




ZearalaTest and ZearalaTest WB

Instruction Manual

(for HPLC use)

VICAM®

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1.0 INTRODUCTION

1.1 INTENDED USER

ZearalaTest™ and ZearalaTest™ WB are quantitative methods for the detection of zearalenone in corn, wheat, and grains. These products are safe and simple to use. Sample cleanup with ZearalaTest immunoaffinity columns takes as little time as 15 minutes. The methods described in this manual are intended for HPLC systems.

1.2 PRINCIPLE

Zearalenone is an estrogenic mycotoxin produced by the fungus Fusarium graminearum. Zearalenone induces feminization in animals at concentrations in feed of about 1 ppm. Higher concentrations (50-100 ppm) can interfere with conception, ovulation, implantation, fetal development, and the viability of newborn animals.

Zearalenone testing is performed by mixing a representative sample of a given commodity with an extraction solution, followed by blending and filtering. The extract is then applied to the ZearalaTest or ZearalaTest WB column, which contains specific antibodies to zearalenone. At this stage, the zearalenone binds to the antibody on the column. After the column is washed to remove impurities, the zearalenone is released from the antibody by passing methanol through the column. The resulting solution is then injected into an HPLC system to measure the concentration of zearalenone in the sample. These steps are outlined in sections 1.7, ZearalaTest Overview and 1.8, ZearalaTest WB Overview.

1.3 APPLICABILITY

ZearalaTest and ZearalaTest WB have been validated for quantitative measurement of zearalenone in corn. The Table of Contents lists the testing protocols developed for specific commodities as of the publication date of this manual. For assistance with measuring zearalenone in other commodities, please contact our Technical Assistance Department.

1.4 LIMITATIONS

These tests have been designed for use with the procedures and reagents described on the following pages. Do not use materials beyond the expiration date. Deviation from these instructions may interfere with test 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 Federal Grain Inspection Service (FGIS) publications:

FGIS Grain Inspection Handbook, Book 1, Grain Sampling

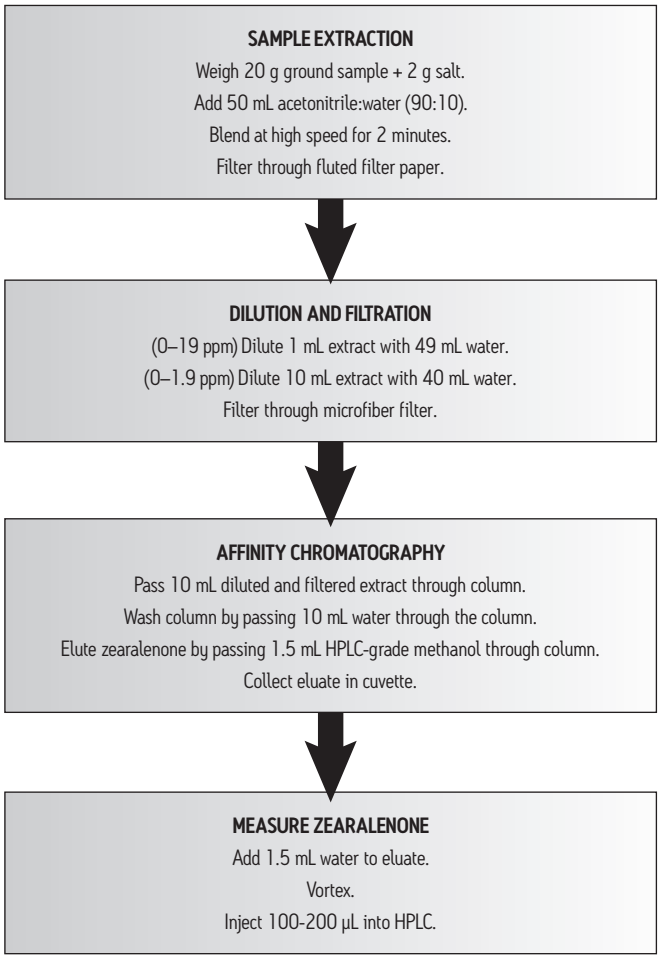
FGIS Mechanical Sampling Systems Handbook

To view these publications online, go to http://www.gipsa.usda.gov. Then click on the “Handbooks” Hot Link.

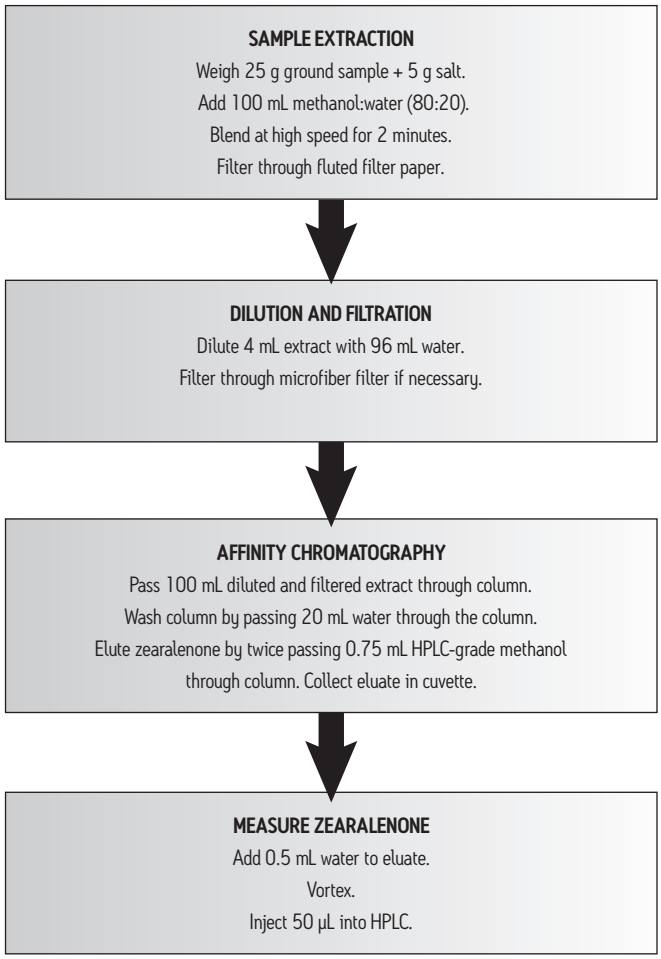
1.6 SHELF LIFE AND STORAGE CONDITIONS

Store columns at room temperature (18–30°C). Storage at temperatures above 30°C for a prolonged time may reduce shelf life. If storage temperatures higher than 30°C are anticipated, all components should be refrigerated (2–8°C). Reagents should be at room temperature (18–22°C) for usage. Check column box or reagent bottle for expiration date.

