





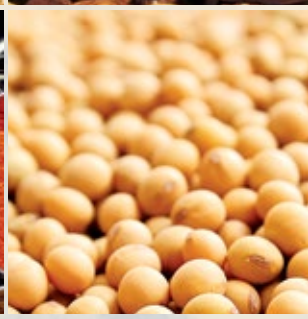


Myco6in1+® LC/MS/MS

Instruction Manual

VICAM®

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1.0 INTRODUCTION

1.1 INTENDED USER

Myco6in1$^+$ is a quantitative method for the simultaneous determination of aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, nivalenol, zearalenone, T-2 and HT-2 toxins. Samples are purified by the VICAM Myco6in1$^+$ immunoaffinity column before being quantitated by liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS).

While LC/ESI-MS/MS was used to generate validation data in corn, other methods of quantitation may be used. The column is designed to capture and allow quantitation of the mycotoxins listed. Any measurement method that is adequate to quantitate these mycotoxins could, in principle, be applied to the immunoaffinity column methanol eluate.

1.2 PRINCIPLE

Samples are prepared by extracting with either a double or single extraction of aqueous based and methanol based solvents. This extraction results in improved recoveries of fumonisin B_1, fumonisin B_2 and DON as compared to a single methanol:water extraction.

For the double extraction procedures, the methanol:water extract is passed through the Myco6in1$^+$ immunoaffinity column. The column is washed with PBS to remove any traces of methanol present and then the PBS extract is passed over the same Myco6in1$^+$ column.

The column is washed with distilled water to remove the PBS and any matrix interfering compounds. Toxins are eluted from the column with two or three applications of methanol or other eluting solution. The column eluate is dried down, reconstituted in an appropriate solution and quantitated by LC/ESI-MS/MS or LC with absorbance and fluorescence detection. These steps are outlined in section 1.8–1.9.

For the single extraction procedure, the sample is blended with water followed by methanol and filtered. The filtered extract is dried slightly and diluted with PBS. The diluted extract is then passed over the column, column washed with water then eluted with methanol and water. The eluate is dried, reconstituted in mobile phase and quantitated by LC/MS/MS. These steps are outlined in section 1.10.

1.3 APPLICABILITY AND APPROVALS

Myco6in1$^+$ has been optimized for quantitative measurement of aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, nivalenol, zearalenone, T-2 and HT-2 toxins in corn. The procedures discussed in the Lattanzio paper (see References) meet European Committee for Standardization (CEN) criteria for mycotoxin analysis methods. The single extraction method for Myco6in1$^+$ has been optimized for cereals and derived products. Assistance in analyzing 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.**

Click on “Federal Grain Inspection” then “Publications” on the left.

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

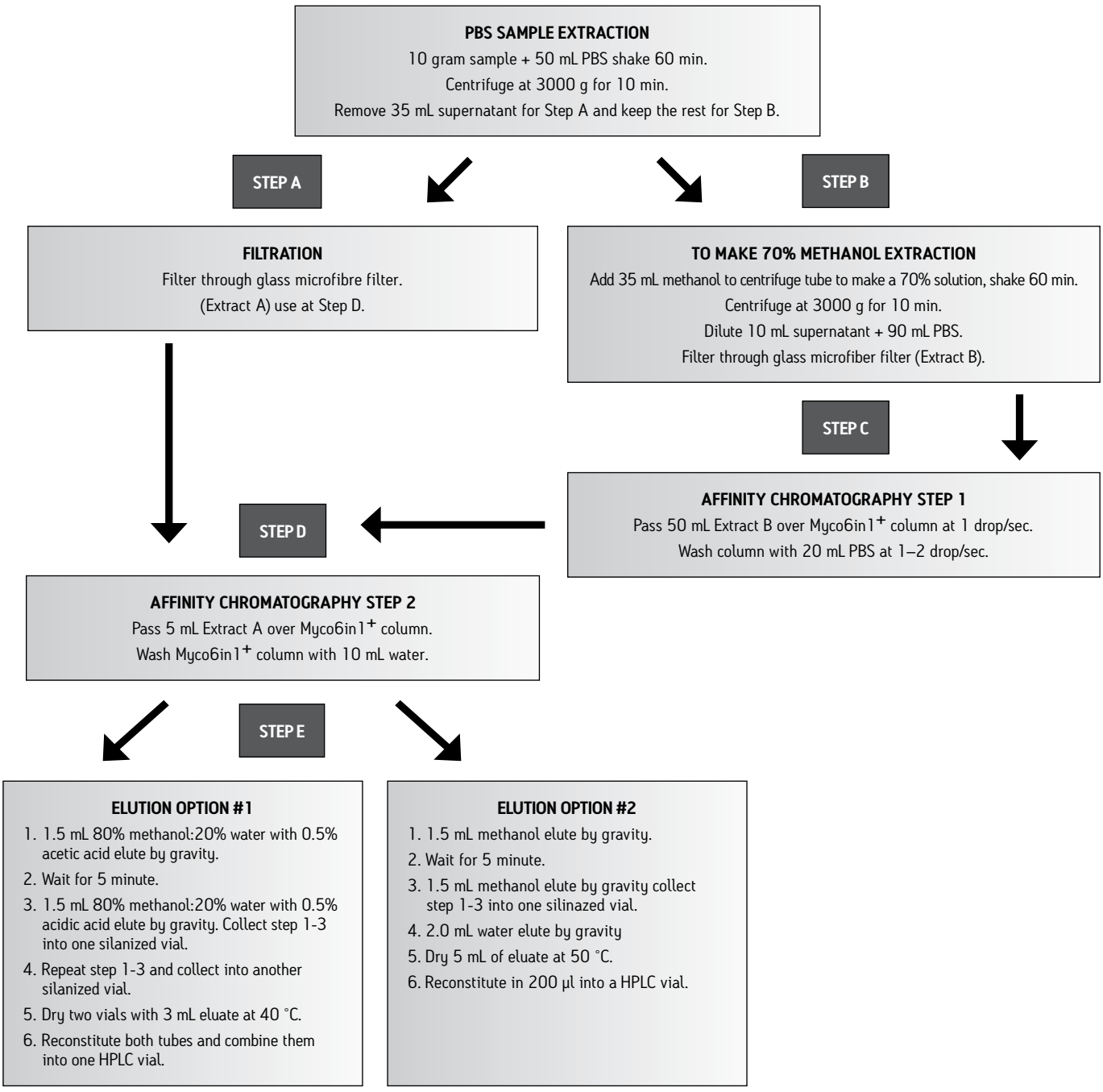
Store Myco6in1$^+$ columns at refrigerated temperature (2–8 °C) up until the expiration date on the box of columns. Columns are good for one year from production date. It is recommended that all reagents and columns be at room temperature (18–22 °C) for usage.

