USER GUIDE

ECALBIO® Ochratoxin A Immunoaffinity Column

CAT#: IAC1009

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1 General

Ochratoxin is a mycotoxin produced by the fungus Aspergillus ochraceous and also by several species of Penicillium fungi. Ochratoxin has been known to cause kidney damage and decreased egg production in chickens. It is an immunosuppressant and is considered a potential carcinogen.

Ochratoxin A immunoaffinity columns can be used with AOAC Official Methods for the measurement of ochratoxin in baby food,barley, beer, wine,green coffee and roasted coffee.

2 Principle

To measure Ochratoxin levels, samples are prepared by mixing with an extraction solution, followed by blending and filtering. The extract is then applied to the Ochratoxin A column, which contains specific antibodies for Ochratoxin A. At this stage, the ochratoxin binds to the antibody on the column. The column is then washed to rid the immunoaffinity column of impurities. By passing methanol through the column, the ochratoxin is removed from the antibody. The methanol can then be injected into an HPLC or LC-MS system.

3 Intended Use

Ochra Test is quantitative method for the detection of ochratoxin A in a variety of commodities. Such as grain, feed, soybean, rapeseed, vegetable oil, soy sauce, vinegar, jam and liquor.These products are safe and simple. Sample clean up can be performed in less than 15min and determined by HPLC or LC-MS. It is a fast, simple, safe and highly accurate method for quantitatively measuring Ochratoxin A.

4 Preparation of solutions

4.1 Extracting solution:

Extracting solution1

Methanol-water(8+2,V/V): 80mL Methanol+20mL water, mixing blending.

Extracting solution2

Take 150g NaCl, 20g $NaHCO_3$ dissolved in pure water 1000mL.

4.2 PBS solution(pH=7):

8.0NaCl+2.90gNa_2HPO_4. $12H_2O$+0.24g KH_2PO_4+0.2g KCl, dissolve in approximately 990 mL purified water, adjust the pH to 7.0 with concentrated HCl, adjust 1.0L with purified water.

4.3 Mobile Phase :

acetonitrile-water-acetic acid (99+99+2,v/v), 495mL acetonitrile and 10ml acetic acid, bring to 1 liter with purified water, filtered by 0.22um Nylon filter paper.

4.4 Standard solution:

Dilute OchratoxinA stock solution with mobile phase.(e.g. take 100uL 10ug/mL OchratoxinA stock solution to 10mL volumetric flask, add mobile phase to 10mL, then the standard solution is 100ng/mL.) (2-8 ℃ storage, valid for 7days)

4.5 Washing liquor:

25g NaCl, 5g $NaHCO_3$ dissolved in pure water 1000mL.

4.6 Buffer solution:

25g NaCl, 5g $NaHCO_3$ and 0.1mL Tween-20, dissolved in pure water 1000mL.

The column capacity of IAC -OTA (maximum adsorption amount of Ochratoxin) is 300ng, when Ochratoxin in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5 METHOD: IAC-OTA Test procedure -Grain, Cereal, Soybean, Rapeseed, Vegetable oil.

5.1 Sample Extraction:

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

5.1.2 Add to jar 125 mL, methanol: water (80:20).

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 and Filtration

5.2.1 Transfer 10mL filtered extract into another clean vessel.

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

5.2.3 Filter diluted extract through glass microfibre filter and collect filtrate in a clean container.

5.3 Column Chromatography:

5.3.1 Pass 10mL filtered diluted extract completely through IAC-OTA at a rate of about 1 drops/second until air comes through column.

5.3.2 Pass 10mL of pH=7.0 PBS through the column at a rate of about 1-2 drops/second.

5.3.3 Pass 10mL purified water through the column at a rate of 1-2 drops/second until air comes through the column.

5.3.4 Method 1:

Elute Ochratoxin column at flow rate of 1 drops per second with 1.5 mL HPLC grade methanol and collect in a clean glass cuvette. Dry down eluate under an Nitrogen stream at 50°C. Reconstitute with 1000µL mobile phase.

Method 2:

Elute Ochratoxin column at flow rate of 1 drops per second with 1.5 mL HPLC grade methanol and collect in a clean glass cuvette. Add 1.5 mL water in the glass cuvette, mix.

5.3.5 Injected into HPLC or LC-MS.

6. METHOD: METHOD: IAC-OTA Test procedure for feed, chilli

6.1 Sample Extraction:

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

6.1.2 Add to jar 80mL methanol: water (80:20).

6.1.3 Cover blender jar and blend at high speed for 1 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 5 mL filtered extract into a clean vessel.

6.2.2 Dilute extract with 45mL of pH=7.0 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=0.4 ml sample equivalent) completely through IAC at a rate of about 1 drops/second until air comes through column.

6.3.2 Pass 10mL 10mL of buffer solution(4.6) through the column at a rate of about 2 drops/second.

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

6.3.4 Method 1:

Elute Ochratoxin column at flow rate of 1 drops per second with 1.5 mL HPLC grade methanol and collect in a clean glass cuvette. Dry down eluate under an Nitrogen stream at 50°C. Reconstitute with 1000µL mobile phase.

Method 2:

Elute Ochratoxin column at flow rate of 1 drops per second with 1.5 mL HPLC grade methanol and collect in a clean glass cuvette. Add 1.5 mL water in the glass cuvette, mix.