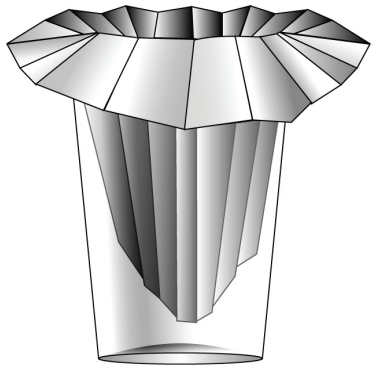
1.7 ZEARALATEST HPLC PROCEDURE OVERVIEW



1.8 ZEARALATEST™ WB PROCEDURE OVERVIEW



2.1 FILTRATION STEPS



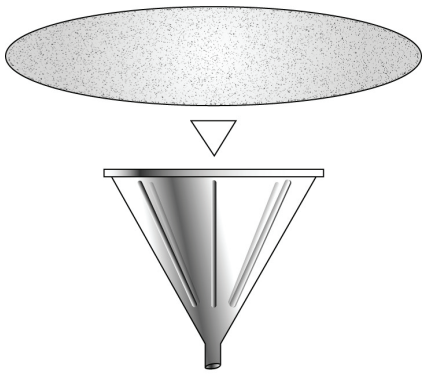
2.1.1 Fluted Filter

The fluted filter paper is used to separate the extract solution from the coarse particulate solids in the prepared sample. The extract solution is collected in a clean vessel.

1. Carefully open one fluted filter paper and insert it into clean vessel. (Optional: a funnel may be used to hold the filter.)

2. Fold edges of filter over rim of cup to secure it. The fluted folds of the filter paper are designed to maximize surface area and should be left in place to maximize the speed of filtration.

3. Pour prepared sample into filter-lined vessel. When the desired amount of extract has been collected, continue with the next step.

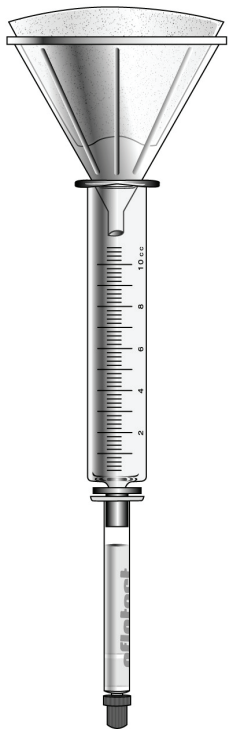


2.1.2 Microfiber Filter

The microfiber filter is used to remove precipitates from the extract solution. This step is performed just before affinity chromatography to facilitate flow of the extract solution through the affinity column.

1. Place a small funnel in top outlet of syringe barrel or clean vessel.

2. Place one microfiber filter in small funnel and gently press the filter into place with your index finger. Be careful not to rip or puncture the filter. Pour solution into filter-lined funnel.



2.2 PUMP STAND SETUP

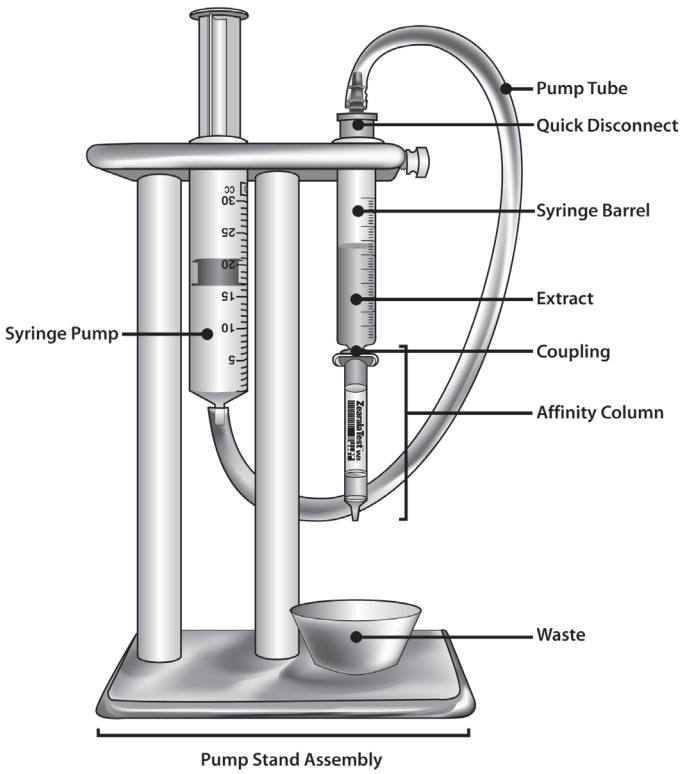
The ZearalaTest HPLC affinity column attaches to a pump stand with a 10 mL syringe barrel for collecting sample extract. A manually operated syringe pump pushes liquids into the column through the pump tube. An adjustable air pump (part # 20650) that works without hand pumping may be substituted. Two-position pump stands (part # 21030), 4-position (part # 21045), and 12-position (part # G1104) pump stands with air pumps are available for running multiple samples at one time. Alternatively, a vacuum manifold can be used to pull liquid through the affinity column.