1.7 CALIBRATION STANDARDS FOR LC/MS/MS

Calibrate instrument for LC/MS/MS using matrix matched calibration standards or internal calibration standards to account for ion suppression or enhancement.

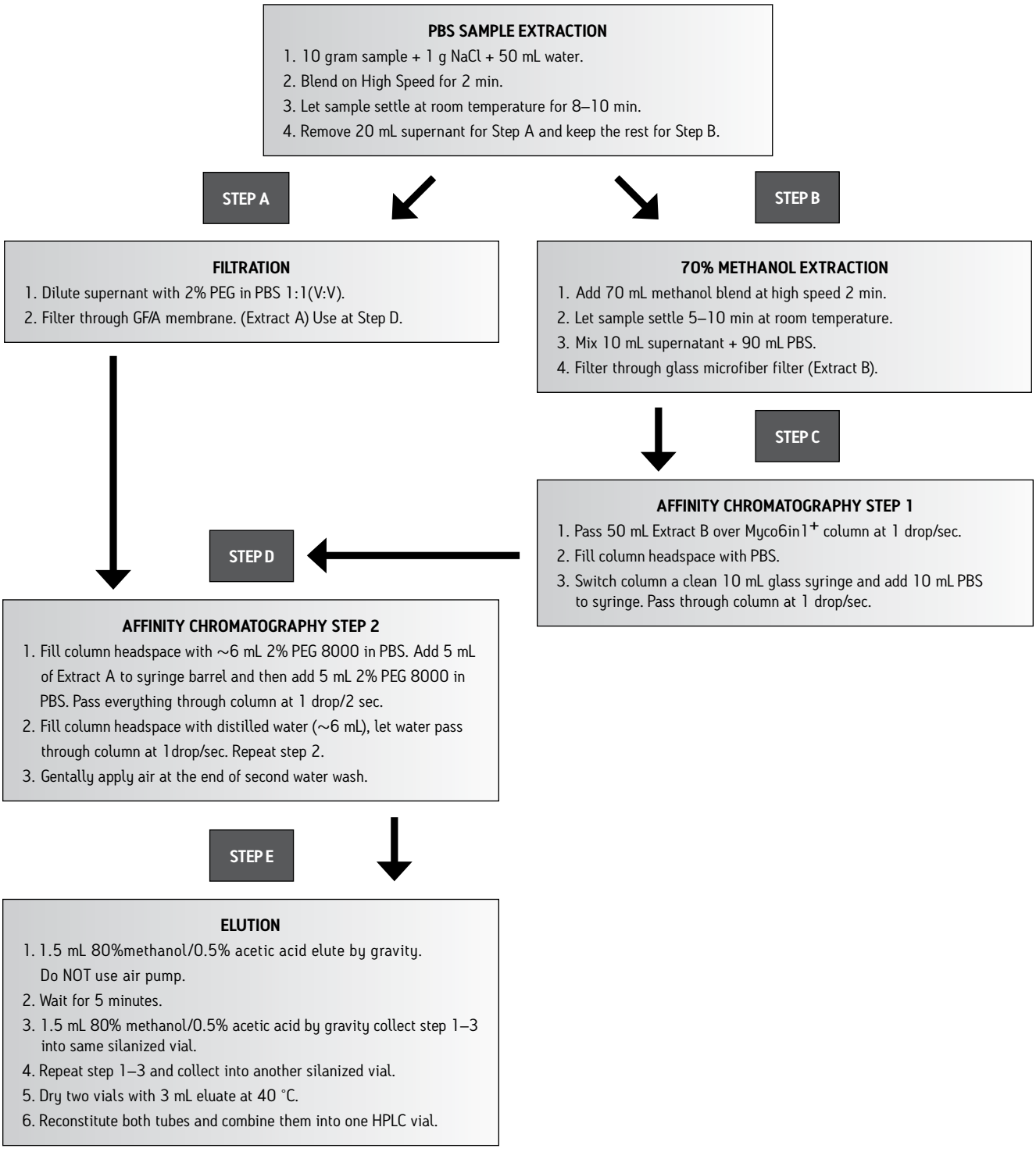
1.8 MYCO6in1$^+$ DOUBLE EXTRACTION PROCEDURE WITH SHAKER FOR LC MS/MS

(1 gram equivalent)



