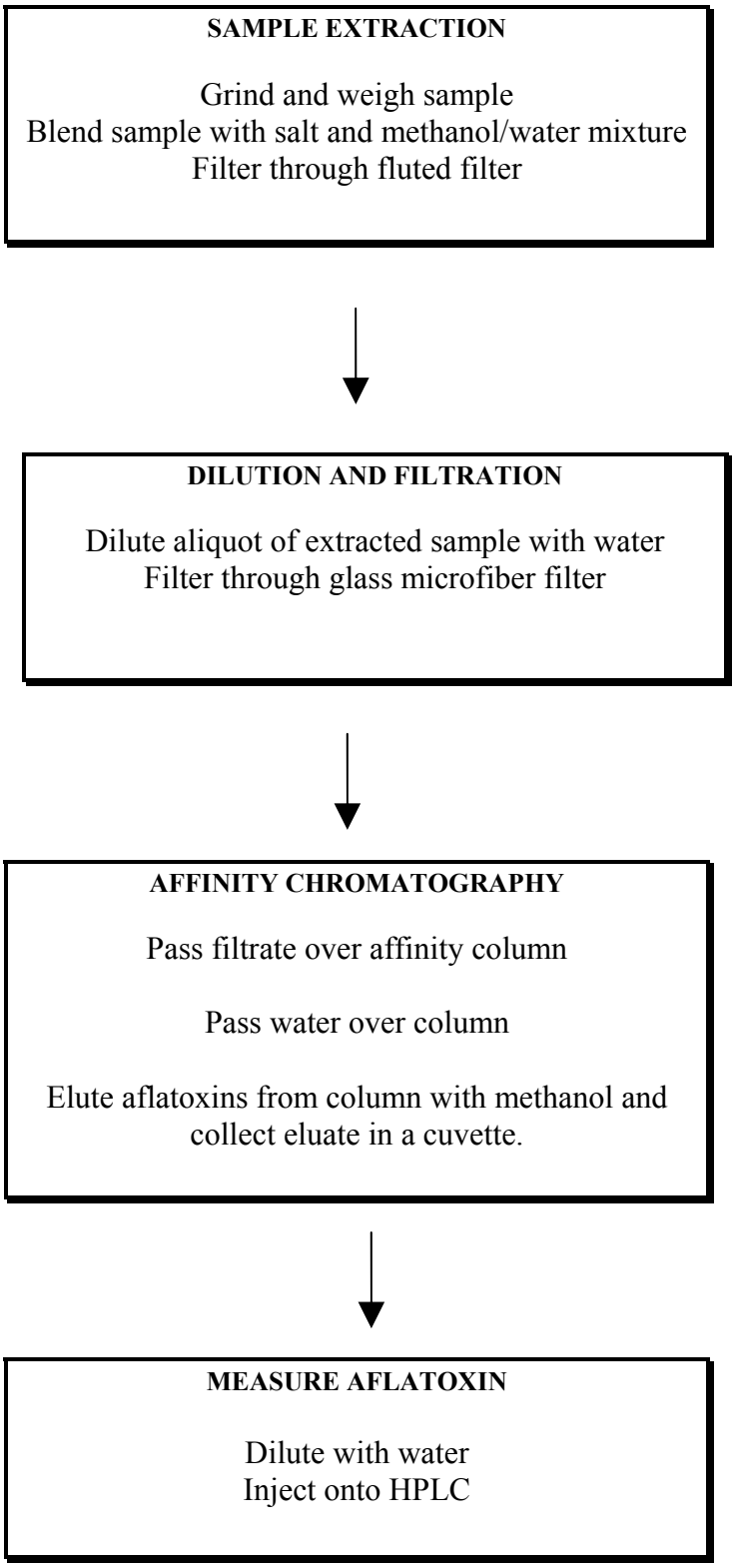
These can be viewed online at www.gipsa.usda.gov.

