




T-2/ HT-2 HPLC

Instruction Manual

VICAM®

A Waters Business

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1.1 INTENDED USER

T-2/HT-2 HPLC™ method is a quantitative assay intended for use by trained customers in the food processing industry who need to test samples for the presence of both T-2 and HT-2 toxins with a method that is safe, simple, fast, and which works reproducibly and accurately.

1.2 PRINCIPLE

T-2 and HT-2 mycotoxins are produced by *Fusarium* and other mold species that can commonly attack grains and can grow at temperatures from slightly above freezing to about 86°F (30°C). All domestic animals are susceptible to injury by dietary intake of T-2 in the range of a few ppm. In poultry, feed contaminated with 1.0 to 3.5 ppm of T-2 has produced lesions at the edges of the beaks, abnormal feathering in chicks, a drastic and sudden drop in egg production, eggs with thin shells, reduced weight gains, and mortality.

VICAM's T-2/HT-2 WB column can effectively bind both T-2 and HT-2 toxins in various matrices. To measure T-2 and HT-2 levels, samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the T-2/HT-2 HPLC column which contains specific antibodies that recognize both T-2 and HT-2. At this stage, the T-2 and HT-2 bind to the antibody on the column. The column is then washed to rid the column of impurities. The T-2 and HT-2 are removed from the antibody by passing methanol through the column. The T-2 and HT-2 can then be quantified by injection into an HPLC system. These steps are outlined in section 1.6, T-2/HT-2 HPLC Overview.

1.3 APPLICABILITY

T-2/HT-2 HPLC has been optimized for quantitative measurement of T-2 and HT-2 in oats and other grains.