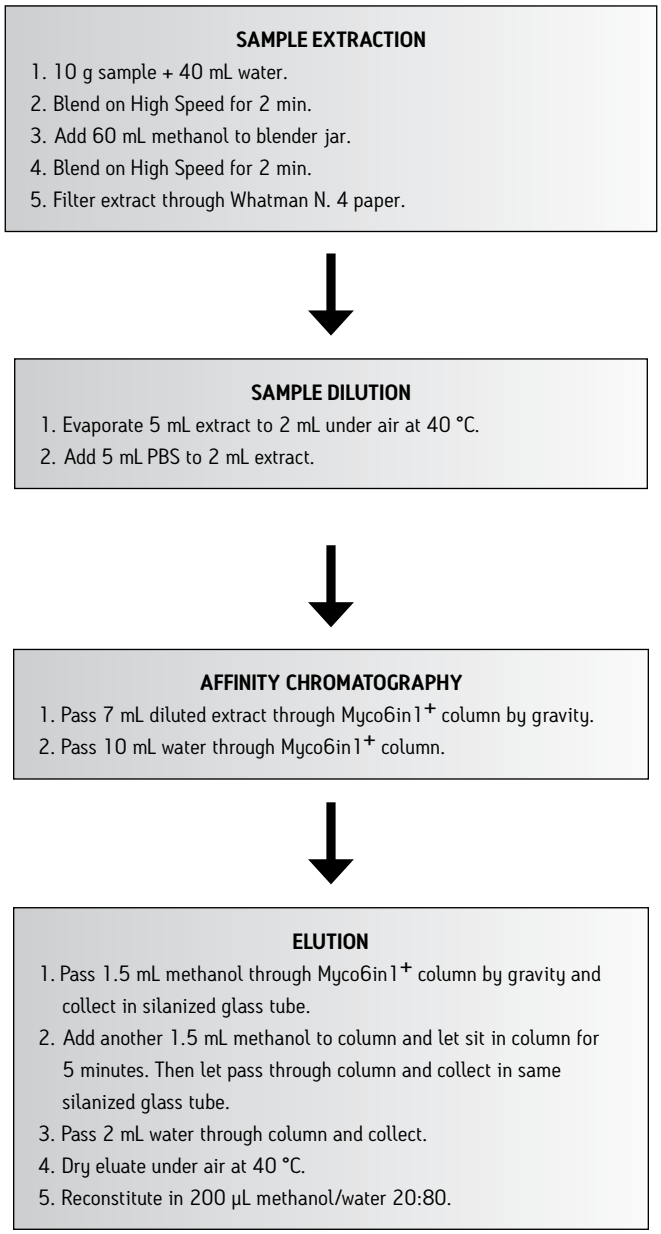
1.9 MYCO6in1+ DOUBLE EXTRACTION PROCEDURE WITH BLENDER FOR LC WITH ABSORBANCE/FLUORESCENCE DETECTION

(0.5 gram equivalent)



1.10 MYCO6in1+ SINGLE EXTRACTION PROCEDURE CHART

(0.5 gram equivalent)



2.0 EQUIPMENT CALIBRATION AND PREPARATION

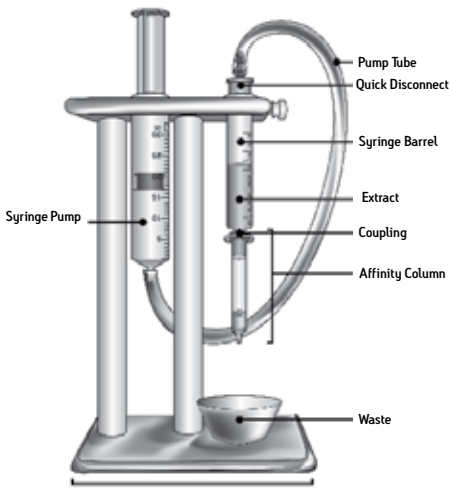
2.1 PUMP STAND SETUP

Myco6in1+ affinity chromatography is easily performed with the Myco6in1+ 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 #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 (VICAM #21030), four-position pump stands with aquarium pumps (VICAM #21045), and twelve-position pump stands with aquarium pumps (VICAM #G1104) are available for running multiple samples at one time. Pump stands with 50 mL plastic syringe barrels are also available (part #G4051 and G4046).

When using a pump stand:

1. Remove large top cap from the column.
2. VICAM WB Column Coupling (part #G1118) provides a reusable coupling for attaching the column to the syringe barrel reservoir.
3. Place waste collection cup under column outlet. Keep bottom cap on column.
4. Place desired amount of extract in glass syringe barrel.
5. Pull up on the plastic syringe piston.
6. Insert 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 the flow rate specified in the procedure. Repeat for wash and elution steps (see procedures).

Affinity Column Syringe Barrel Connection



Pump Stand Assembly

Vacuum Manifold

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.

Alternatively, a vacuum manifold can be used.

2.2 CLEANING EQUIPMENT

Before Starting Myco6in1+ Testing:

Before using the equipment, it should be washed with a mild detergent solution and then rinsed thoroughly with purified water. 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 extraction containers. Rinse all equipment carefully to remove detergent residue from glassware since mass spectrometry can be adversely affected by the presence of trace amounts of detergent.

Between Assays:

After each assay, any equipment that will be reused to hold, collect or transfer samples or sample extracts needs to be washed with a mild detergent solution and rinsed thoroughly with purified water.

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:

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 materials into the sample and thereby affect results.

3.0 REAGENT PREPARATION

Phosphate Buffered Saline (pH 7.4)

0.20 g KCl

0.20 g KH_2PO_4

2.92 g $Na_2HPO_4 \bullet 12H_2O$

8.00 g NaCl

Dissolve in 900 mL purified water. Adjust to pH 7.4 with 0.1M HCl or 0.1M NaOH and dilute to 1000 mL.

Commercial buffered saline tablets may also be used. A 10X concentrate of PBS may also be purchased from VICAM (#G1113). 10X PBS Concentrate should be diluted to 1X with purified water as needed — i.e. dilute 100 mL of 10X concentrate with 900 mL purified water.

