Immunoaffinity Column of Zearalenone (IAC-ZEN)

Instruction Manual (C/N: IAC 105)

1. GENERAL

Zearalenone is a kind of mycotoxins,producing by Fusarium Species.They mainly exist in corn, wheat, oats ,barley, animal feed and other related products. It proved has great harm to human and animal liver, esophagus and other tissues and organs. Zearalenone contamination can occur in plant growth, harvest and processing, storage, transportation in the process.The timely detection of pollution sources is the best way to prevent Zearalenone contamination.

2. INTENDED USE

A simple and efficient extraction and purification procedure for Zearalenones was developed by means of the immunoaffinity column (IAC-SEP® ZEN) as a cleanup tool. Zearalenones content in grain feed 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 Zearalenones.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering.The extract is then applied to the Zearalenones immunoaffinity column bound with specific antibodies to Zearalenones． At this stage, the Zearalenones 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 Zearalenones 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（8+2,V/V）：80mL acetonitrile+20mLwater, mix well.
Acetonitrile-water（9+1,V/V）：90mL acetonitrile+10mLwater, mix well.

4.2 pH=7.0 PBS：

8.0 g 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 with purified water.

4.3 Mobile Phase: Acetonitrile-water-methanol (46+46+8, V/V) :
460mL Acetonitrile + 460mL water + 80mL methanol,mix well.

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

The column capacity of IAC-SEP ® ZEN (maximum adsorption amount of Zearalenone) is 1500 ng, when Zearalenones in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC-ZENTest procedure for Grain, peanuts and its products, soybean, vinegar, vegetable oil, sauce.

5.1 Sample Extraction:

5.1.1 Weigh 40g sample with 5g NaCl and place in blender jar.

5.1.2 Add to jar 100 mL acetonitrile-water (9+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 10mL filtered extract into a clean vessel.

5.2.2 Dilute extract with 40mL of purified water. Mix well.

5.2.3 Filter dilute extract through glass microfibre filter into a clean vessel.

5.3 Column Chromatography

5.3.1 Pass 10mL filtered diluted extract (10mL = 0.8g 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 Repeat step 5.3.2 once more until air comes through the column.

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

5.3.5 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0mL) in a glass cuvette.

5.3.6 Add 1.5 mL of purified water to eluate. Mix well. Inject 5-100µL into HPLC.

6. METHOD: IAC-ZENTest procedure for Feed.

6.1 Sample Extraction:

6.1.1 Weigh 40g sample with 5g NaCl and place in blender jar.

6.1.2 Add to jar 100 mL acetonitrile-water (8+2).

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

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

6.2 Extract Dilution

6.2.1 Pipet or pour 10mL filtered extract into a clean vessel.

6.2.2 Dilute extract with 40mL of pH=7.0 PBS(4.2). Mix well.

6.2.3 Filter dilute extract through glass microfibre filter into a clean vessel.

6.3 Column Chromatography

6.3.1 Pass 10mL filtered diluted extract (10mL = 0.8g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

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

6.3.3 Repeat step 6.3.2 once more until air comes through the column.

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

6.3.5 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0mL) in a glass cuvette.

6.3.6 Add 1.5 mL of purified water to eluate. Mix well. Inject 5-100µL into HPLC.

7. METHOD: IAC-ZENTest procedure for Soy sauce, Vinegar, Liquor.

7.1 Sample Extraction:

7.1.1 Weigh 25g sample into 100mL volumetric flask.

7.1.2 Bring to 100mL with acetonitrile and transfer it to a blender jar.

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

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

7.2 Extract Dilution

7.2.1 Pipet or pour 10mL filtered extract into a clean vessel.

7.2.2 Dilute extract with 40mL of pH=7.0 PBS(4.2). Mix well.

7.2.3 Filter dilute extract through glass microfibre filter into a clean vessel.

7.3 Column Chromatography

7.3.1 Pass 10mL filtered diluted extract (10mL = 0.5g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

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

7.3.3 Repeat step 7.3.2 once more until air comes through the column.

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

7.3.5 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0mL) in a glass cuvette.

7.3.6 Add 1.5mL of purified water to eluate. Mix well. Inject 5-100 µL into HPLC.

8. HPLC Set up:

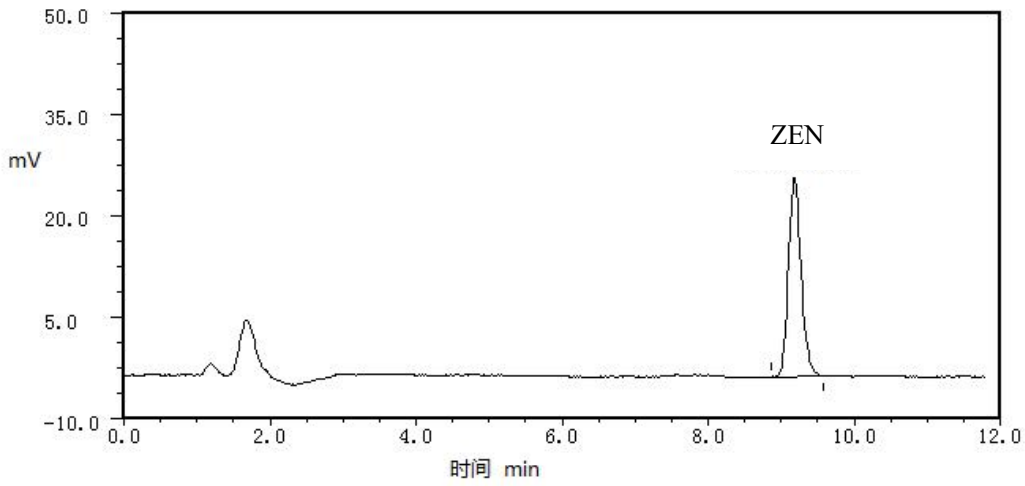
8.1 Column: Cloversil-C18,4.6×150mm（5um）or 4.6*250mm（5um）

8.2 Flow rate: 0.8 mL/min.

8.3 Detector: Fluorescence detector Excitation wavelength: 274 nm, Emission wavelength:440 nm
Ultraviolet detector:wavelength: 265 nm

8.4 Sample loop: 5-100 µL.

8.5 Mobile Phase:Acetonitrile-water-methanol(46+46+8,V/V).



HPLC chromatogram of ZEN standard

9. IMPORTANT NOTES

9.1 Storage: IAC ZEN should be stored at 2-8 ºC. Do not freeze.

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

9.3 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 acetonitrile concentration <20%,or the adsorption capacity of immunoaffinity column will be influenced.

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

9.5 When dry the eluate do be careful the speed of Nitrogen gas flow ,or there will be a loss of ZEN.