Immunoaffinity column of Deoxynivalenol (IAC-DON)

Instruction Manual (C/N: IAC 111)

1. GENERAL

Deoxynivalenol (DON) is one of several mycotoxins produced by certain Fusarium species that frequently infect corn, wheat, oats, barley, rice, and other grains in the field or during storage. Vomiting toxins have a very strong toxicity for both people and animals.It can cause human and animal vomiting, diarrhea, skin irritation, lack of apptite, neurological disorders, miscarriage, stillbirth,etc. Deoxynivalenol 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 Deoxynivalenol contamination.

2. INTENDED USE

A simple and efficient extraction and purification procedure for Deoxynivalenol was developed by means of the immunoaffinity column (IAC-SEP ® DON) as a cleanup tool. Deoxynivalenol content in corn, wheat, oats, barley, rice, and other grains 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 DON.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering.The extract is then applied to the Deoxynivalenol immunoaffinity column bound with specific antibodies to Deoxynivalenol. At this stage, the Deoxynivalenol binds to the antibody on the column.The column is then washed with water to remove the impurities.By passing methanol through the column, the Deoxynivalenol is 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:** Acetonitrile-water（1+9,V/V）：100mL Acetonitrile+900mLwater, mix .
Methanol-water（2+8,V/V）：200mL Methanol+800mLwater, mix .

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 1 liter with purified water.

4.3 **Standard solution:** Dilute Deoxynivalenol stock solution with mobile phase.(2-8 ℃ storage, valid for 7days)

The column capacity of IAC-SEP ® DON (maximum adsorption amount of Deoxynivalenol) is 2000 ng, when Deoxynivalenol in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC-DONTest procedure for Grain, peanuts and its products, Feed.

5.1 Sample Extraction:

5.1.1 Weigh 25g sample with 5g polyethylene glycol 8000(PEG) and place in blender jar.

5.1.2 Add to jar 100mL purified water.

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

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

5.2 Extract Filtration:

5.2.1 Place a gently folded microfibre filter inside a small funnel and set funnel in clean glass syringe barrel. Use a new microfibre filter for each test.

5.2.2 Filter extract through glass microfibre filter into a clean container.

5.3 Column Chromatography

5.3.1 Pass 1mL filtered diluted extract (1mL = 0.25g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

5.3.2 Pass 5mL 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 1.0mL 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 Dry down eluate under an Nitrogen stream at 50℃. Reconstitute with 500µL mobile phase.. Mix well. Inject 5-100µL into HPLC.

6. METHOD: IAC-DONTest procedure for Soy sauce, Vinegar, Liquor.

6.1 Sample Extraction:

6.1.1 Weigh 25g sample into 100mL volumetric flask

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

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 Filtration:

6.2.1 Place a gently folded microfibre filter inside a small funnel and set funnel in clean glass syringe barrel. Use a new microfibre filter for each test.

6.2.2 Filter extract through glass microfibre filter into a clean container.

6.3 Column Chromatography

6.3.1 Pass 1mL filtered diluted extract (1mL = 0.25g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

6.3.2 Pass 5mL 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 1.0mL HPLC grade methanol into glass syringe barrel.

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

6.3.6 Dry down eluate under an Nitrogen stream at 50℃. Reconstitute with 500μL mobile phase.. Mix well. Inject 5-100 μL into HPLC.

7. HPLC Set up:

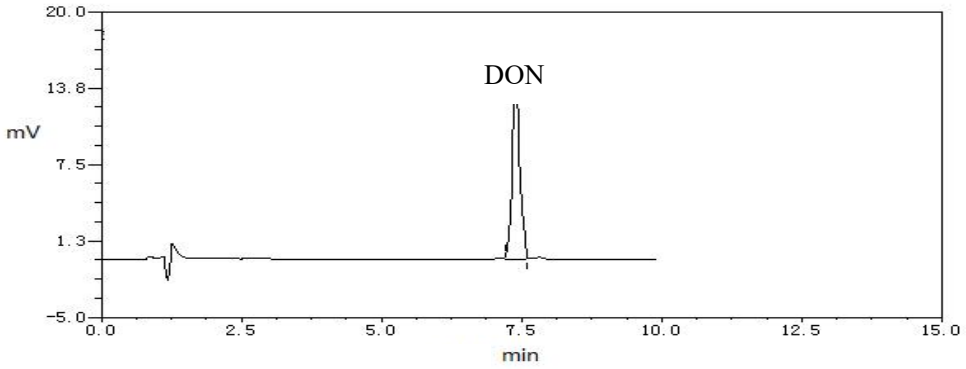
7.1 Column: Cloversil-C18,4.6×250mm(5um) or 4.6*250mm (5um)

7.2 Flow rate: 0.8 mL/min.

7.3 Detector: Ultraviolet detector:wavelength: 218 nm

7.4 Sample loop: 20-100 μL

7.5 Mobile Phase :Acetonitrile-water (1+9,V/V) or Methanol-water (2+8,V/V)



HPLC chromatogram of DON standard

8. IMPORTANT NOTES

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

8.2 Shelf Life: IAC DON columns and kits are stable for 18 months from date 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 <15%,or the adsorption capacity of immunoaffinity column will be influenced.

8.4 When pass water through the column ,the flow rate must < 1-2 drops per second,or there will be a loss of DON.

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

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