4.0 SAMPLE EXTRACTION PROCEDURES

4.1 MATERIALS AND EQUIPMENT REQUIRED

Materials Required	
Description	**Part #**
Myco6in1+ LC/MS/MS Columns (25/box)	100000176
Phosphate Buffered Saline, 10X concentrate (150 mL)	G1113
Disposable Cuvettes (250)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25)	36010
Whatman GF/A microfibre filters	
Acetic acid, glacial (HPLC grade)	
Ammonium acetate (mass spectrometry grade)	
Distilled, reverse osmosis or deionized water	
PEG 8000 for blender method	

Equipment Required	
Description	**Part #**
Graduated Cylinder, 50 mL	20050
Digital Scale with AC Adapter	20100
Micro-pipettor, 1.0 mL	G4033
Micro-pipette Tips for 1 mL Micro-pipettor (100)	20656
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
VICAM WB Column Coupling (6 per pack)	G1118
Filter Funnel, 65 mm (10 per pack)	36020
Orbital shaker	
Centrifuge capable of obtaining 3000 x g Relative Centrifugal Force	
LC/MS/MS System or HPLC System	

Aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, ochratoxin A, fumonisin B_1, fumonisin B_2, deoxynivalenol, nivalenol, zearalenone, T-2 and HT-2 toxins are available for sale from Sigma-Aldrich (www.sigmaaldrich.com). The sale of some of these mycotoxins may be restricted in certain countries.

4.2 MYCO6in1+ DOUBLE EXTRACTION PROCEDURE WITH SHAKER FOR CORN FOR LC MS/MS

(1 gram equivalent)

1.0 LC/MS/MS Conditions:

Example, See Section 5.1

2.0 Sample Extraction, Dilution and Filtration:

2.1 Add 50 mL PBS to 10 g ground corn and shake for 60 minutes on an orbital shaker.

2.2 Centrifuge sample at 3000 g for 10 minutes. Remove 35 mL PBS extract and reserve the solid material for further extraction.

2.3 Filter the PBS extract through a Whatman GF/A glass microfibre filter (extract A).

2.4 Add 35 mL of HPLC grade methanol to the remaining solid material and shake again for 60 minutes on an orbital shaker.

2.5 Centrifuge sample at 3000 g for 10 minutes and remove the methanol:PBS extract.

2.6 Dilute 10 mL of the methanol:PBS extract with 90 mL of PBS. Mix and then filter this through a Whatman GF/A glass microfibre filter (extract B).

3.0 Column Chromatography

3.1 Remove large top cap from column. VICAM WB Column Coupling (part #G1118) provides a reusable coupling for attaching the column to the syringe barrel reservoir on the pumpstand.

3.2 Pass 50 mL of extract B (1.0 gram equivalent) completely through Myco6in1+ affinity column at a rate of about 1 drop/second or by gravity.

3.3 Pass 20 mL of PBS through the column at a rate of about 1–2 drops/second to wash any remaining methanol from the column.

3.4 Pass 5 mL of extract A (1.0 gram equivalent) completely through Myco6in1+ affinity column at a rate of about 1 drop/second.

3.5 Pass 10 mL of purified water through the column at a rate of about 1–2 drops/second until air comes through column to remove any PBS residue and matrix interfering compounds.

3.6 Place a silanized glass cuvette under Myco6in1+ column. Add 1.5 mL HPLC grade methanol into the column headspace and reattached column to the glass syringe barrel.

3.7 Elute column at a rate of 1 drop/second or by gravity by passing the methanol through the column and collecting all of the sample eluate in the silanized glass cuvette. When most of the methanol has passed through the column but the top of the resin bed is not yet dry, stop applying air pressure to the column and allow it to sit undisturbed for 5 minutes.

3.8 Add an additional 1.5 mL HPLC grade methanol into the column headspace and reattach column to the glass syringe barrel. Continue to elute column at a rate of 1 drop/second and collect all of the sample eluate in the same glass cuvette.

3.9 Add 2 mL water to the column headspace, pass through column at a rate of 1 drop/second and collect in the same glass cuvette.

3.10 Dry down total eluate under an air stream at 50 °C. Reconstitute in 200 µL appropriate LC mobile phase. Analyze 20 µL (equivalent to 100 mg corn sample) by LC/MS/MS.

4.0 Limit of Detection:

(values are dependent upon the detection system used*)

Toxin	Limit of Detection (ppb)
Aflatoxin B_1	0.6
Aflatoxin B_2	0.3
Aflatoxin G_1	0.4
Aflatoxin G_2	0.8
Deoxynivalenol	4.2
Fumonisin B_1	1.1
Fumonisin B_2	0.4
Ochratoxin-A	0.6
T-2*	1.5
HT-2*	1.9
Zearalenone	0.7

* See Lattanzio reference for details.

4.3 MYCO6in1+ DOUBLE EXTRACTION PROCEDURE WITH BLENDER FOR CORN FOR LC WITH ABSORBANCE/ FLUORESCENCE DETECTION

(0.5 gram equivalent)

1.0 HPLC Conditions:

Example, See Section 5.2

2.0 Sample Extraction, Dilution and Filtration:

2.1 Weigh 10 g ground corn, add 1 g NaCl and add 50 mL water. Blend at high speed 2 minutes and let settle at room temperature for 8–10 min.

2.2 Take top 20 mL into a container without disturbing the bottom layer. Dilute with 20 mL of 2% PEG in PBS at 1:1 (V/V) ratio and filter through GF/A filter paper. (Extract A).

2.3 Add 70 mL 100% methanol to remaining solids in blender jar, blend at high speed 2 min. and let settle 5–10 min at room temperature.

2.4 Take top 10 mL of extract and dilute with PBS at 1:10 (10 mL extract plus 90 mL PBS). Filter through GF/A filter paper. Filtrate is extract B. (If running more replicates, dilute more extract with PBS: i.e. take top 20 mL of extract and add 180 mL PBS, and then filter through GF/A).

3.0 Column Chromatography

3.1 Remove large top cap from column. VICAM WB Column Coupling (part #G1118) provides a reusable coupling for attaching the column to the syringe barrel reservoir on the pump stand.

3.2 Pass 50 mL of extract B (0.5 gram equivalent) completely through Myco6in1$^+$ affinity column at a rate of about 1 drop/second until air comes through column.