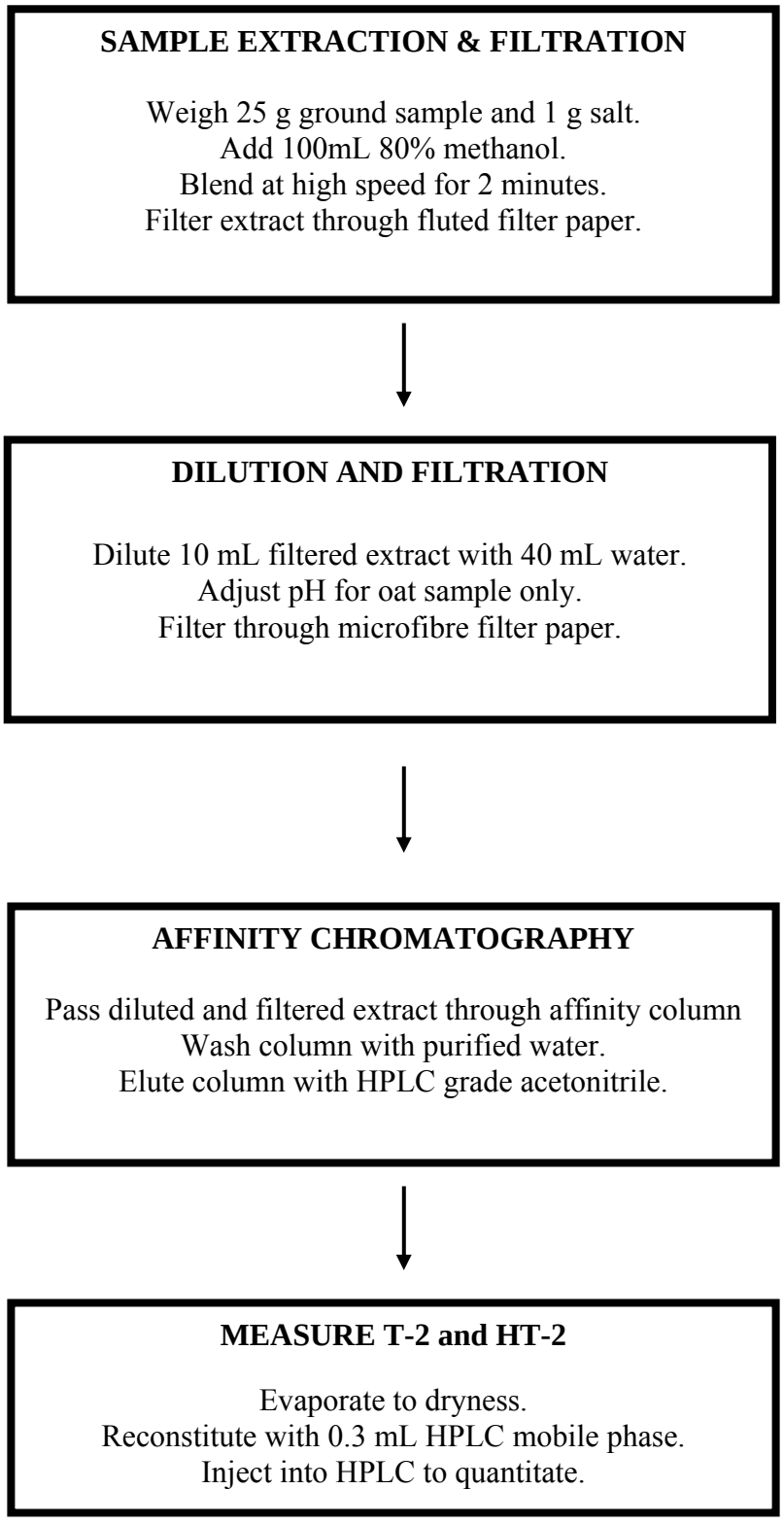
1.4 LIMITATIONS

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

1.5 SHELF LIFE AND STORAGE CONDITIONS

Store at room temperature (18-25 °C, 64-73 °F) until the expiration date printed on the label. 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.

1.6 T-2/HT-2 HPLC™ PROCEDURE OVERVIEW



2.1 PUMP STAND SETUP

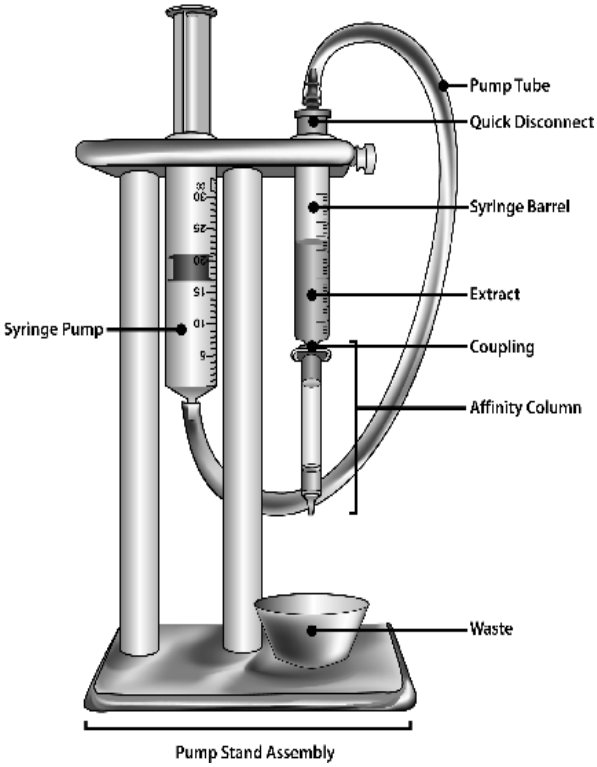
T-2/HT-2 HPLC affinity chromatography is easily performed with the T-2/HT-2 HPLC affinity column attached to a pump stand. The stand has a 10 mL glass syringe barrel that serves as a reservoir for the column. A large plastic syringe with tubing and coupling provides air pressure to manually push liquids 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 T-2/HT-2 HPLC 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 T-2/HT-2 HPLC™ 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 barrels 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.

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.

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, acetonitrile, 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.

2.3 MATERIALS AND EQUIPMENT REQUIRED

Materials Required

Description	Part #
T-2/HT-2 HPLC™ Columns (25/box)	176000207
Disposable Plastic Pipets, 1 mL (50)	20652
DON Fluted Filter Paper, 24 cm (100)	31242
OchraTest™ Eluting Solution	32016
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25)	36010
Acetonitrile, HPLC Grade (4 x 4 L)	G1130
Noniodized sodium chloride (salt, NaCl)	G1124
Whatman GF/A glass microfibre filters	
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
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
Micro-pipettor, 1.0 mL	G4033
Micro-pipette Tips for 1 mL Micro-pipettor (100)	20656
Silanized glass cuvettes (example: E P Scientific # CTS-1275)	
HPLC System as specified in procedure	

3.0 T-2/HT-2 HPLC™ PROCEDURE FOR OATS (OR OTHER CEREAL GRAINS)

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 (55:45, v:v) isocratic, degassed
1.3 Flow rate: 1.0 mL/min
1.4 Absorbance detector: Waters 2995, λ = 208nm
1.5 Injection volume: 100 µL
1.6 Retention time: HT-2 ~5 min; T-2 ~ 9 min.

2.0 Sample Extraction:

2.1 Weigh 25 g ground sample into a blender jar. Add 1 g NaCl.
2.2 Add 100 mL methanol:water (80:20, v/v).
2.3 Cover blender jar and blend at high speed for 2 minutes.
2.4 Remove cover from jar and pour extract into DON fluted filter paper (VICAM part # 31242). Collect filtrate in a clean vessel.

