Immunoaffinity Column of Aflatoxin-M1 (IAC-AFLA M1)

Instruction Manual (C/N: IAC 102)

1. GENERAL

Aflatoxin is a kind of fungus toxic metabolites. They mainly exist in the peanut, grains, nuts, seeds and some food, animal feed and other related products. AflatoxinM_1 is the metabolic product of aflatoxin B_1. If cows intake feed that contain aflatoxinB_1, they're milk will containing aflatoxinM_1. Due to aflatoxin M_1 are quite stable, pasteurization cannot kill it, so we need to strictly detection of aflatoxin M_1 in milk and products.

2. INTENDED USE

A simple and efficient extraction and purification procedure for Aflatoxins was developed by means of the immunoaffinity column (IAC-SEP ® AFLA M_1) as a cleanup tool. This method do not use toxic solvents such as chloroform and methylene chloride. AflatoxinM_1 content in milk, fermented milk, powder, infant formula powder, cheese and cream 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 aflatoxin M_1.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution(or just centrifuging), blending and filtering. The extract is then applied to the Aflatoxin M_1 immunoaffinity column bound with specific antibodies to Aflatoxin M_1. At this stage, the Aflatoxin M_1 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 Aflatoxin M_1 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 Mobile Phase: Water-Acetonitrile-Methanol(68+24+8,V/V): 680mL water+240mL acetonitrile +80mL methanol, mixing blending.

4.2 Standard Solution: Dilute aflatoxins stock solution with mobile phase.(2-8℃ storage, valid for 24 h).

4.3 0.5 mol/L NaOH Solution (for Fermented milk): 2.0g NaOH bring to 100mL with purified water.

4.4 Acetonitrile -Water(1+4,V/V)（for cheese）: 800mLwater+200mL acetonitrile, mixing blending.

The column capacity of IAC - SEP ® AFLA M_1 (maximum adsorption amount of aflatoxin) is 150 ng, when aflatoxin M_1 in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC- AFLAM_1Test procedure for Milk and Powdered Milk

5.1 Powder Milk Sample Preparation :

5.1.1 Weigh 10g of powder milk and place into a 250mL beaker.

5.1.2 Heat 100 mL purified water to 30-40 °C.

5.1.3 Add 80mL preheated water in small amounts into the milk powder.

5.1.4 Mix continually until a homogeneous mixture is obtained.

5.1.5 Transfer milk mixture into a 250mL graduate cylinder and add 20mL of remaining preheated water. And Cooled it to room temperature. It is called as a recovery milk.

5.2 Sample Extraction

5.2.1 Measure 60.0mL of fluid milk or recovery milk and place into two 50mL polypropylene centrifuge tubes.

5.2.2 Centrifugate at 4000RPM for 5min.

5.2.3 Separate fat (top) layer from defatted (skim) layer. Use defatted (skim) milk for further analysis.

5.3 Affinity Chromatography:

5.3.1 Remove two end caps from IAC

5.3.2 Pass 50mL of defatted (skim) milk sample through the column at a steady slow flow rate of about 1 drops per second.

5.3.3 After extract has completely passed through column, pass 10mL of purified water through column at a flow rate of about 1-2 drops per second.

5.3.4 Elute IAC column at a flow rate of 1 drops per second with 1.0mL HPLC grade methanol and collect it in a clean glass cuvette.

5.3.5 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100μL into HPLC.

6. METHOD: IAC- AFLA M_1Test procedure for Fermented milk:

6.1 Sample Extraction

6.1.1 Weigh 100g of Fermented milk and place into a 250 mL beaker.

6.1.2 Adjust pH to 7.0 with 0.5 mol/L NaOH Solution, mix well.

6.1.3 Centrifugate at 4000RPM for 5min.

6.1.4 Collect the upper levels 50mL.

6.2 Affinity Chromatography:

6.2.1 Remove two end caps from IAC

6.2.2 Pass 50mL solution(6.1.4) through the column at a steady slow flow rate of about 1 drops per second.

6.2.3 After extract has completely passed through column, pass 10mL of purified water through column at a flow rate of about 1-2 drops per second.

6.2.4 Elute IAC column at a flow rate of 1 drops per second with 1.0mL HPLC grade methanol and collect it in a clean glass cuvette.