3.3 Remove column and connect to a clean 10 mL glass syringe barrel. Fill column headspace with PBS and add 10 mL PBS to syringe. Let PBS pass through column at 1 drop/second.

3.4 Fill column headspace with ~6 mL 2% PEG 8000 in PBS and attach column to glass syringe barrel. Add 5 mL of extract A (0.5 gram equivalent) to syringe barrel, and then add 5 mL 2% PEG 8000 in PBS. Let it go through column at a rate of about 1 drop/two seconds until air comes through column.

3.5 Remove column and fill column headspace with distilled water (~6 mL), let water pass through column at 1 drop/second. Then fill column headspace with distilled water (~6 mL) once again, let water pass through column at 1 drop/second. Gently apply air at the end to remove water.

3.6 Place a silanized glass cuvette under Myco6in1$^+$ column. Add 1.5 mL of a solution of 80% methanol: 20% water with 0.5% acetic acid added (freshly prepared) directly into column headspace. Let it elute into a silanized glass cuvette by gravity. After methanol runs through resin bed, do not apply any air pressure, let column stand there for 5 min.

3.7 After 5 min, add another 1.5 mL 80% methanol: 20% water with 0.5% acetic acid directly into column headspace and collect into the same cuvette by gravity (~3 mL at this step in total).

3.8 Once the second 1.5 mL elution buffer completely runs through resin bed, replace the silanized glass cuvette and add two separate 1.5 mL portions of 80% methanol: 20% water solution with 0.5% acetic acid directly into column headspace. Collect the eluate into the cuvette by gravity. Apply strong air pressure to collect all the eluate at the end (elution volume in this cuvette is ~3 mL, but the total volume for a sample is ~6 mL).

3.9 Dry elutes at vacuum with medium heater (~3 hours). Adjust volume after drying 1.5-2 hours (because elute in one cuvette might dry faster than the other).

3.10 Add 125 µL of methanol:water (50:50) to each sample cuvette (each sample has two cuvettes), vortex well and let sit 10 min (vortex occasionally during incubation). Mix the elution of each split sample cuvette (total ~250 µL for each sample). Inject 100 µL on HPLC for analysis.

4.4 MYCO6in1$^+$ SINGLE EXTRACTION PROCEDURE WITH BLENDER FOR CEREALS AND DERIVED PRODUCTS* (MAIZE, WHEAT, CORN FLAKES AND MAIZE CRACKERS)

(0.5 gram equivalent)

1.0 LC/MS/MS Conditions:
Example, See Section 5.1

2.0 Sample Extraction, Dilution and Filtration:

2.1 Weigh 10g of ground sample into a blender jar. Add 40 mL purified water and blend for 2 minutes at high speed.

2.2 Add 60 mL of methanol to the blender jar (without removing the first extract) and blend again for 2 minutes at high speed.

2.3 Filter extract through paper filter (Whatman N. 4).

2.4 Evaporate 5 mL (0.5 g sample) of filtered extract in 20 mL acid washed glass tube under air stream at about 40 °C to reduce the volume to approximately 2 mL.

2.5 Add 5 mL PBS to remaining evaporated extract.

3.0 Column Chromatography

3.1 Remove large top cap from Myco6in1$^+$ column. VICAM WB Column Coupling (part #G1118) provides a reusable coupling for attaching the column to the syringe barrel reservoir on the pumpstand or vacuum manifold.

3.2 Pass extract (approximately 7 mL) through Myco6in1$^+$ immunoaffinity column at a flow rate of about 1 drop per second. Column can flow by gravity, but not more than 1 drop/second. If it is necessary, use stopcock to control flow rate.

3.3 Wash column by passing 10 mL purified water though Myco6in1$^+$ column, then gently pass a few seconds of air through column to dry.

3.4 Place a silanized 10 mL glass tube under Myco6in1$^+$ column to collect eluate. Pass 1.5 mL methanol through the column by gravity (slower than 1 drop/2 seconds) and collect in the silanized glass tube. Apply a second portion of 1.5 mL methanol to the column then close off the bottom of the column with a stopcock to allow the methanol to sit in the column bed for 5 minutes. Allow column to flow again by gravity and collect the 1.5 mL methanol in the silanized glass tube. Pass 2 mL of purified water through the Myco6in1$^+$ column and collect in the same silanized glass tube. Pass a few seconds of air through the column to dry.

3.5 Dry eluate under air (or nitrogen) stream at 40 °C. Reconstitute with 200 µL methanol/water 20:80 and vortex at least three times for 10 seconds each. Inject 20 µL (equivalent to 50 ng sample) into the LC-MS/MS apparatus.

*Lattanzio, V., Ciasca, B., Powers, S., Visconti, A., Improved method for the simultaneous determination of aflatoxins, ochratoxin A and *Fusarium* toxins in cereals and derived products by liquid chromatography-tandem mass spectrometry after multi-toxin immunoaffinity clean up, Journal of Chromatography A, 2014; 1354:139-143.

5.0 DETECTION METHOD

5.1 LC/MS/MS CONDITIONS

System: Waters UPLC H-class with Xevo TQD
Mobile phase A: Water + 0.1 % formic acid
Mobile phase B: Acetonitrile + 0.1% formic acid
Column: Waters, ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Column temp: 40 °C
Injection volume: 10 µL

Gradient:

