Immunoaffinity Column of Fumonisins (IAC-Fumo)

Instruction Manual (C/N: IAC 107)

1. GENERAL

Fumonisins are mycotoxins produced by the fungi Fusarium verticilliodes (= F.moniliforme) and F.proliferatum. Fusarium species are a frequent, almost universal, inhabitant of corn. Fumonisins are present in most corn samples tested. Fumonisin is thought to cause equine leukoencephalomalacia in horses, swine pulmonary edema, and human esophageal cancer.

2. INTENDED USE

A simple and efficient extraction and purification procedure for Fumonisins was developed by means of the immunoaffinity column (IAC-SEP®Fumo) as a cleanup tool. Fumonisins content in Rice, Corn, Wheat, Nuts, Spices, Peanuts Peanuts products, Vegetable oil, Feed samples are cleaned up by IAC and determined by HPLC or LC-MS/MS. It is a fast,simple, safe and highly accurate method for quantitatively measuring FumonisinB1,B2,B3.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering.The extract is then applied to the Fumonisins immunoaffinity column bound with specific antibodies to Fumonisins . At this stage, the Fumonisins bind to the antibody on the column.The column is then washed with water to remove the impurities.By passing methanol through the column, the Fumonisins are removed from the antibody. This methanol solution can then be injected into HPLC or LC-MS/MS system.

4. PREPARATION OF SOLUTIONS

4.1 Extracting solution:

Acetonitrile -water（5+5,V/V）：500mL Acetonitrile +500mLwater，mixing blending.

4.2 pH=7.0 PBS：

8.0g NaCl ＋ 1.44 g Na_2HPO_4. $12H_2O$ ＋ 0.24g KH_2PO_4 ＋0.2 g KCl

dissolve in approximately 990 mL purified water,adjust the pH to 7.0, bring to 1L purified water.

4.3 0.1%Tween-20 pH7.0 PBS: Add 1.0mL Tween-20 into 1L pH =7.0 PBS, mixing blending.

4.4 0.1 M sodium tetraborate solution ($Na_2B_4O_7$): weigh 3.8 g $Na_2B_4O_7$ .10 H_2O in a 100 mL volumetric flask, bring to 100 ml with purified water, mixing blending.

4.5 OPA reagent A : Completely dissolve 40 mg o-Phthaldialdehyde in 1mL methanol, Dilute with 5 mL 0.1 M $Na_2B_4O_7$ solution（4.4）,Add 50 µL 2-Mercaptoethanol. Mix it well and store in the dark for up to one week at room temperature in a capped amber vial.

4.6 0.05M sodium tetraborate solution ($Na_2B_4O_7$): weigh 19.1 g $Na_2B_4O_7$ .10 H_2O in a 980 mL volumetric flask, adjust the pH to 10.5 with 1M NaOH solution,bring to 1000mL with purified water, mixing blending.

4.7 OPA reagent B: Completely dissolve 2000 mg o-Phthaldialdehyde in 20mL methanol, Dilute with 400ml 0.05M $Na_2B_4O_7$ solution （4.6）,Add 500 µL 2-Mercaptoethanol，bring to 500mL with 0.05M $Na_2B_4O_7$ solution（4.6）, Mix it well and store in the dark for up to one week at room temperature in a capped amber vial.

The column capacity of IAC-Fumo(maximum adsorption amount of Fumonisins) is 5000 ng, when Fumonisins in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC-FumoTest procedure for Rice, Corn, Wheat, Nuts, Spices, Peanuts Peanuts products, Vegetable oil, Feed.

5.1 Sample Extraction:

5.1.1 Weigh 25g sample with 5g NaCl and place in blender jar.

5.1.2 Add to jar 100mL Acetonitrile -water（5+5,V/V）(4.1).

5.1.3 Cover blender jar and blend at high speed for 2 minute.

5.1.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

5.2 Extract Dilution

5.2.1 Pipet or pour 2mL filtered extract into a clean vessel.

5.2.2 Dilute extract with 48mL of 0.1%Tween-20 pH=7.0 PBS(4.3). Mix well.

5.3 Column Chromatography

5.3.1 Pass 50mL filtered diluted extract (50mL = 0.5g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

5.3.2 Pass 10mL of purified water through the column at a rate of about 2 drops/second.

5.3.3 Place glass cuvette under IAC and add 1.5mL HPLC grade methanol into glass syringe barrel.

5.3.4 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.5 mL) in a glass cuvette.

5.3.5 Dry down eluate under an Nitrogen stream at 50 ℃. Redissolve eluate in 500µL Acetonitrile -water（5+5, V/V）,it is for Precolumn Derivatization(HPLC) Test.

Or redissolve eluate in 500µL Acetonitrile -water(2+8, V/V), it is for Post-column Derivatization(HPLC) Test.

6. Precolumn Derivatization(HPLC) Test

6.1 Transfer 50 µL aliquots(5.3.5) to bottom of 1 ml test tube, and add 50 µL OPA reagent A(see section 4.5). Mix solution for 30 seconds with vortex mixer.

