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Method of Test for Mycotoxins in Foods-Test of Aflatoxin M_1

1. Scope

This method is applicable to the determination of aflatoxin M_1 in liquid milk, milk powder and food for infant and young child.

2. Method

After extraction and purification, aflatoxin M_1 is determined by high performance liquid chromatography (HPLC).

2.1. Equipment

2.1.1. High performance liquid chromatograph.

2.1.1.1. Detector: fluorescence detector.

2.1.1.2. Column: RP-18, 5 μm, 4.6 mm i.d. x 25 cm, or an equivalent product.

2.1.2. Centrifuge: centrifugal force > 2500 ×g.

2.1.3. Nitrogen evaporator.

2.1.4. Ultrasonicator.

2.1.5. Solid phase vacuum extraction manifold.

2.2. Chemicals

Acetonitrile, HPLC grade;

Methanol, HPLC grade;

Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);

Aflatoxin M_1 (0.5 μg/mL in acetonitrile), reference standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 1 mL and 2 mL, amber.

2.3.3. Immunoaffinity column: VICAM column containing the monoclonal antibody specific for aflatoxin M_1, or an equivalent product.

2.3.4. Membrane filter: 0.45 μm, PTFE.

2.4. Mobile phase

Mix deionized water, acetonitrile and methanol at the ratio of 17:6:2 (v/v/v), and filter with a membrane filter.

2.5. Standard solution preparation

Dilute appropriate volume of aflatoxin M_1 reference standard with mobile phase to 0.25-2 ng/mL, as the standard solutions.

2.6. Sample solution preparation

2.6.1. Extraction

2.6.1.1. Liquid milk

Transfer about 50 g of the mixed-well sample accurately weighed into a centrifuge tube, and centrifuge at 2500 ×g for 15 min at 4°C. Remove the upper layer of fat, and take the lower layer for purification.

2.6.1.2. Milk powder

Transfer about 5 g of the mixed-well sample accurately weighed into a 50-mL volumetric flask, make up to volume with deionized water, transfer into a centrifuge tube, and centrifuge at 2500 ×g for 15 min at 4°C. Remove the upper layer of fat, and take the lower layer for purification.

2.6.1.3. Food for infant and young child

Modulate the sample according to the proportions indicated on the label. Transfer about 50 g of the modulated sample accurately weighed into a centrifuge tube, and centrifuge at 2500 ×g for 15 min at 4°C. Remove the upper layer of fat, and take the lower layer for purification.

2.6.2. Purification

Transfer the solution for purification from section 2.6.1 into the immunoaffinity column (flow rate controlled at 1 drop/second), and discard the eluent. Wash the centrifuge tube with a small amount of deionized water, and add the washings into the immunoaffinity column. Wash the immunoaffinity column twice with 10 mL of deionized water (flow rate controlled at 1 drop/second), and discard the eluent. Dry the column by vacuum suction if necessary. Add 4 mL of acetonitrile (flow rate controlled at 1 drop/second), and collect the eluent. Evaporate the eluent to dryness by gently flushing with a stream of nitrogen at 50°C. Dissolve the residue, and dilute with mobile phase to 1 mL. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7. Identification and quantification

Accurately inject 100 μL of the sample solution and the standard solutions into HPLC separately, and operate according to the following conditions. Identify aflatoxin M_1 based on the retention time. Calculate the amount of aflatoxin M_1 in the sample by the following formula:

$$\text{The amount of aflatoxin } M_1 \text{ in the sample } (\mu g/kg) = \frac{C \times V}{M}$$

Where,

C: the concentration of aflatoxin M_1 in the sample solution calculated by the standard curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

HPLC operating conditions (note):

Fluorescence detector: excitation wavelength, 365 nm; emission wavelength, 435 nm.

Column: RP-18, 5 μm, 4.6 mm i.d. × 25 cm.

Injection volume: 100 μL.

Mobile phase: prepared as section 2.4.

Flow rate: 1.0 mL/min.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limits of quantification (LOQ) for aflatoxin M_1 is 0.005 μg/kg in liquid milk and food for infant and young child, and 0.05 μg/kg in milk powder.
2. Further validation should be performed when interfering compounds appear in samples.

References

1. Mao, J., Lei, S., Liu, Y., Xiao, D., Fu, C., Zhong, L. and Ouyang, H. 2015. Quantification of aflatoxin M_1 in raw milk by a core-shell column on a conventional HPLC with large volume injection and step gradient elution. Food Control 51: 156-162.
2. Wu, S. H., Chiu, J. Y., Yu, M. C., Lwo, C. H., Chang, T. P., Chen, J. H. and Shih, W. C. 2019. Development and validation of analytical methods for the determination of mycotoxins and PAHs in foods. Commissioned Research Report of Taiwan Food and Drug Administration.