6.2.5 Add 1.0mL of purified water to eluate. Mix well. Inject 5-100μL into HPLC.

7. METHOD: IAC- AFLA M_1Test procedure for Cream

7.1 Sample Extraction

7.1.1 Weigh 2.0g of cream into a 50mL centrifuge tube.

7.1.2 Add 8mL petroleum ether, dissolve the cream, add 9mL of water and 11mL of methanol, shake for 30min.

7.1.3 Stand for 5min.

7.1.4 Collect the lower layer solution 10mL, add 20mL of water, mix well.

7.2 Affinity Chromatography:

7.2.1 Remove two end caps from IAC

7.2.2 Pass 30mL solution(7.1.4) through the column at a steady slow flow rate of about 1 drops per second.

7.2.3 After extract has completely passed through column, pass 10mL of purified water

through column at a flow rate of about 1-2 drops per second.

7.2.4 Elute IAC column at a flow rate of 1 drops per second with 1.0mL HPLC grade methanol and collect it in a clean glass cuvette.

7.2.5 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100μL into HPLC.

8. METHOD: IAC- AFLAM$_1$Test procedure for Cheese

8.1 Sample Extraction

8.1.1 Weigh 2.0g Cheese (chopped) into a 50mL centrifuge tube.

8.1.2 Add add 1 mL of water and 19mL of methanol, shake for 60min.

8.1.3 Centrifugate at 4000rpm for 5min.

8.1.4 Collect the upper levels 10mL, add 40mL of water, mix well.

8.2 Affinity Chromatography:

8.2.1 Remove two end caps from IAC

8.2.2 Pass 50mL solution(8.1.4) through the column at a steady slow flow rate of about 1 drops per second.

8.2.3 After extract has completely passed through column, pass 10mL of purified water through column at a flow rate of about 1-2 drops per second.

8.2.4 Elute IAC column at a flow rate of 1 drops per second with 1.0mL HPLC grade methanol and collect it in a clean glass cuvette.

8.2.5 Add 1.0mL of purified water to eluate. Mix well. Inject 5-100μL into HPLC.

9. HPLC Set up:

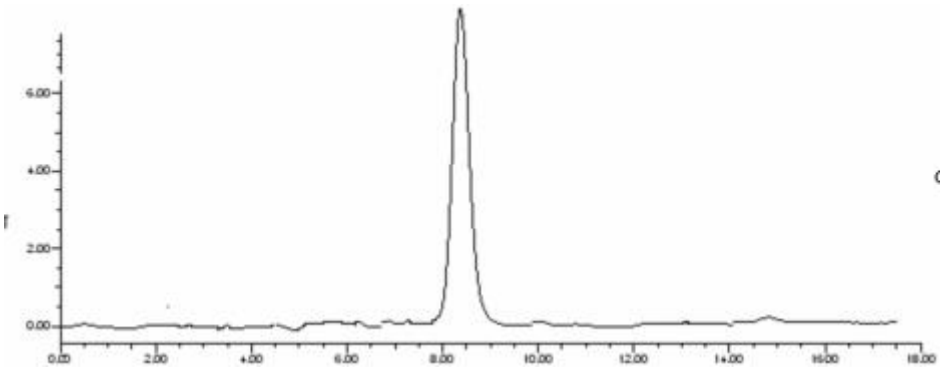
9.1 Column: Cloversil-C18,4.6×150mm（5um）or 4.6*250mm（5um）

9.2 Flow rate: 0.8 mL/min.

9.3 Detector: Fluorescence detector Excitation wavelength: 360 nm, Emission wavelength:440 nm

9.4 Sample loop: 20-100 μL.

9.5 Mobile Phase:Water-Acetonitrile-Methanol(68+24+8,V/V)



HPLC chromatogram of AFLA M_1 standard

10. IMPORTANT NOTES

10.1 Storage: IAC-AFLA M_1 should be stored at 2-8 ºC. Do not freeze.

10.2 Shelf Life: IAC-AFLA M_1 columns and kits are stable for 18 months if stored at 2-8 ºC.

10.3 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 <5%,or the adsorption capacity of immunoaffinity column will be influenced.

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

10.5 When dry the eluate do be careful the speed of Nitrogen gas flow ,or there will be a loss of AFLA M_1.