When using a pump stand:

1. Remove large top cap from column. Cut 1/8-inch off the bottom with scissors or a blade to make a reusable coupling for attaching column to the sample syringe barrel. (For the WB column coupling, order part # G1118.)
2. Attach coupling to column and place waste collection cup underneath. Keep bottom cap on.
3. Pour extract through microfiber filter (see previous section) and collect desired amount of extract in sample syringe barrel.
4. Pull up on piston of syringe pump barrel.
5. Attach pump tube to syringe barrel. Remove column bottom cap.
6. Apply pressure to piston of syringe pump. Push liquid into column, maintaining a flow rate of 1–2 drops per second, until the sample syringe is empty. Note: Liquid may flow at desired rate without additional hand pumping.
7. Repeat for wash and elution steps. (See Procedures.)

Note: To avoid displacing antibody-coated beads, do not pull up on piston while syringe pump is connected to sample syringe barrel. Doing so may affect test results.

Affinity Column Syringe Barrel Connection



2.3 CLEANING EQUIPMENT

Before Testing

To eliminate contaminants that might cause background fluorescence, thoroughly clean all equipment. This step is particularly important when equipment is brand new or has been in storage for extended periods. The lubricant on new syringe barrels should be removed by thorough washing with a brush, a mild detergent, and water, and then rinsed with purified water and methanol. Graduated cylinders, funnels, and blender jars should be washed with soap and rinsed thoroughly with purified water; 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 dispensers with soap. Methanol bottle dispenser needs only to be refilled with methanol.

Between each assay, rinse the syringe barrel reservoir once with methanol and then rinse it again with purified water to prevent cross-contamination of samples. After testing a large number of samples, wash the syringe with a brush and mild detergent and then rinse well with purified water.

Cuvettes are designed for one-time use and should be discarded, rather than washed and reused.

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.

2.4 EXTRACTION SOLUTION

The ZearalaTest procedure uses an acetonitrile:water (90:10) or a methanol:water (80:20) solution to extract zearalenone from dry samples.

Prepare extraction solution every week or as needed. The formula below will yield 1 liter of solution. Solution volume may be increased or decreased as needed, provided the proportion of reagents is kept consistent. Use reagent-grade (or better, i.e., HPLC-grade) acetonitrile or methanol when preparing extraction solutions.

SOLVENT	SOLVENT AMOUNT (mL)	PURIFIED WATER (mL)	TOTAL VOLUME (mL)
Acetonitrile	900	100	1000 (1 liter)
Methanol	800	200	1000 (1 liter)

CAUTION: Extraction solvent is flammable. Keep container tightly capped when not in use.

3.0 HPLC PROCEDURES

3.1 MATERIALS AND EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Consumables Required

DESCRIPTION	PART #
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfiber Filters, 1.5 mm, 11 cm (100)	31955
Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC-Grade (4 X 4 L)	35016
ZearalaTest Columns HPLC (25)	G1012
Or ZearalaTest HPLC Kit - 100 columns and 1 set standards	G1058
ZearalaTest WB Columns (25 per box)	G1026
Or ZearalaTest WB HPLC Kit - 100 Columns and 1 set Stds	100000189
Acetonitrile HPLC-Grade	G1130
Noniodized sodium chloride (salt, NaCl) (100 g)	G1124
Distilled, reverse osmosis, or deionized water	–

Equipment Required

DESCRIPTION	PART #
Graduated Cylinder, 100 mL	G4009
Digital Scale with AC Adapter	20100
Commercial Blender with Stainless Steel Container	20200
Glass Blender Jars	20300
Graduated Cylinder, 250 mL	20250
Wash Bottle, 500 mL	20700
Cuvette Rack	21010
Single Position Pump Stand	21020
or 2- Position Pump Stand w air pump (10 mL)	21030
or 4-Position Pump Stand w/2 air pump (10 mL)	21045
or 12-Position Pump Stand w/6 air pump (10 mL)	G1104
Filter Funnel, 65 mm (10 per pack)	36020
Handheld Micropipettor, 1.0 mL	G4033

DESCRIPTION	PART #
Vortex Mixer	23040
Filter Funnel, 105 mm (4 per pack)	36022
Adjustable Air Pump	20650

3.2 ZEARALATEST HPLC PROCEDURE FOR CORN, 17% PROTEIN POULTRY FEED, AND MILO (1:50 dilution) SAMPLE (0-19 PPM)

3.2.1 HPLC Setup

See HPLC Condition #1.

3.2.2 Sample Extraction

1. Weigh 20 g ground sample with 2 g salt (NaCl) and add to a glass blender jar.
2. Begin extraction procedure by adding 50 mL acetonitrile:water (90:10).

   NOTE: To ensure adequate blending for this procedure, use the glass blender jar with the amounts specified above. If only a stainless steel blender jar is available, use 40 g sample, 4 g salt, and 100 mL extraction solvent.
3. Cover blender jar and blend at high speed for 2 minutes.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.2.3 Extract Dilution

1. Remove two end caps from ZearalaTest affinity column.
2. Cut off tip of column top cap to use as a coupling. Attach column to outlet of 10 mL syringe barrel on pump stand.
3. Transfer 1 mL filtered extract into another clean vessel.
4. Dilute extract with 49 mL distilled, reverse osmosis, or deionized water. Mix well.
5. Filter dilute extract through microfiber filter (part # 31955) into a clean vessel.

3.2.4 Column Chromatography

1. Pass 10 mL (10 mL = 0.08 g equivalent) diluted and filtered extract completely through ZearalaTest affinity column at a rate of about 1-2 drops/second until air comes through column.
2. Wash column by passing 10 mL of distilled, reverse osmosis, or deionized water through the column at a rate of 1-2 drops/second until air comes through the column.
3. Elute affinity column by passing 1.5 mL HPLC-grade methanol through column at a rate of about 1 drop/second, collecting all of the sample eluate (1.5 mL) in a glass cuvette.
4. Add 1.5 mL water to eluate. Vortex.
5. Inject 100-200 µL into HPLC.