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 ® WB SR HPLC PROCEDURE OVERVIEW



2.1 PUMP STAND SETUP

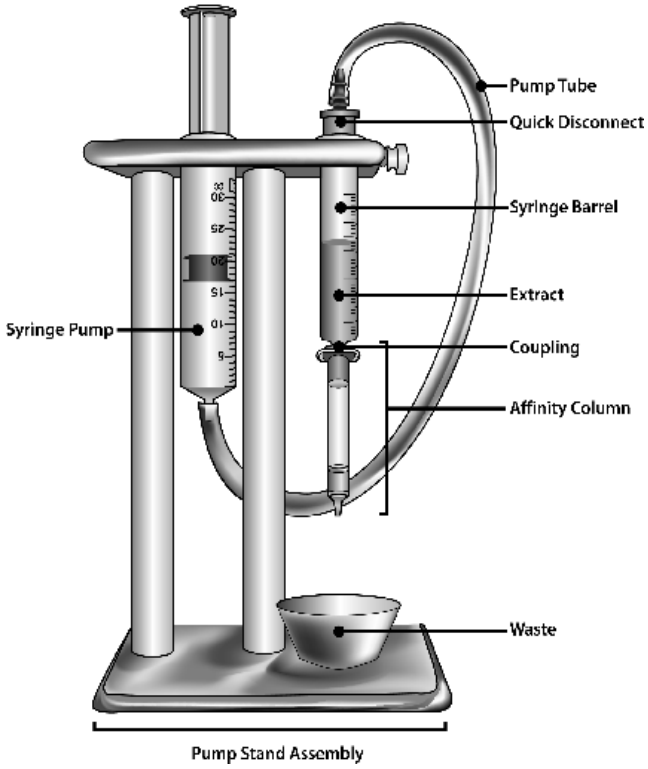
AflaTest® WB SR affinity chromatography is easily performed with the AflaTest® WB SR affinity column attached to a pump stand. The stand has a 10 mL glass syringe barrel that serves as a reservoir for the column. A large plastic syringe with tubing and coupling provides air pressure to manually push 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. Alternatively, a vacuum manifold can be used to pull liquid through the affinity column provided that the equipment will allow control of the flow rate of individual columns as specified in the AflaTest® WB SR procedure.

When using a pump stand:

1. Remove large top cap from column.
2. VICAM WB Column Coupling (part # G1118) provides a reusable coupling for attaching the column to the syringe barrel reservoir.
3. Attach column to coupling and place waste collection cup under column outlet. Keep bottom cap on column.
4. Measure extract into 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® WB SR Testing

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. Wash new syringe barrel for pump stands using a brush with soap and water. Then rinse with purified water and methanol before using. Other pieces of equipment that need to be cleaned with detergent before using are graduated cylinders, funnels and blender jars. Bottle dispensers 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 bottle dispenser with soap. Methanol bottle dispenser 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.

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.

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 MATERIALS AND EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Materials Required

Description	Part #
AflaTest® WB SR Columns (25/box)	G1068
Disposable Plastic Pipets, 1 mL (50)	20652
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfiber Filters, 1.5µm, 11 cm (100)	31955
Disposable Cuvettes (250)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25)	36010
Noniodized sodium chloride (salt, NaCl)	G1124
Distilled, reverse osmosis or deionized water	

Equipment Required

Description	Part #
Graduated Cylinder, 50 mL	20050
Digital Scale with AC Adapter	20100
Commercial Blender with Stainless Steel Container	20200
Graduated Cylinder, 250 mL	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
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

3.2 AFLATEST ® WB SR HPLC PROCEDURE FOR ALMONDS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 500 PPB)

1.0 HPLC Set Up*:

- **1.1** **Column**: NovaPak C18, 4µm, 3.0 x 150 mm (Waters #WAT086344) with NovaPak C18 guard column (Waters #WAT044380)
- **1.2** **Mobile phase**: methanol:water (45:55, v:v)
- **1.3** **Flow rate**: 0.8 mL/min
- **1.4** **Fluorescence detector**: Waters 2475 fluorescence detector, excitation 360 nm, emission 440 nm
- **1.5** **Post column**: Photochemical reactor (VICAM #G8500)

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 diluted extract through 1.5µm microfibre filter (VICAM # 31955) into a clean vessel.

4.0 Column Chromatography

- **4.1** Pass 15 mL filtered diluted extract (15 mL = 1g sample equivalent) completely through AflaTest ® WB SR affinity column at a rate of about 1 drop/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 # 34000) under AflaTest ® WB SR column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® WB SR 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 Recovery: On Certificate of Analysis supplied with columns.

*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.