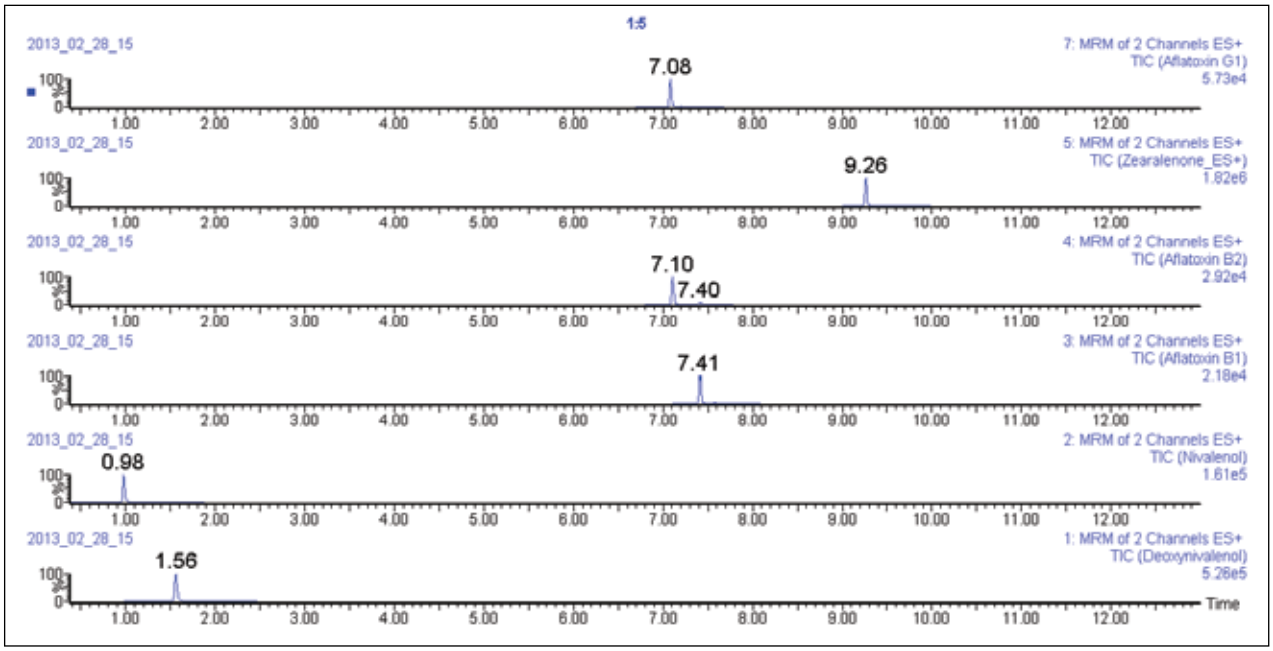
Time (min)	Flow	%A	%B
0	0.4	90	10
3	0.4	90	10
10	0.4	30	70
10.1	0.4	10	90
12	0.4	10	90
12.1	0.4	90	10
15	0.4	90	10