3.2.5 Limit of Detection: 0.10 ppm

3.2.6 Recovery: Greater than 85% zearalenone at 1500 ng

3.3 ZEARALATEST HPLC PROCEDURE FOR CORN (1:5 dilution) SAMPLE (0-1.9 PPM)

3.3.1 HPLC Setup

See HPLC Condition #1.

3.3.2 Sample Extraction

1. Weigh 20 g ground sample with 2 g salt (NaCl) and add to a glass blender jar.
2. Begin extraction procedure by adding 50 mL acetonitrile:water (90:10).

   NOTE: To ensure adequate blending for this procedure, use the glass blender jar with the amounts specified above. If only a stainless steel blender jar is available, use 40 g sample, 4 g salt, and 100 mL extraction solvent.
3. Cover blender jar and blend at high speed for 2 minutes.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.3.3 Extract Dilution

1. Remove two end caps from ZearalaTest affinity column.
2. Cut off tip of column top cap to use as a coupling. Attach column to outlet of 10 mL syringe barrel on pump stand.
3. Transfer 10 mL filtered extract into another clean vessel.
4. Dilute extract with 40 mL distilled, reverse osmosis, or deionized water. Mix well.
5. Filter dilute extract through microfiber filter (part # 31955) into a clean vessel.

3.3.4 Column Chromatography

1. Pass 10 mL (10 mL = 0.8 g equivalent) diluted and filtered extract completely through ZearalaTest affinity column at a rate of about 1-2 drops/second until air comes through column.
2. Wash column by passing 10 mL of distilled, reverse osmosis, or deionized water through the column at a rate of 1-2 drops/second until air comes through the column.
3. Elute affinity column by passing 1.5 mL HPLC-grade methanol through column at a rate of about 1 drop/second, collecting all of the sample eluate (1.5 mL) in a glass cuvette.
4. Add 1.5 mL water to eluate. Vortex.
5. Inject 100-200 µL into HPLC.

3.3.5 Limit of Detection: 0.010 ppm

3.3.6 Recovery: Greater than 85% zearalenone at 1500 ng

3.4 ZEARALATEST HPLC PROCEDURE FOR CORN (A. Visconti's Method) 10 mL SAMPLE (0-5.0 PPM)

3.4.1 HPLC Setup

See HPLC Condition #1.

3.4.2 Sample Extraction

1. Weigh 20 g ground corn sample with 2 g KCl (potassium chloride) and add to a glass blender jar.
2. Begin extraction procedure by adding 50 mL acetonitrile:water (90:10).

   Note: To ensure adequate blending for this procedure, use the glass blender jar with the amounts specified above. If only a stainless steel blender jar is available, use 40 g sample, 4 g salt, and 100 mL extraction solvent.
3. Cover blender jar and blend at high speed for 2 minutes.
4. Remove cover from jar and pour extract through an 11 mm filter paper (Whatman #1) into a clean vessel.

3.4.3 Extract Dilution

1. Remove two end caps from ZearalaTest affinity column.
2. Cut off tip of column top cap to use as a coupling. Attach column to outlet of 10 mL syringe barrel on pump stand.
3. Transfer 1 mL filtered extract into another clean vessel.
4. Dilute extract with 49 mL distilled, reverse osmosis, or deionized water. Mix well.
5. Filter dilute extract through microfiber filter (part # 31955) into a clean vessel.

3.4.4 Column Chromatography

1. Pass 10 mL (10 mL = 0.08 g equivalent) diluted and filtered extract completely through ZearalaTest affinity column at a rate of about 1–2 drops/second until air comes through column.
2. Wash column by passing 10 mL distilled, reverse osmosis, or deionized water through the column at a rate of 1–2 drops per second until air comes through the column.
3. Elute affinity column by passing 1.5 mL HPLC-grade methanol through column at a rate of about 1 drop/second, collecting all of the sample eluate (1.5 mL) in a glass cuvette.
4. Dry samples using a concentrator (SpeedVac from Savant).
5. Re-suspend in 250 µL water:methanol:acetonitrile (70:20:10, v:v:v).
6. Mix well and add to autosampler vial.
7 Inject 100 µL into HPLC system.

3.4.5 Limit of detection: Less than 0.10 ppm

3.4.6 Recovery: Greater than 85% zearalenone at 1500 ng

3.5 ZEARALATEST WB HPLC PROCEDURE FOR CORN (0–1.5 PPM)

3.5.1 HPLC Setup

See HPLC Condition #2.

3.5.2 Sample Extraction

1. Weigh 25 g ground sample with 5 g salt (NaCl) and add to a glass blender jar.
2. Begin extraction procedure by adding 100 mL methanol:water (80:20).
3. Cover blender jar and blend at high speed for 2 minutes or shake 30 minutes at 150 shakes per minute.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.5.3 Extract Dilution and Filtration

1. Remove two end caps from ZearalaTest WB affinity column.
2. Attach column to outlet of 10 mL syringe barrel on pump stand or vacuum manifold.
3. Transfer 4 mL filtered extract into another clean vessel.
4. Dilute extract with 96 mL distilled, reverse osmosis, or deionized water. Mix well. Filter through a microfiber filter if necessary

3.5.4 Column Chromatography

1. Pass all 100 mL diluted extract completely through ZearalaTest WB affinity column (100 mL = 1 g equivalent) at a rate of about 1-2 drops/second until air comes through column.
2. Wash column by passing 20 mL of distilled, reverse osmosis, or deionized water through the column at a rate of 1-2 drops/second until air comes through the column.
3. Elute affinity column by twice passing 0.75 mL HPLC-grade methanol through column at a rate of about 1 drop/second, collecting all of the sample eluate (1.5 mL) in a glass cuvette. Add 0.5 mL water to eluate. Vortex.
5. Inject 50 µL into HPLC.

3.5.5 Limit of Detection: 1.9 µg/kg

3.5.6 Limit of Quantitation: 5.8 µg/kg

3.5.7 Recovery: Greater than 85% zearalenone at 1500 ng

3.6 ALTERNATIVE ZEARALATEST WB HPLC PROCEDURE FOR CORN (1:5 dilution) SAMPLE (0-1.9 PPM)

3.6.1 HPLC Setup

See HPLC Condition #1.

