Immunoaffinity Column of Citrinin (IAC-CIT)

Instruction Manual (C/N: IAC 109)

1. GENERAL

Citrinin is a naturally occurring fungal metabolite produced by several species of the genera Penicillium and Aspergillus which causes kidney and liver damage. Citrinin has been found to be mutagenic in hepatocytes and has been implicated as a potential cause of human Endemic Balkan Nephropathy as well as porcine nephropathy.

2. INTENDED USE

A simple and efficient extraction and purification procedure for Citrinin was developed by means of the immunoaffinity column (IAC-SEP® IAC-CIT) as a cleanup tool. Citrinin content in Kogi Red Rice, Rice, Corn and Chili 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 Citrinin.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the Citrinin immunoaffinity column bound with specific antibodies to Citrinin. At this stage, the Citrinin 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 Citrinin 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:**

Methanol-water（7+3,V/V）：70mL Methanol+30mLwater，mixing blending.

4.2 **pH7.0 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 990mL purified water, adjust the pH to 7.0,bring to 1L with 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% Phosphoric acid (85%) solution:** Add 1.0mL Phosphoric acid (85%) into 1L purified water, mixing blending.

4.5 **Elution solution**: 70mL Methanol+30mL 0.1% Phosphoric acid (85%) solution，mixing blending.

The column capacity of IAC-CIT(maximum adsorption amount of Citrinin) is 100 ng, when Citrinin in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC-CIT Test procedure for Red Rice

5.1 Sample Extraction:

5.1.1 Weigh 5g sample with 1g NaCl and place in blender jar.

5.1.2 Add to jar 100 mL methanol: water (70:30).

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 1 mL filtered extract into a clean vessel.

5.2.2 Dilute extract with 39 mL of pH=7.0 PBS. 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 = 0.0125g 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 0.1%Tween-20 pH=7.0 PBS(4.3) through the column at a rate of about 2 drops/second.

5.3.3 Pass 10mL of purified water through the column at a rate of about 2 drops/second, until air comes through the column.

5.3.4 Place glass cuvette under IAC and add 1.0mL Elution solution(4.5) into glass syringe barrel.

5.3.5 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, Mix well, Inject 20-100μL into HPLC.

6. METHOD: IAC-CIT Test procedure for Rice, Corn and Chili

6.1 Sample Extraction:

6.1.1 Weigh 20g sample with 5g NaCl and place in blender jar.

6.1.2 Add to jar 100 mL methanol: water (70:30).

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 1 mL filtered extract into a clean vessel.

6.2.2 Dilute extract with 49 mL of pH7.0 PBS. 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 = 0.04g 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 0.1%Tween-20 pH=7.0 PBS(4.3) through the column at a rate of about 2 drops/second.

6.3.3 Pass 10mL of purified water through the column at a rate of about 2 drops/second, until air comes through the column.

6.3.4 Place glass cuvette under IAC and add 1.0mL Elution solution(4.5) 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, Mix well, Inject 20-100μL into HPLC.

7. HPLC Set up:

7.1 Column: Cloversil-C18,4.6×150mm（5um）

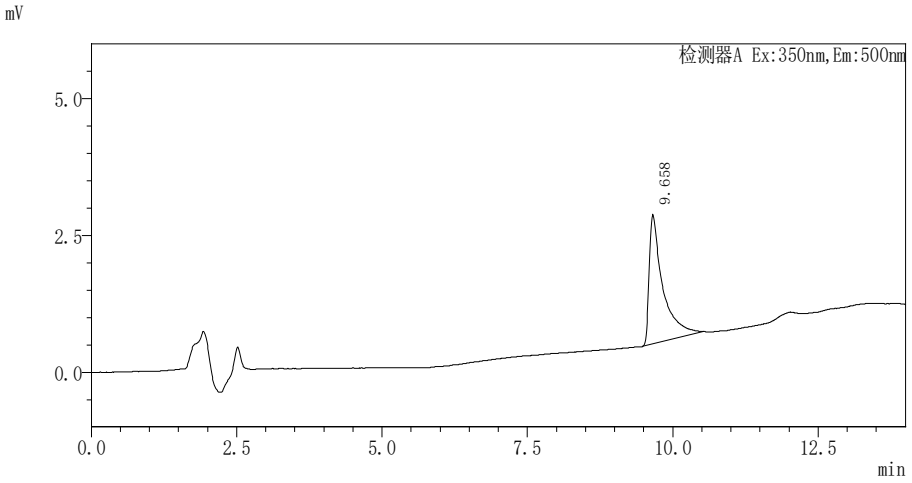
7.2 Flow rate: 0.8 mL/min.

7.3 Detector: Fluorescence detector Excitation wavelength: 350 nm, Emission wavelength:500 nm

7.4 Sample loop: 20-100 μL.

7.5 Mobile Phase: Solution A = Acetonitrile Solution B = 0.1% Phosphoric acid (85%) solution.

Time	Mobile Phase A	Mobile Phase B
0	40%	60%
1	40%	60%
7	90%	10%
9	90%	10%
10	40%	60%
15	40%	60%



HPLC chromatogram of Citrinin standard

8. IMPORTANT NOTES

8.1 Storage: IAC-CIT should be stored at 2-8℃. Do not freeze.

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

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 <5%, 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.