3.3 AFLATEST ® WB SR HPLC PROCEDURE FOR CORN (1.0 GRAM SAMPLE EQUIVALENT, 0 - 100 PPB)

1.0 HPLC Set Up*:

- **1.1** **Column**: NovaPak C18, 4µm, 3.0 x 150 mm (Waters #WAT086344) with NovaPak C18 guard column (Waters #WAT044380)
- **1.2** **Mobile phase**: methanol:water (45:55, v:v)
- **1.3** **Flow rate**: 0.8 mL/min
- **1.4** **Fluorescence detector**: Waters 2475 fluorescence detector, excitation 360 nm, emission 440 nm
- **1.5** **Post column**: Photochemical reactor (VICAM #G8500)

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 diluted extract through 1.5µm microfibre filter (VICAM # 31955) into a clean vessel.

4.0 Column Chromatography

- **4.1** Pass 10 mL filtered diluted extract (10 mL = 1g sample equivalent) completely through AflaTest ® WB SR 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 # 34000) under AflaTest ® WB SR column and add 1.0 mL HPLC grade methanol into glass syringe barrel.
- **4.5** Elute AflaTest ® WB SR 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 Recovery: On Certificate of Analysis supplied with columns.

*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.

3.4 OTHER PUBLISHED HPLC PROCEDURES USING IMMUNOAFFINITY COLUMNS

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 B_1 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 B_1 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 M_1 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.

4.1 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)
2. Post-column iodine or Pyridinium hydrobromide perbromide (PBPB) derivatization
3. Post-column electrochemically generated bromine (KOBRA or COBRA cell)
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.

4.2 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 ampoules. 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 ampoule 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.

4.2.1. 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.

4.2.2. Spiking sample with aflatoxin at 26 ppb level

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

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

4.2.3. 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

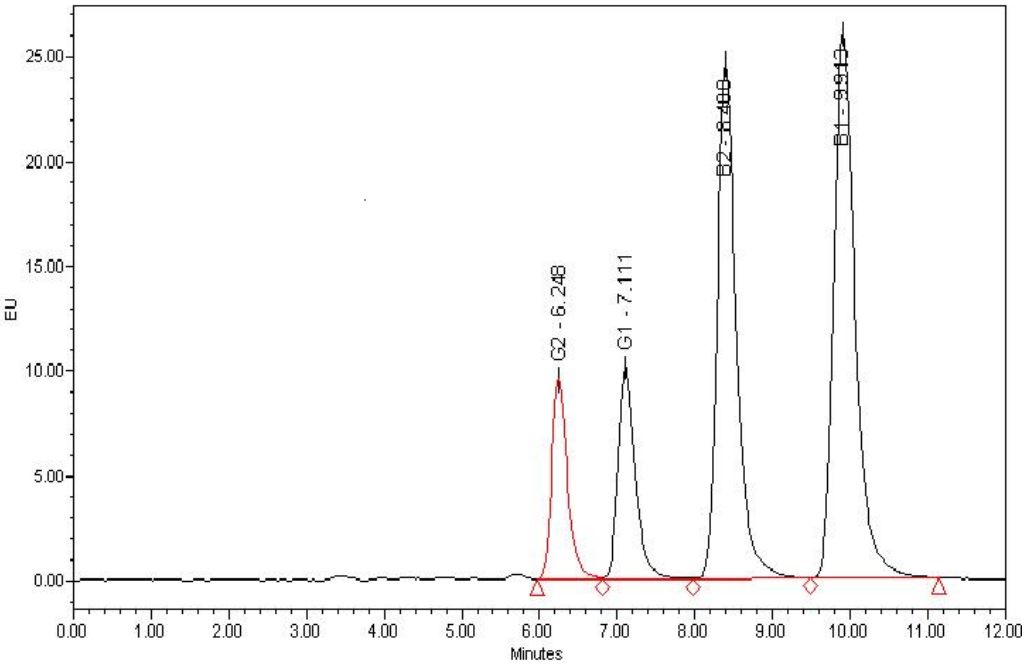
2.6 ppb (1.0 B_1:0.3 B_2:1.0 G_1:0.3 G_2 ng/g) X 1g = 2.6 ng
2.6 ng ÷ 0.026ng/µL standard = 100µL
100µL 0.026ng/µL standard added to 900µl methanol