3.6.2 Sample Extraction

1. Weigh 20 g ground sample with 2 g salt (NaCl) into a glass blender jar.
2. Add to glass blender jar 50 mL acetonitrile:water (90:10, v/v).
3. Cover blender jar and blend at high speed for 2 minutes.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.6.3 Extract Dilution and Filtration

1. Remove two end caps from ZearalaTest WB affinity column.
2. Attach column to outlet of 10 mL reservoir on pump stand.
3. Transfer 10 mL filtered extract into another clean vessel.
4. Dilute extract with 40 mL distilled, reverse osmosis, or deionized water. Mix well.
5. Filter dilute extract through microfiber filter (VICAM part # 31955) into a clean vessel (or directly into syringe barrel).

3.6.4 ZearalaTest WB Affinity Chromatography

1. Pour 10 mL diluted and filtered extract (10 mL = 0.8 g equivalent) into the syringe barrel and pass completely through ZearalaTest WB affinity column at a flow rate of 1-2 drops per second until air comes through column.
2. After extract has completely passed through column, pass 10 mL distilled, reverse osmosis, or deionized water completely through the column at 1-2 drops/second.
3. Elute ZearalaTest WB affinity column at ~1 drop/second with 1.5 mL HPLC-grade methanol into a clean glass cuvette.
4. Add 1.5 mL distilled, reverse osmosis, or deionized water to the 1.5 mL methanol eluate. Vortex.
5. Inject 100-200 µL into HPLC.

3.7 OTHER PUBLISHED HPLC PROCEDURES

BABY FOOD AND ANIMAL FEED

Arranz, I., Mischke, C., and Stroka, J. (2007) Journal of AOAC International, Liquid Chromatographic Method for the Quantification of Zearalenone in Baby Food and Animal Feed: Interlaboratory Study 90 (6), 1598-1609.

BARLEY, MAIZE AND WHEAT FLOUR, POLENTA, AND BABYFOOD

MacDonald, S., Anderson, S., Brereton, P. (2005) Journal of AOAC International, Determination of Zearalenone in Barley, Maize and Wheat Flour, Polenta, and Maize-Based Baby Food by Immunoaffinity Column Cleanup with Liquid Chromatography: Interlaboratory Study 88 (6), 1733-1740.

BOTANICAL DIETARY SUPPLEMENTS, SOYBEANS, GRAINS, AND GRAIN PRODUCTS

Trucksess, M., Fu W., Oles, C., White, K. (2011) Journal of AOAC International, Determination of Zearalenone in Botanical Dietary Supplements, Soybeans, Grains, and Grain Products by Immunoaffinity Column Cleanup and Liquid Chromatography: Single-Laboratory Validation 94 (2), 589-595.

CEREAL GRAINS, ANIMAL FEED, AND FEED INGREDIENTS

Campbell, H., and Armstrong, J. (2007) Journal of AOAC International, Zearalenone in Cereal Grains, Animal Feed and Feed Ingredients by Immunoaffinity Column Cleanup and Liquid Chromatography: Interlaboratory Study 90 (6), 1610-1622.

CORN

Kruger, S., Kohn, B., Ramsey, C., and Prioli, R. (1999) Journal of AOAC International, Rapid Immunoaffinity-Based Method for Determination Zearalenone in Corn by Fluorometer and HPLC 82 (6), 1364-1368.

Visconti, A., and Pascale, M. (1998) Journal of Chromatography A, Determination of Zearalenone in Corn by Means of Immunoaffinity Cleanup and High-Performance Liquid Chromatography with Fluorescence Detection 815, 133-140.

CORN, RICE, AND WHEAT GRAINS

Llorens, A., et al. (2002) Food Additives and Contaminants, Comparison of Extraction and Cleanup Procedures for Analysis of Zearalenone in Corn, Rice and Wheat Grains by High-Performance Liquid Chromatography with Photodiode Array and Fluorescence Detection 19 (3), 272-281.

CROSS-REACTIVITIES FOR α-ZEARALENOL β-ZEARALENOL, ZEARALENONE, α-ZEARALENOL, AND β-ZEARALENOL

Erbs, M., et al. (2007) Journal of AOAC International, Determination of Cross-Reactivities for α-Zearalenol, β-Zearalenol, Zearalenone, α-Zearalenol, and β-Zearalenol on Three Commercial Immunoaffinity Columns Targeting Zearalenone 90 (4), 1197-1202.

INFANT CEREAL FOODS

Lombaert, G., et al. (2003) Food Additives and Contaminants, Mycotoxins in Infant Cereal Foods from the Canadian Retail Market 20 (5), 494-504.

TRADITIONAL CHINESE MEDICINAL PLANTS AND RELATED PRODUCTS

Zang, Xiaofei, et al. (2011) Food Additives and Contaminants Part A: Chemistry, Analysis, Control, Exposure, and Risk, Determination of Zearalenone in Chinese Medicinal Plants and Related Products by HPLC-FLD.

3.8 HPLC CONDITIONS

3.8.1 HPLC Condition 1:

1. Column: Reverse phase, 4 mm column 3.9 mm X 150 mm, C18 (Waters Nova-Pak (Waters WAT086344)
2. Mobile phase: acetonitrile:water:methanol (46:46:8)
3. Flow rate: 1.0 mL/min
4. Injection volume: 100-200 µL
5. Fluorescence detector: Waters 474 Scanning Fluorescence Detector or Absorbance detector: Waters 486 Tunable Absorbance Detector
6. Detection wavelength:

   Fluorescence: 274 nm excitation and 440 nm emission

   Absorption: 236 nm

3.8.2 HPLC Condition 2:

1. Column: LUNA RP-18(2), 250 X 4.6 mm with guard column, Phenomenex USA
2. Temperature: 35°C
3. Mobile phase: methanol/0.01 M acetic acid 3:1 (v/v)
4. Flow rate: 1.0 mL/min. isocratic
5. Injection volume: 50 µL
6. Fluorescence detector: Jasco Corp., Japan
7. Detection wavelength: 274 nm excitation and 446 nm emission