3.0 Extract Dilution and Filtration:

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 Oat samples will need to have the pH of this diluted extract adjusted so that it is approximately 6.8. Add 300 µL of OchraTest Eluting Solution (VICAM # 32016) or 0.1 N NaOH to adjust the pH. Mix well. It is not necessary to adjust the pH if the sample is corn or wheat.
3.4 Filter diluted extract through Whatman GF/A glass microfibre filter into a clean vessel.

4.0 Column Chromatography:

4.1 Load 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) into a syringe barrel connected to a T-2/HT-2 HPLC™ affinity column.
4.1 Remove the column endcap and allow extract to flow by gravity pressure (~ 1 drop/second). If the flow rate slows add pressure to keep the extract flowing through the column at a rate of about 1 drop/second until air comes through column.
4.2 Remove the column from the syringe barrel and completely fill the column headspace with purified water. Replace the column on the syringe barrel and add 10 mL purified water to the syringe barrel. Pass the water through the column at a rate of 1-2 drops/second until air comes through the column.
4.3 Repeat the previous step once more until air comes through the column.
4.4 Remove the column from the syringe barrel. Clean the column headspace with a Kimwipe to remove any remaining water.
4.5 Add 1.5 mL HPLC grade acetonitrile directly into the column headspace. Elute the column into a silanized glass cuvette by gravity flow rate or at a rate of 1 drop/second until air comes through the column.
4.6 Evaporate the eluate to dryness under a vacuum at room temperature. Moderate heating (up to 50 °C) may also be used.

4.7 Reconstitute eluate in 300µl of HPLC mobile phase (methanol:water, 55:45). Mix well and inject 100 µL onto HPLC.

5.0 Limit of Quantitation: 100 ng

6.0 Assay Range: 100 - 1000 ng each of T-2 and HT-2

7.0 Recovery: ≥ 75% for HT-2 and ≥84% for T-2.

4.0 GENERAL PRECAUTIONS

Always use good, clean equipment and reagents (HPLC grade methanol for sample elution and distilled, reverse osmosis or deionized water for extraction and washing).

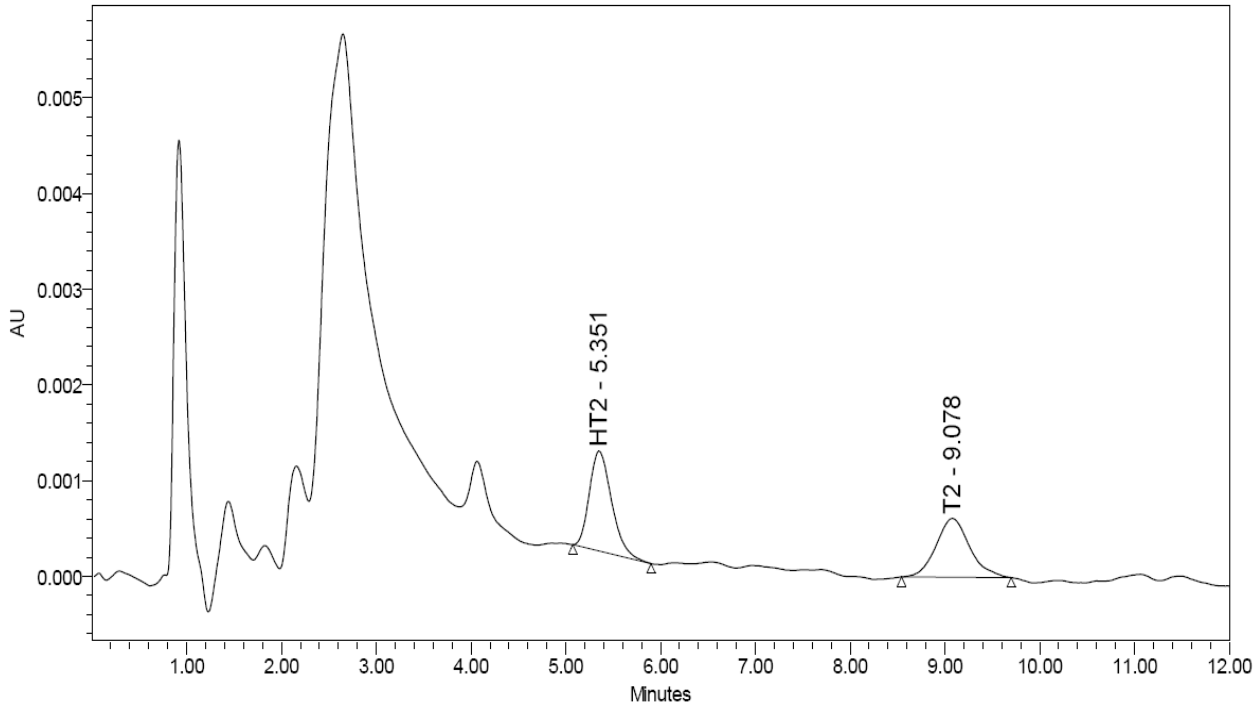
Maintain the flow rate through the T-2/HT-2 HPLC column as specified in the procedure. Faster flow rates may cause incorrect results.