6.3.5 Injected into HPLC or LC-MS.

7 METHOD: IAC-OTA Test procedure for liquor

7.1 Sample Extraction:

7.1.1 Place 20 g of sample into 25mL volumetric flask.(If sample contain CO_2, 4℃refrigerate for 30min, then ultrasonic treatment)

7.1.2 Add extracting solution 2 to 25mL, mix well.

7.2 Extract Dilution and Filtration

7.2.1 Transfer 10mL filtered extract into another clean vessel.

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

7.2.3 Filter diluted extract through glass microfibre filter and collect filtrate in a clean container.

7.3 Affinity Chromatography:

7.3.1 Pass 10mL filtered diluted extract completely through IAC-OTA affinity column at a rate of about 1drop/second until air comes through column.

7.3.2 Pass 10mL of washing liquor(4.5)through the column at a rate of 1-2 drops/second until air comes through the column.

7.3.3 Pass 10mL purified water through the column at a rate of 1-2 drops/second until air comes through the column.

7.3.4 Method 1:

Elute Ochratoxin column at flow rate of 1 drops per second with 1.5mL HPLC grade methanol and collect in a clean glass cuvette. Dry down eluate under an Nitrogen stream at 50°C. Reconstitute with 1000µL mobile phase.

Method 2: Elute Ochratoxin column at flow rate of 1 drops per second with 1.5mL HPLC grade methanol and collect in a clean glass cuvette. Add 1.5mLwater in the glass cuvette, mix.

7.3.5 Injected into HPLC or LC-MS.

8. METHOD: IAC-OTA Test procedure for for soy sauce, vinegar, jam and jam products.

8. 1 Sample Extraction

8.1.1 Place 25 g of sample into 50mL volumetric flask.

8.1.2 Add methanol/water(80:20,v/v) to 50mL, then ultrasonic treatment 2min.

8.2 Extract Dilution and Filtration

8.2.1 Transfer 10mL filtered extract into another clean vessel.

8.2.2 Dilute extract with 40mL of pH7.0 PBS. Mix well.

8.2.3 Filter diluted extract through glass microfibre filter and collect filtrate in a clean container.

8.3 Affinity Chromatography:

8.3.1 Pass 10mL filtered diluted extract completely through IAC-OTA affinity column at a rate of about 1 drop/second until air comes through column.

8.3.2 Pass 10mL of washing liquor(4.5)through the column at a rate of 1-2 drops/second until air comes through the column.

8.3.3 Pass 10mL purified water through the column at a rate of 1-2 drops/second until air comes through the column.

8.3.4 Method 1:

Elute Ochratoxin column at flow rate of 1 drops per second with 1.5mL HPLC grade methanol and collect in a clean glass cuvette. Dry down eluate under an Nitrogen stream at 50°C. Reconstitute with 1000µL mobile phase.

Method 2:

Elute Ochratoxin column at flow rate of 1drops per second with 1.5mL HPLC grade methanol and collect in a clean glass cuvette. Add 1.5mL water in the glass cuvette, mix.

8.3.5 Injected into HPLC or LC-MS.

9 HPLC Set up:

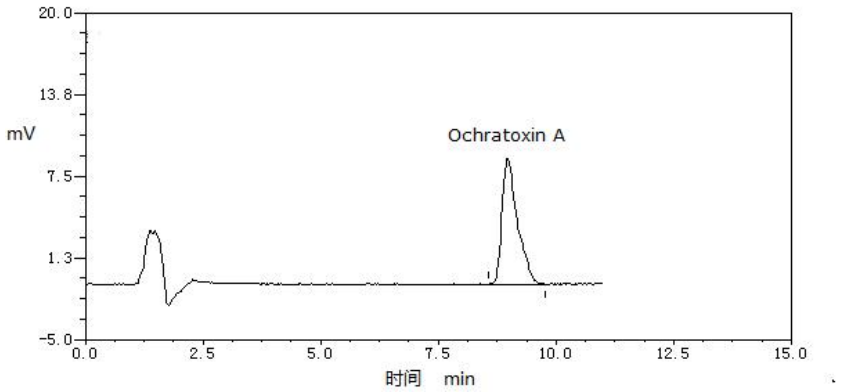
9.1 Column: Cloversil-C18,4.6×150mm（5um）or 4.6*250mm（5um）

9.2 Flow rate: 0.8mL/min.

9.3 Detection: FLD, Ex=333nm, Em=477nm

9.4 Sample loop: 20-100 µL

9.5 Mobile Phase : acetonitrile-water-acetic acid (99+99+2,v/v)



HPLC chromatogram of OTA standard

10 Important Notes

10.1 Ochratoxin may be lost if eluate is passed through nylon disc filter.

10.2 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.

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

10.4 control the flow rate when Affinity Chromatography, do not too fast, may cause the ochratoxin lost.

10.5 If standard substance recycling test is needed,make sure acetonitrile concentration <20%,or the adsorption capacity of immunoaffinity column will be influenced.

10.6 Make sure the equipment is clean and not contaminated with materials that might cause background Fluorescence

10.7 Control the Nitrogen gas flow when Drying down eluate under an Nitrogen stream at 50℃.Ochratoxin may be lost if the flow is too large.