3.8.3 HPLC Condition 3:

1. Column: Reverse phase, 4 mm column 3.9 mm X 150 mm,
   C18 (Waters Nova-Pak (Waters WAT086344)
2. Mobile phase: water:methanol:acetonitrile (40:30:30, isocratic degassed)
3. Flow rate: 0.8 mL/min.
4. Injection volume: 100 µL
5. Absorbance detector: Waters 486 Tunable Absorbance Detector
6. Detection wavelength: 254 nm

3.9 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 zearalenone standard (Supelco product # 46916-U) comes in sealed ampoules. The concentration of this zearalenone standard stock solution is approximately 50 µg/mL. This standard is prepared according to AOAC Official methods. The certificate of analysis will show the exact concentration. An opened ampoule should be usable for as long as two weeks when stored at 2–8 °C.

3.9.1 Zearalenone Working Solution

Prepare a 5 µg/mL zearalenone standard by adding 100 µL of the 50 µg/mL zearalenone standard stock solution to 900 mL acetonitrile.

3.9.2 Spiking corn sample with zearalenone at 0.25 ppm level

0.25 ppm (µg/g) X 20 g sample – 50 µg/mL (undiluted standard) = 0.1 mL = 100 µL

Add 100 µL of the zearalenone standard to 20 g sample.

3.9.3 Preparing Zearalenone HPLC Standards for 0.8 g Sample Equivalent Procedures (methods on page 12 and 15)

0.25 ppm (µg/g) X 0.8 g sample equivalent = 0.2 µg;

0.2 µg ÷ 5 µg/mL (diluted standard) = 0.04mL = 40 µL (diluted standard)

Add 40 µL diluted standard to 1460 µL methanol.

0.5 ppm (µg/g) X 0.8g sample equivalent = 0.4 µg;

0.4 µg ÷ 5 µg/mL (diluted standard) = 0.08mL = 80 µL (diluted standard)

Add 80 µL diluted standard to 1420 µL methanol.

1.25 ppm (µg/g) X 0.8 g sample equivalent = 1 µg;

1 µg ÷ 5 µg/mL (diluted standard) = 0.2 mL = 200 µL (diluted standard)

Add 200 µL diluted standard to 1300 µL methanol.

2.5 ppm (µg/g) X 0.8 g sample equivalent = 2 µg;

2 µg ÷ 50 µg/mL (undiluted standard) = 0.04 mL = 40 µL (undiluted standard)

Add 40 µL undiluted standard to 1460 µL methanol.

Add 1.5 mL water to all standards and vortex to make solvent composition for the standards similar to the mobile phase.

