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MOHWT0005.02

Method of Test for Mycotoxins in Foods- Test of Patulin

1. Scope

This method is applicable to the determination of patulin in apple juice, apple puree, baby foods, and apple juice and solid apple products including apple compote and apple puree for infant and young child.

2. Method

After enzyme hydrolysis and extraction, patulin is determined by high performance liquid chromatography (HPLC).

2.1. Equipment

2.1.1. High performance liquid chromatograph.

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: Inertsil ODS-2, 5 μm, 4.6 mm i.d. x 15 cm or an equivalent product.

2.1.2. Blender.

2.1.3. Vortex mixer

2.1.4. Horizontal shaking bath: capable of controlling temperature at ± 1°C

2.1.5. Shaker.

2.1.6. Centrifuge: centrifugal force > 2000 ×g.

2.1.7. Rotary evaporator.

2.1.8. Nitrogen evaporator.

2.1.9. Ultrasonicator.

2.1.10. pH meter.

2.2. Chemicals

Acetonitrile, HPLC grade;
Ethyl acetate, HPLC grade;
Anhydrous sodium sulfate, reagent grade;
Acetic acid, reagent grade;
Sodium carbonate, reagent grade;
Glycerol, reagent grade;
Pectinase, 16 U/mg;
Deionized water, resistivity ≥ 18 MΩ•cm (at 25°C);
Patulin, reference standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 15 mL and 50 mL, PP.

2.3.2. Concentration flask: 100 mL.

2.3.3. Membrane filter: 0.45 μm, PVDF.

2.3.4. Filter paper: Whatman No.4, diameter 11 cm, or an equivalent product.

2.4. Reagents preparation

2.4.1. 1.5% sodium carbonate

Dissolve and dilute 1.5 g of sodium carbonate with deionized water to 100 mL.

2.4.2. pH 4.0 acetic acid solution

Take 100 mL of deionized water, and adjust to pH 4.0 with acetic acid.

2.4.3. 50% glycerol

Dilute 5 mL of glycerol with deionized water to 10 mL.

2.4.4. Pectinase solution

Dilute the pectinase with deionized water to the concentration of 1.4 U/mg

2.5. Mobile phase

Mix deionized water and acetonitrile at the ratio of 9:1 (v/v), and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 5 mg of patulin reference standard accurately weighed to a 50-mL volumetric flask. Dissolve and dilute to volume with ethyl acetate as the standard stock solution. Store at 4°C. When to use, take approximate amount of the standard stock solution, evaporate to dryness by gently flushing with a stream of nitrogen, dissolve and dilute the residue with pH 4.0 acetic acid solution to 50 - 500 ng/mL as the standard solutions(note).

Note: The standard solutions should be used within 1 week.

2.7. Sample solution preparation

Transfer about 5 g of the homogenized sample or the modulated baby food sample according to the proportions indicated on the label accurately weighed into a 50-mL centrifuge tube. Add 75 μL of the pectinase solution and 5 mL of deionized water to react in a water bath at 40°C for 2 hr. Add 10 mL of ethyl acetate, shake for 1 min, and centrifuge at 2000 ×g for 3 min. Collect the upper layer, add 10 mL of ethyl acetate to the lower layer, and repeat the above extraction procedure. Combine the upper layer, add 2 mL of 1.5% of sodium carbonate, shake for 1 min, and centrifuge at 2000 ×g for 3 min. Transfer the upper layer into another 50-mL centrifuge tube, add 5 mL of ethyl

acetate to the lower layer, and repeat the above extraction procedure. Combine the upper layer, add 1 g of anhydrous sodium sulfate, vortex for 30 sec, filter with a filter paper, and collect the filtrate. Wash the centrifuge tube with 4 mL of ethyl acetate twice, combine the washing liquid with the supernatant into a concentration flask, and concentrate to about 1-2 mL by a rotary evaporator under reduced pressure at 40°C. Transfer the concentrate into a 15-mL centrifuge tube, wash the concentration flask with 2 mL of ethyl acetate twice, collect the washing liquid into the original 15-mL centrifuge tube, and evaporate to dryness by gently flushing with a stream of nitrogen. Dissolve the residue with 0.5 mL of pH 4.0 acetic acid solution, vortex-mix for 30 sec, and ultrasonicate for 5 min. Centrifuge at 2000 ×g for 3 min, and filter the supernatant with a membrane filter. Take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 50 μL of the sample solution and the standard solutions into HPLC separately, and operate according to the following conditions. Identify patulin based on the retention time and the absorption spectrum. Calculate the amount of patulin in the sample by the following formula:

$$\text{The amount of patulin in the sample (μg/kg)} = \frac{C \times V}{M}$$

Where,

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

V: the final volume used to dissolve the sample (mL)

M: the weight of the sample (g)

HPLC operating conditions(note):

Photodiode array detector: quantitative wavelength, 276 nm.

Column: Inertsil ODS-2, 5 μm, 4.6 mm i.d. x 15 cm.

Mobile phase: prepared as section 2.5.

Flow rate: 0.5 mL/min.

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

Remark

1. Limit of quantification (LOQ) for patulin is 5 μg/kg.
2. Further validation should be performed when interfering compounds appear in samples.

References

1. Sadok, I., Szmagara, A. and Staniszewska, M. M. 2018. The validated and sensitive HPLC-DAD method for determination of patulin in strawberries. Food Chem. 245: 364-370.
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.