6.2 Inject 20 µL derivatized solution into HPLC system exactly 3 minutes after adding OPA reagent.

6.3 HPLC Set up (Precolumn Derivatization):

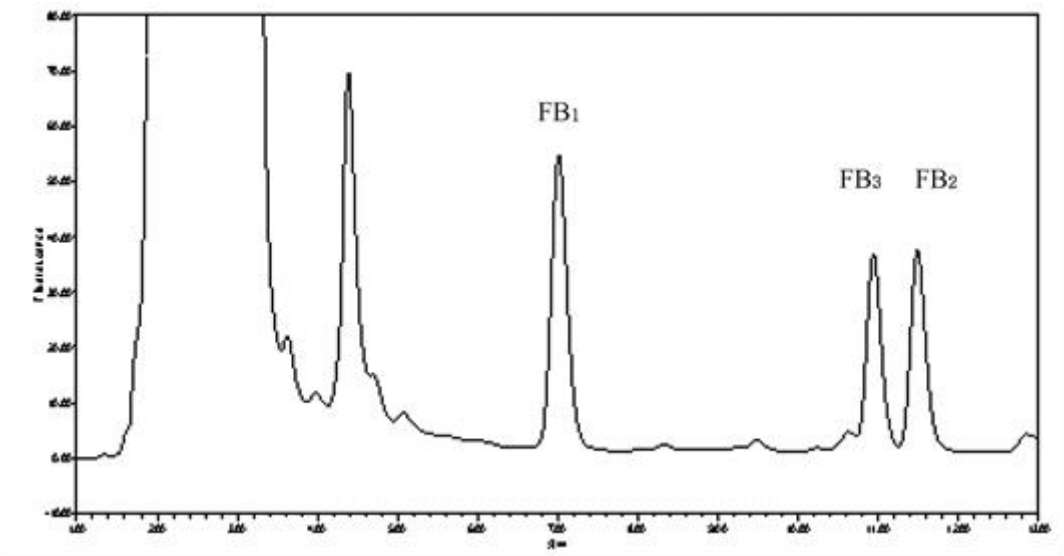
6.3.1 Column: Cloversil-C18, 4.6×250mm（5um）.

6.3.2 Flow rate: 1.0 mL/min.

6.3.3 Detector: Fluorescence detector Excitation wavelength: 335nm, Emission wavelength: 440nm

6.3.4 Sample loop: = 20 µL

6.3.5 Mobile Phase : 0.1 M NaH2PO4 (77:23, v/v) adjusted to approximately pH =3.35 with phosphoric acid (H_3PO_4).



HPLC chromatogram of Fumonisins standard(Precolumn Derivatization)

7. Post-column Derivatization(HPLC) Test

7.1 HPLC Set up (Post-column Derivatization):

7.1.1 Column: Cloversil-C18,4.6×250mm（5um）.

7.1.2 Flow rate: 1.0 mL/min.

7.1.3 Detector: Fluorescence detector Excitation wavelength: 335 nm, Emission wavelength:440 nm

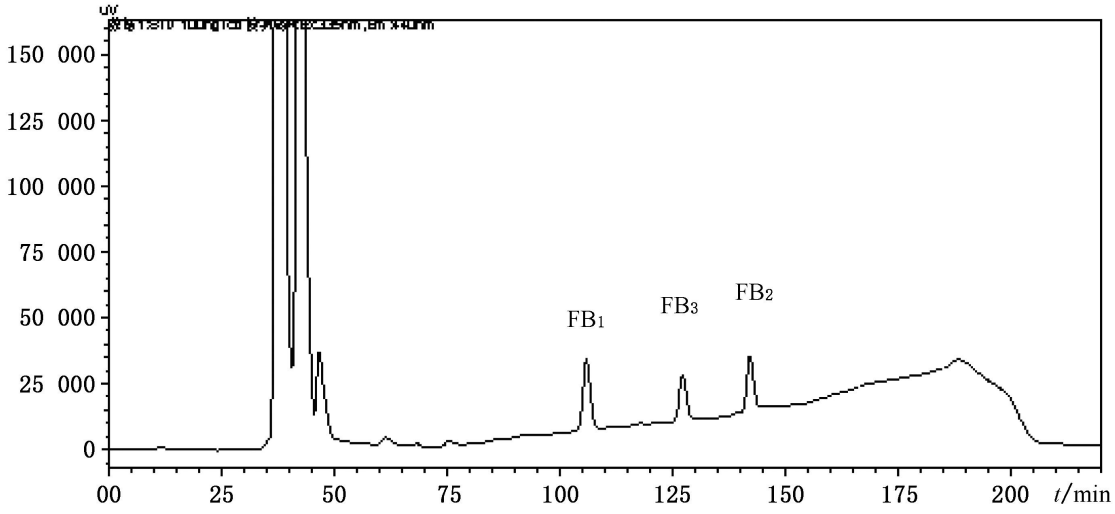
7.1.4 Sample loop: 20-100 μL.

7.1.5 HPLC Mobile Phase : Methanol -water（65+35,V/V）.

7.1.6 Derived pump flow rate: 0.4 mL/min.

7.1.7 Derived pump temperature: 50℃.

7.1.8 Derived pump Mobile Phase : OPA reagent B(4.7).



HPLC chromatogram of Fumonisins standard(Post-column Derivatization)

8. IMPORTANT NOTES

8.1 Storage: IAC-Fumo should be stored at 2-8 ºC. Do not freeze.

8.2 Shelf Life: IAC-Fumo columns and kits are stable for 18 months if stored at 2-8 ºC.

8.3 If Sample recycling test is needed, standard substance should be added to the sample before 2 hours or one night,otherwise, the recovery rate will be low.If standard substance recycling test is needed,make sure methanol concentration <25%,or the adsorption capacity of immunoaffinity column will be influenced.

8.4 If you want to modify the operating instructions of the operation steps, please contact with our technology department.