Perform test from beginning to end without interruptions.

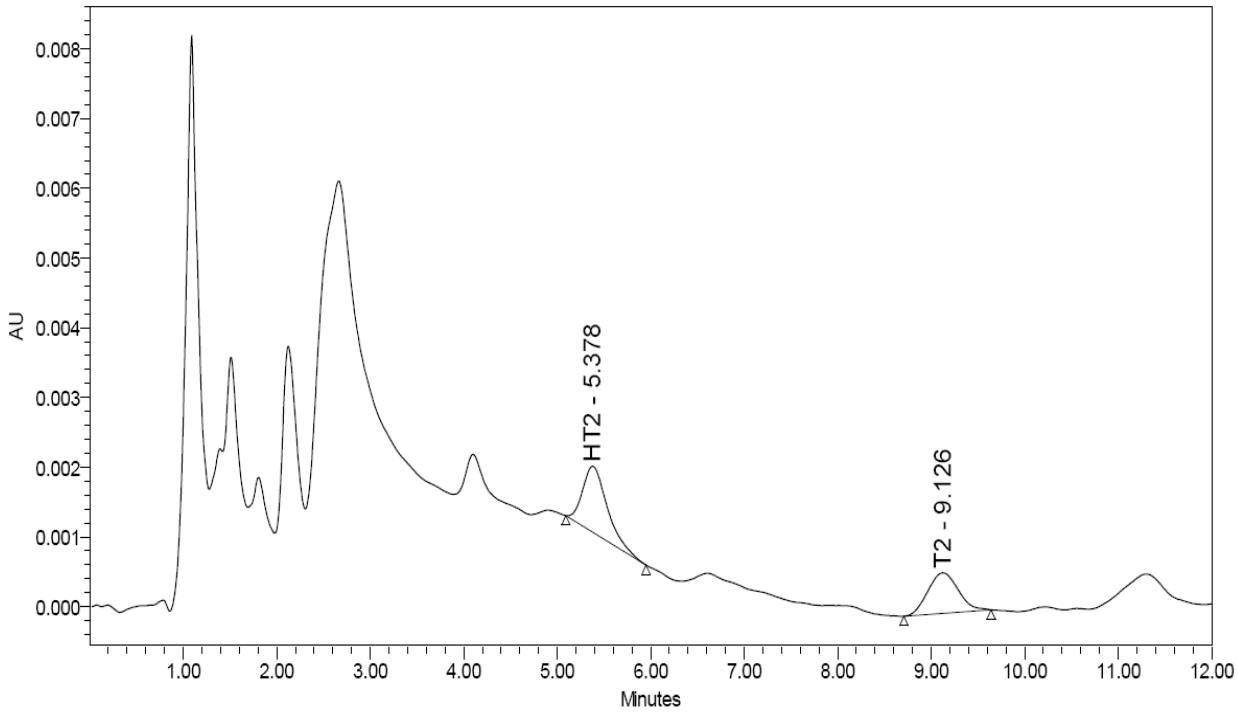
Silanized cuvettes or vials, such as E P Scientific # CTS-1275 are recommended when drying down sample eluate.

5.0 EXAMPLE CHROMATOGRAMS

200 ng of T-2 and HT-2 standard



200 ng of T-2 and HT-2 spiked in oat sample



6.0 LIABILITY

Only individuals with appropriate training should perform the test described in this instruction manual. Materials should be handled and disposed of properly.

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 T-2/HT-2 HPLC analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that T-2/HT-2 HPLC products conform to VICAM's printed specifications and quality control standards. VICAM will as 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 procedure of T-2/HT-2 HPLC product.

Worldwide patents and trademarks protect all VICAM products.

7.0 TECHNICAL ASSISTANCE

If the desired results are not obtained, verify temperatures and methods adhere to the procedure presented in this manual.

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 ORDERING INFORMATION

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

Phone:	877-228-4244	United States and Canada
	800-338-4381	Mexico
	417-725-6588	all International and United States customers
Fax:	417-725-6102	
e-mail:	vicam@vicam.com	

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