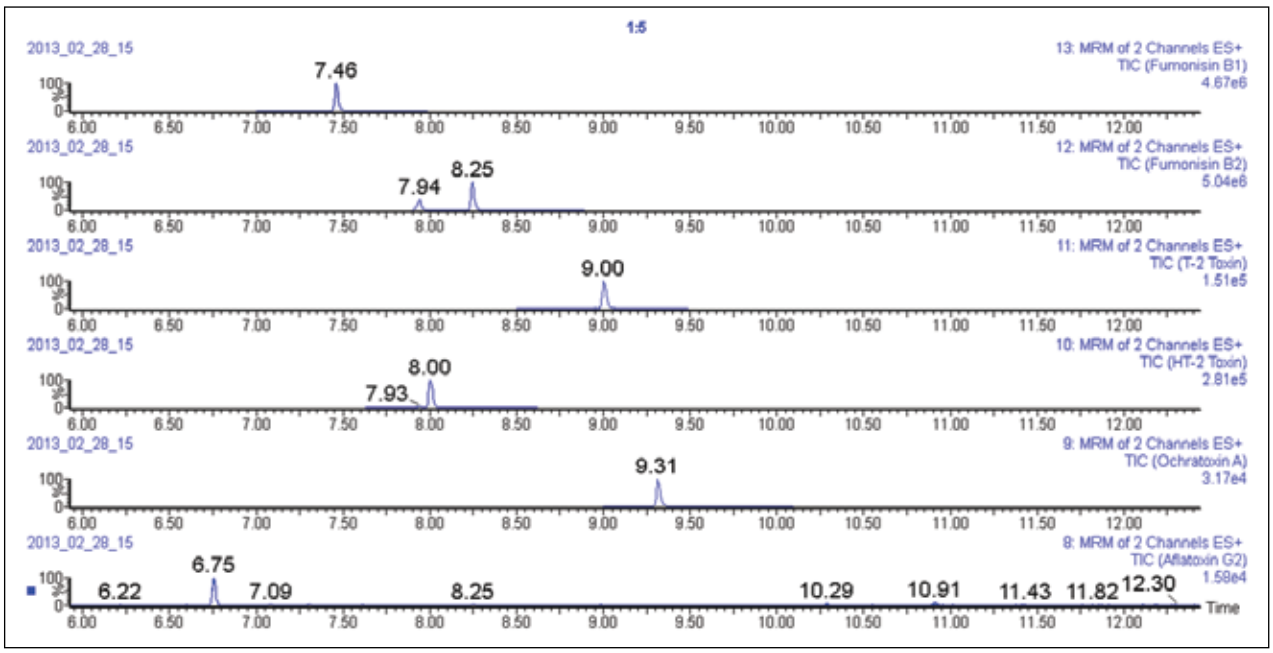
Table 1

Name	Ion Mode	Precursor	Product	Cone Voltage	Collision Energy
Aflatoxin B_1	ES+	313.2	285.1	50	24
Aflatoxin B_1	ES+	313.2	241.1	50	36
Aflatoxin B_2	ES+	315.2	287.1	50	26
Aflatoxin B_2	ES+	315.2	259.1	50	30
Aflatoxin G_1	ES+	329.2	243.1	45	25
Aflatoxin G_1	ES+	329.2	283.1	45	25
Aflatoxin G_2	ES+	331.2	245.1	50	30
Aflatoxin G_2	ES+	331.2	257.1	50	30
Ochratoxin A	ES+	404.2	239.1	30	19
Ochratoxin A	ES+	404.2	358.2	30	14
Deoxynivalenol	ES+	297.1	249.1	25	10
Deoxynivalenol	ES+	297.1	231.1	25	13
Fumonisin B_1	ES+	722.4	334.2	45	40
Fumonisin B_1	ES+	722.4	352.2	45	38
Fumonisin B_2	ES+	706.4	336.2	55	40
Fumonisin B_2	ES+	706.4	318.2	55	40
Nivalenol	ES+	313.2	295.1	13	8
Nivalenol	ES+	313.2	175.1	13	20
T-2 Toxin	ES+	467.3	305.1	10	9
T-2 Toxin	ES+	467.3	245.1	10	9
HT-2 Toxin	ES+	425.2	263.1	15	12
HT-2 Toxin	ES+	425.2	245.1	15	9
Zearalenone	ES+	319.2	187	20	19
Zearalenone	ES+	319.2	185	20	23

Total Ion Chromatogram of corn matrix matched standards for 1 g equivalent method.

Aflatoxin:	4 ppb total using 5:1:3:1 (B_1:B_2:G_1:G_2) mixture of aflatoxins
DON:	500 ppb
Ochratoxin:	4 ppb
Zearalenone:	200 ppb
Fumonisins:	700 ppb total using (5:2:1 B_1:B_2:B_3 mixture of fumonisins)
T-2:HT-2:	250 ppb (using 1:1 ration T-2:HT-2 solution)
Nivalenol:	500 ppb





5.2 HPLC CONDITIONS

The flow sequence of the LC system used in the present study was from HPLC column to PDA detector, to Photochemical Reactor, to post column reactor module (PCRM) and then to fluorescence detector. The reagent manager pump was connected to the post column reactor module and controlled by Empower software. The flow rate for derivatization mixture was 0.5 mL/min. The reagent manager pump was set to pump derivatization mixture at 18.5 min and stop at 23 min in the section of events in the Waters 2795 Separation Module. The column temperature was set to 30 ± 5 °C. The mobile phase consisted of acetonitrile, methanol, and 0.1% phosphoric acid in water. The flow rate was 1.0 mL/min. Gradient elution and the events of the fluorescence detector were shown in Table 1 and 2. A minor adjustment for the timing may be needed due to the differences in instruments.

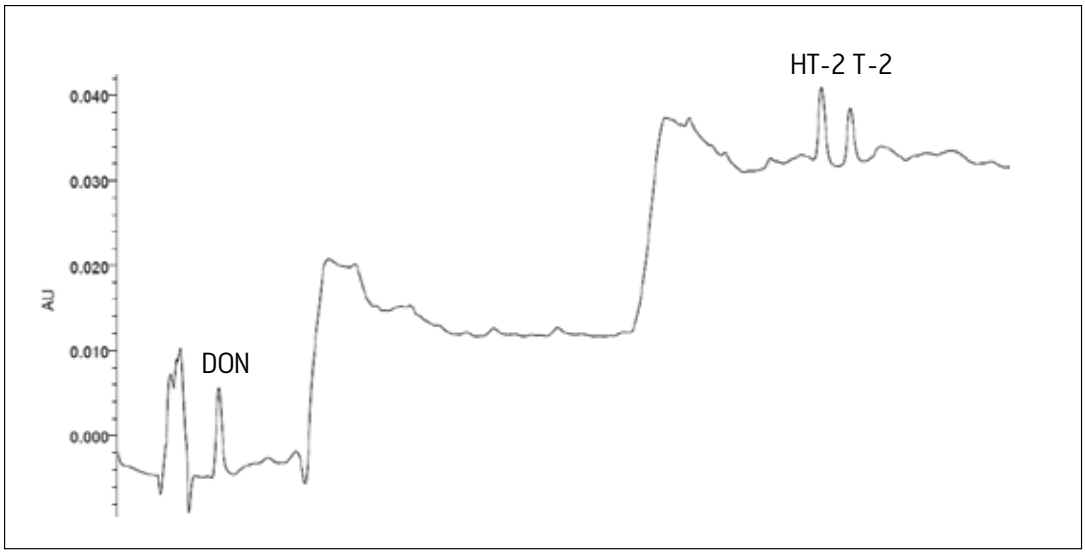
The derivatization of aflatoxins was performed by the photochemical reactor. The derivatization of fumonisins was done using a solution o-phthalaldehyde (OPA) and 2-mercaptoethanol (2ME) pumped into the PCRM by the reagent manager pump. The OPA/2ME solution was made as follows: borate buffer was made by dissolving 19 g of sodium tetraborate decahydrate in 1 liter purified water, filtering through a 0.45 µm membrane and storing at room temperature. Dissolve 150mg of OPA in 5 mL methanol, add to 500 mL borate buffer, add 500 µL of 2-mercaptoethanol, mix well, filter through 0.45 µm membrane and place in a bottle protected from light. The solution for fumonisin derivatization can be used for a week.

Table 1. The gradient elution of mobile phase

Time (min)	Flow	Methanol (%)	0.1% H_3PO_4 (%)	Acetonitrile (%)	Curve
–	1.00	0.0	85.0	15.0	–
4.00	1.00	0.0	85.0	15.0	6
5.00	1.00	25.0	60.0	15.0	6
16.00	1.00	25.0	60.0	15.0	6
17.00	1.00	30.0	40.0	30.0	6
30.01	1.00	0.0	85.0	15.0	11

Table 2. The events of fluorescent detector

Time (min)	Parameter	Wavelength (nm)	Value
0.1	Ex	365	–
–	Em	455	–
–	Gain	–	10
–	Auto zero	–	–
11.0	Auto zero	–	–
18.0	Ex	329	–
–	Em	465	–
20.0	Auto zero	–	–
23.0	Ex	276	–
–	Em	460	–
26.5	Auto zero	–	–
27.0	Gain	–	100
27.9	Ex	329	–
–	Em	460	–
–	Gain	–	10