3.10 ZEARALATEST™ REPRESENTATIVE CHROMATOGRAMS

Using HPLC Condition 1

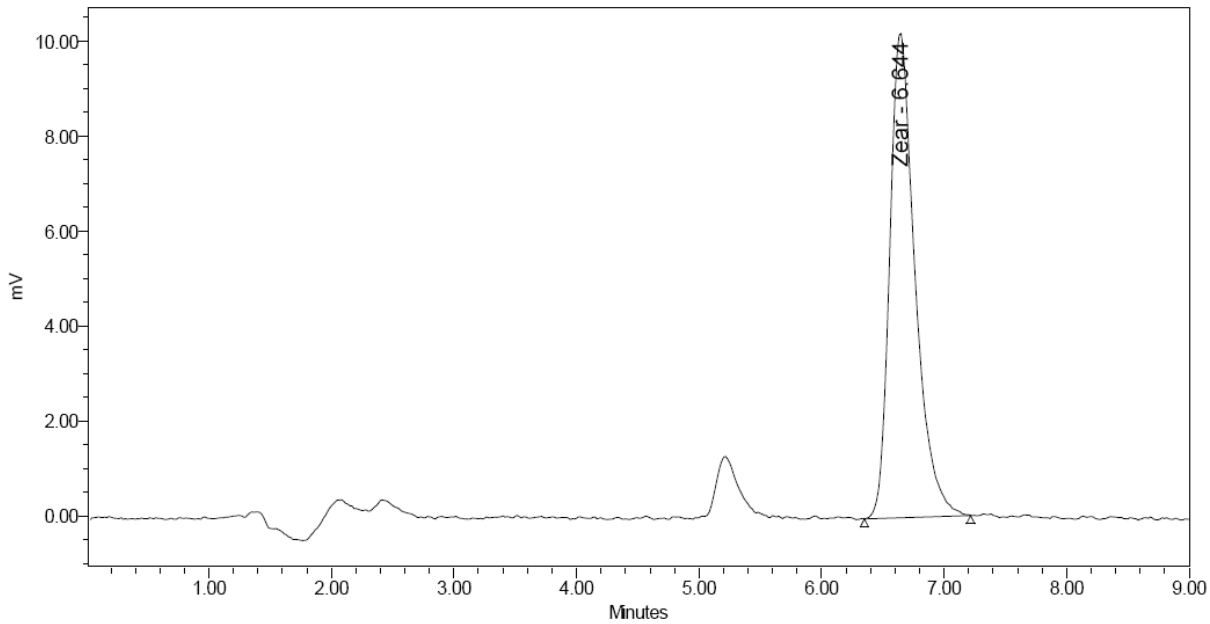


Figure 1: 250 ppb standard with fluorescence detector

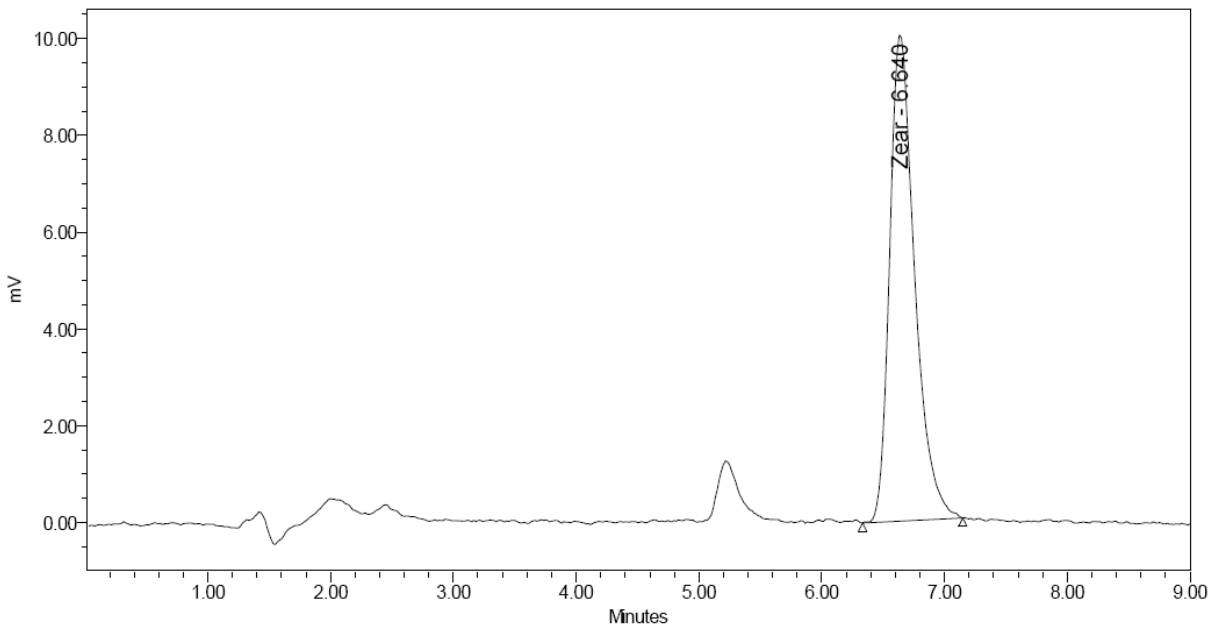


Figure 2: 250 ppb recovery of spiked corn with fluorescence detector

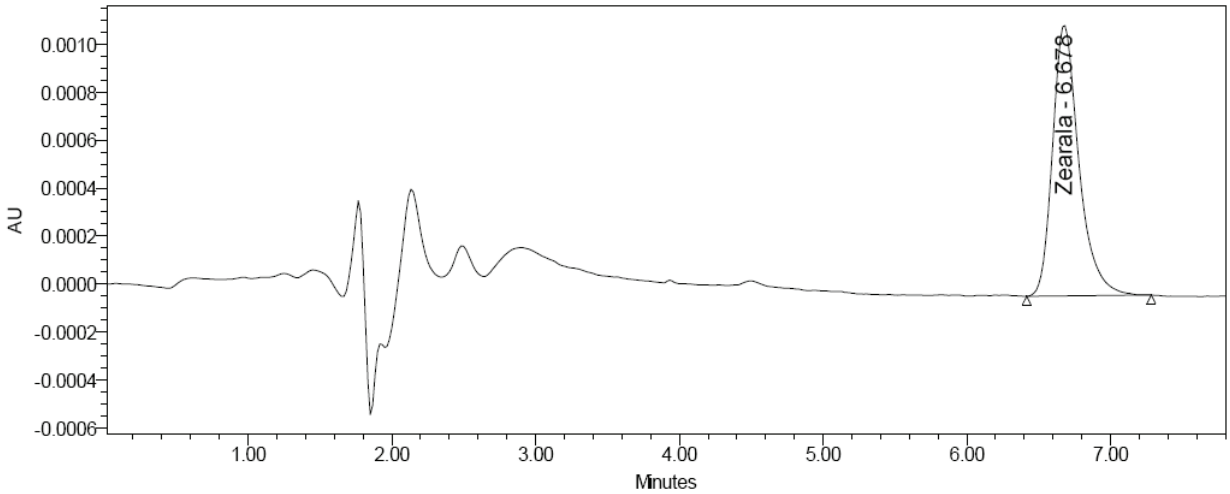


Figure 3: 250 ppb standard with absorbance detector

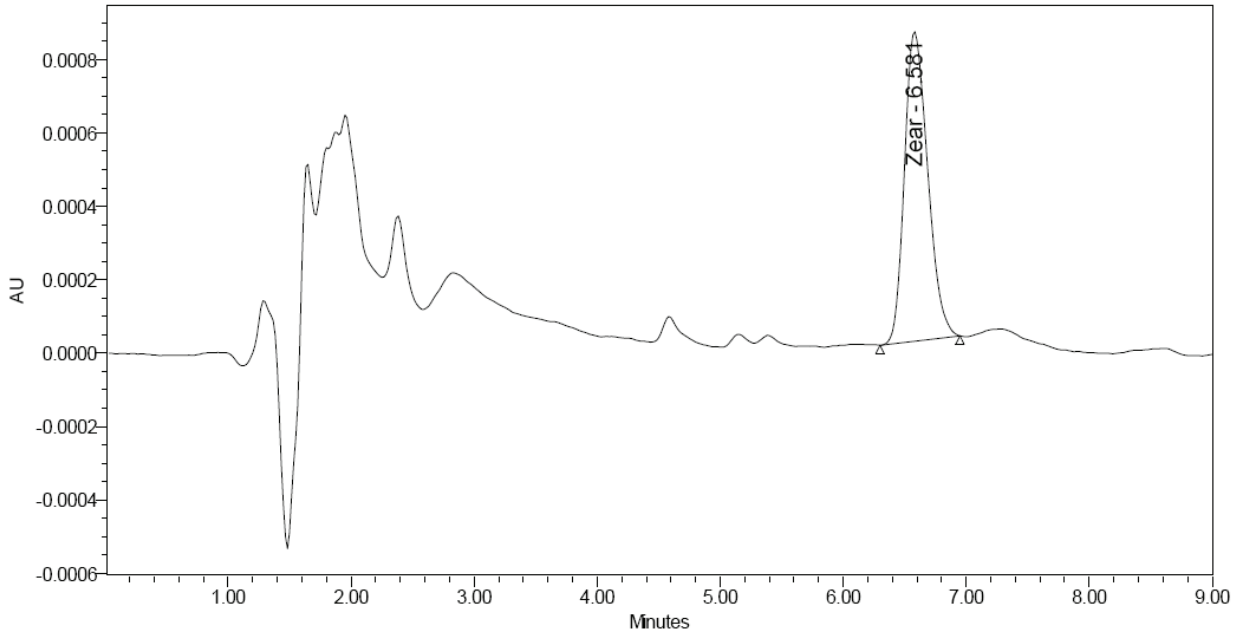


Figure 4: 200 ppb recovery of spiked corn with absorbance detector

Using HPLC Condition 2

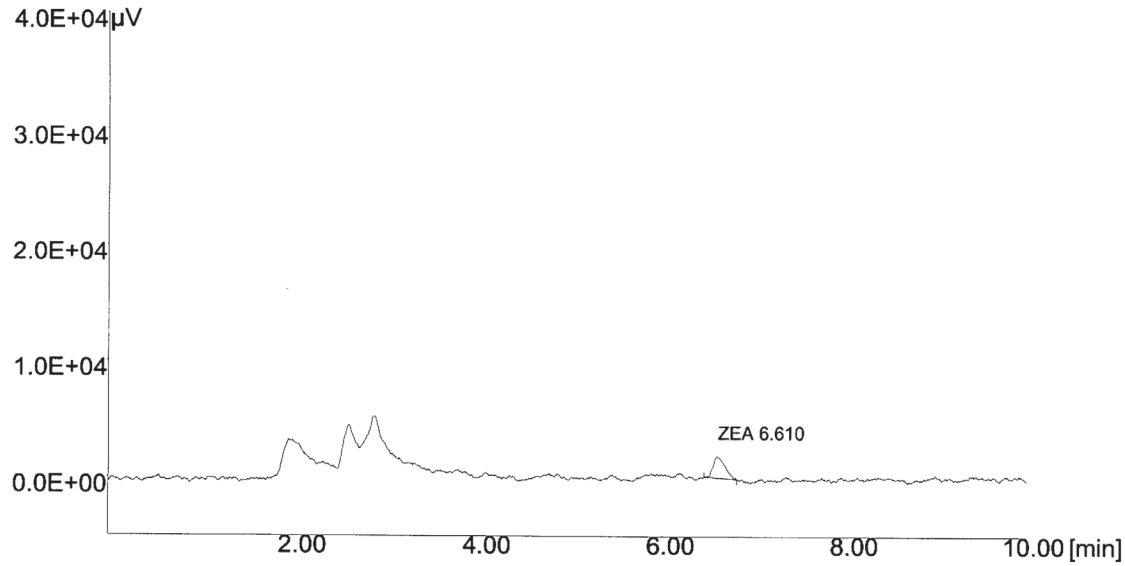