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

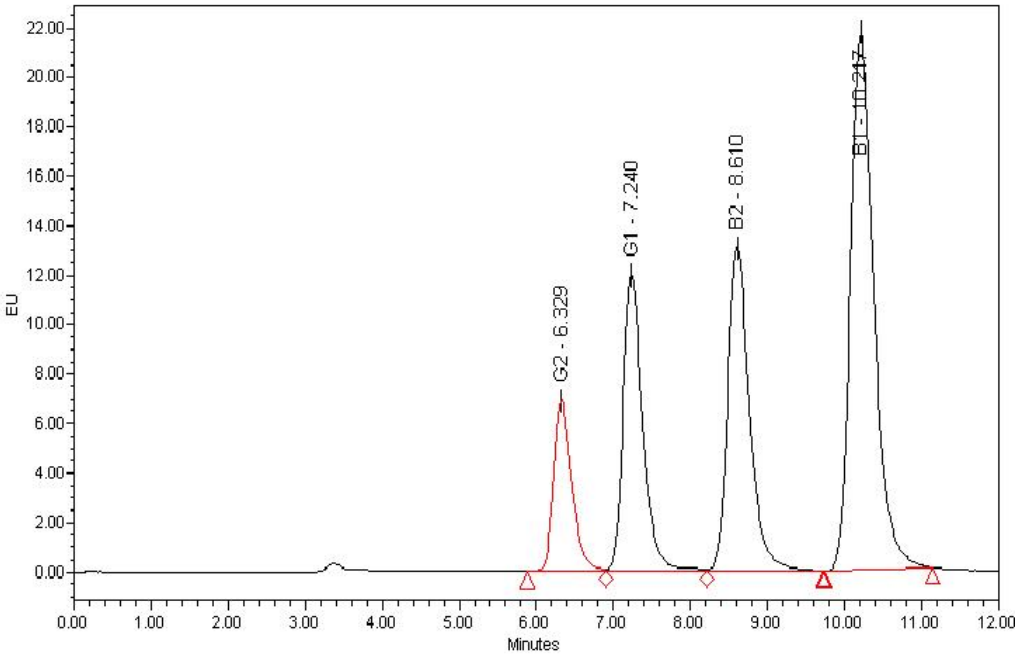
Add 1 mL water to the 1 mL of methanol eluate for all standards and samples before injecting onto the HPLC. Adding water to the standards and samples makes them more similar to the HPLC mobile phase.

4.3 REPRESENTATIVE CHROMATOGRAMS

11 ppb aflatoxin contaminated almonds



10 ppb total aflatoxin standard



5.0 GENERAL PRECAUTIONS FOR HPLC PROCEDURES

Fluorescence 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 silanized vials to avoid irreversible binding of aflatoxins to the tube walls.

The procedures in this manual may involve the use of hazardous and/or corrosive chemicals. Customers are urged to exercise appropriate caution when using and disposing of these chemicals.

6.0 PROCEDURE FOR SILANIZING GLASSWARE

6.1 Make a 2% solution of Dimethyldichlorosilane in toluene.
6.2 Fill glassware with DMDCS/Toluene solution.
6.3 Heat at 40°C for about 30 minutes.
6.4 Rinse three times with toluene.
6.5 Rinse three times with methanol.
6.6 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.

7.0 TECHNICAL ASSISTANCE

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

Phone: 800-338-4381 Canada, Mexico and the United States

508-482-4935 all International and United States customers

Fax: 508-482-4972

e-mail: techservice@vicam.com

8.0 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® WB SR analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that AflaTest® WB SR 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 direct, incidental, consequential or otherwise, to person or property, or for strict liability or negligence arising from or in connection with the use of these assay procedures or AflaTest® WB SR product.

The foregoing notwithstanding, protocols and other products developed by VICAM are periodically improved and revised in order to maximize reliability and optimize customer use and satisfaction. When an improved, new or substitute version of a protocol and product is available, VICAM shall not be held liable or responsible for any earlier protocol or product, even if use of earlier product or protocol be within the expiration date. Please inform yourself about any new protocols by either e-mailing, faxing or phoning VICAM or your local VICAM distributor.

VICAM shall not be liable or responsible for any unsatisfactory or faulty results or performance involving the use of VICAM protocols or products if the testing or sampling in question is not conducted properly. The customer is solely and fully responsible for educating oneself about the proper testing and sampling procedures using VICAM protocols and products.

All VICAM products are protected by worldwide patents and trademarks.

9.0 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
	417-725-6588	all International and United States customers
Fax:	417-725-6102	
e-mail:	orders@vicam.com	

Effective Date: May 1, 2008
715001720 REV A