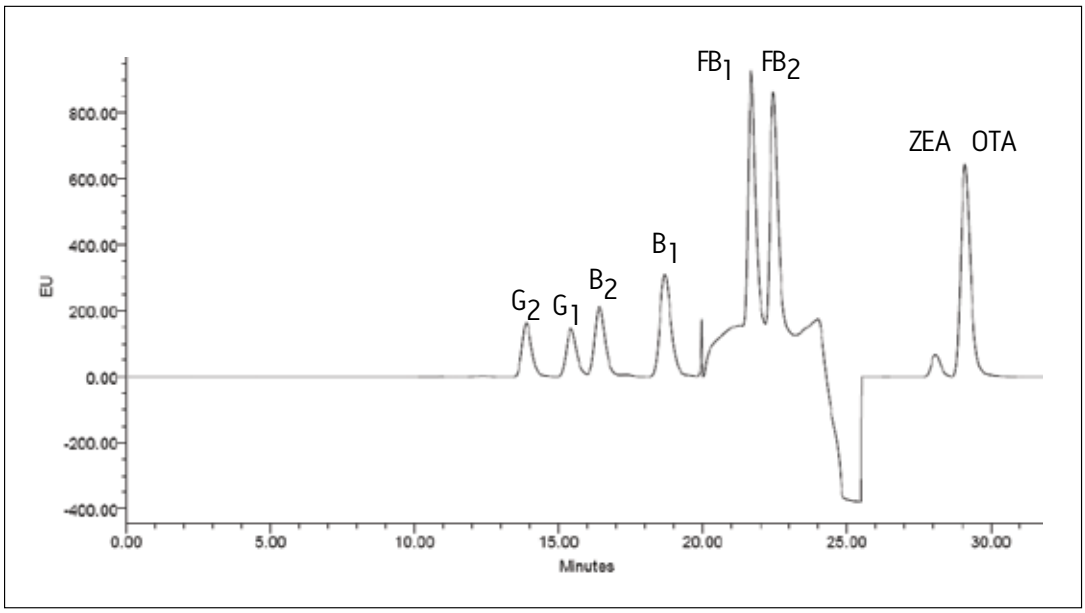
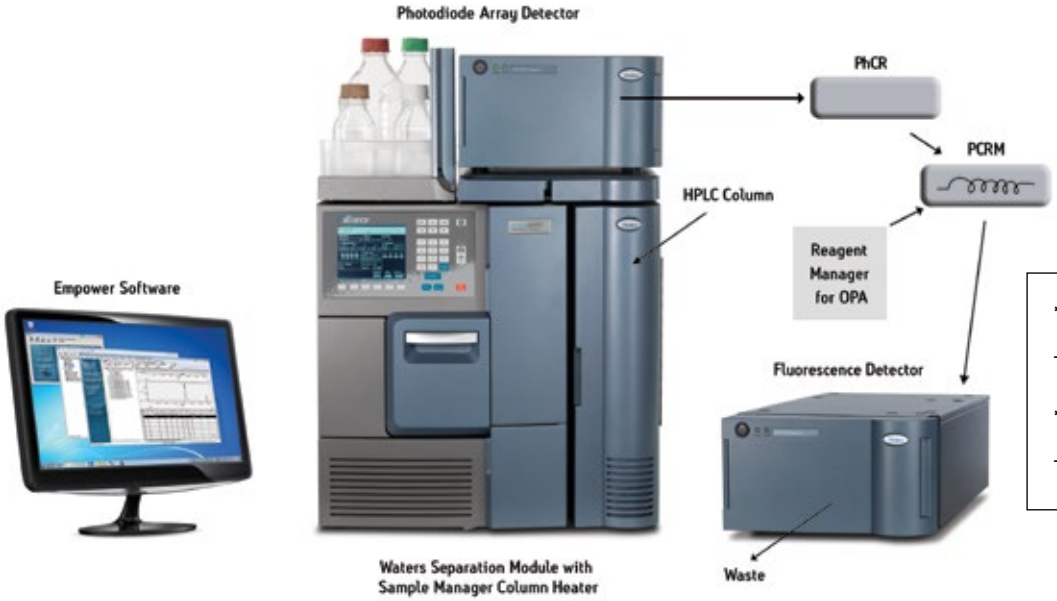


Figure 2. Representative HPLC chromatogram of corn sample containing 11 mycotoxins. The spiking levels in this figure were: 300 ppb for DON, 100 ppb for HT-2 and T-2, 2.23 ppb forAFG_2, 6.45 ppb for AFG_1, 2.48 ppb for AFB_2, 12.93 for AFB_1, 200 ppb for FB_1 and FB_2, 150 ppb for ZEA, and 50 ppb for OTA.

Ordering Information:

Description	Part Number
Separation Column: Waters Symmetry C_{18}, 3.5 µm 3.9 x 150 mm; *Waters*	WAT200632
PhCR: Photochemical Reactor; *Vicam*	600001222
PCRM: Includes the Temperature Control and the Heater; *Waters*	WAT038039
Reaction Coil: Optional*; Waters*	WAT030844
Reagent Manager: *Waters*	725000108
Reagent Manager: *Waters*, *Japanese Version*	725000109

Basic eluent/sample flow for Mycotoxin Setup



***WAT038039**
–include Temperature Control

***WAT030844**
–reaction coil

6.0 REFERENCES

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5. Kim, D.H., Jang, H.S., Choi, G.H., Kim, H.J., Kim, H.J., Kim, H.L., Cho, H.J., Lee C., Occurrence of Mycotoxins in Korean Grains and Their Simultaneous Analysis, Korean J. Food Sci. Technol., 2013; Vol. 45, No 1, pp. 111-119.
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7.0 TECHNICAL ASSISTANCE

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International and United States customers

Telephone: 508-482-4935
Fax: 508-482-4972
E-mail: techservice@vicam.com

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