Figure 5: Sample chromatogram of a corn sample spiked at a level of 10 µg/kg

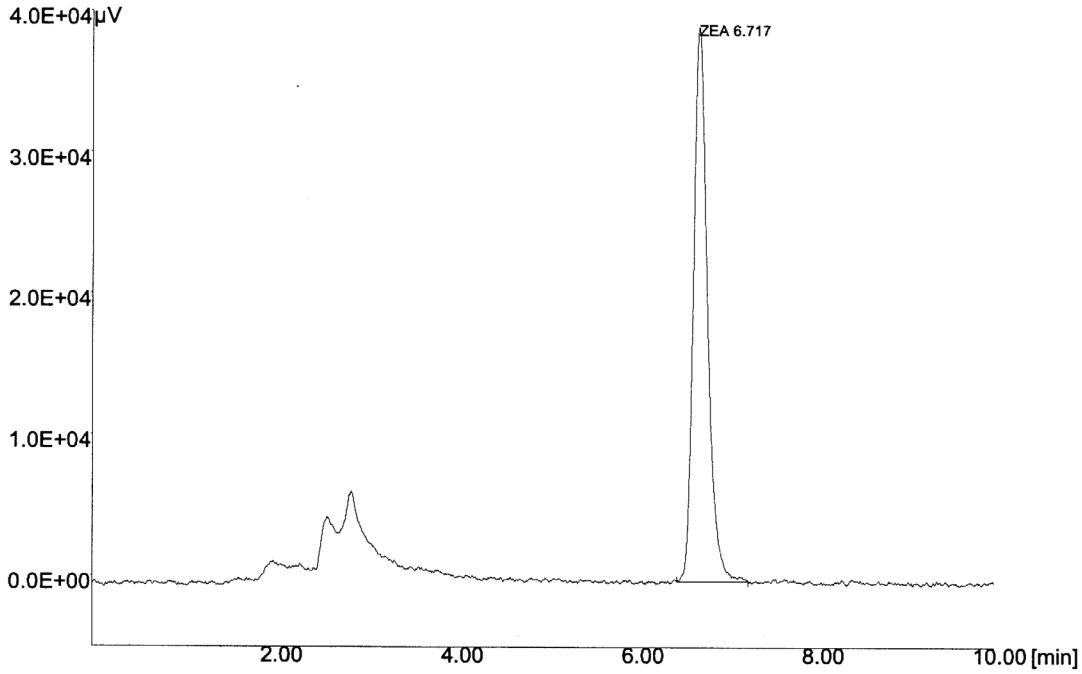


Figure 6: Sample chromatogram of a corn sample spiked at a level of 250 µg/kg

4.0 GENERAL HPLC PRECAUTIONS

Elute column slowly (~1 drop/second) with 1.5 mL HPLC-grade methanol. Add 1.5 mL water to standards and samples before injecting onto the HPLC to make the solvent for the standards and samples similar to the mobile phase.

5.0 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

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6.0 LIABILITY

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