Original: Japanese
Provisional translation

Analytical Method for Narasin (Animal Products)

1. Analyte

Narasin A

2. Application

Animal products

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Reference standard of narasin A: Contains not less than 85% of narasin A.

5. Procedure

1) Extraction

Weigh 10.0 g of sample. Add 50 mL of methanol to the sample and homogenize. Centrifuge at 3,000 rpm for 5 min and collect the methanol layer. Add 30 mL of methanol to the residue, homogenize and centrifuge as described above. Collect the methanol layer, combine it with the previous methanol layer and add methanol to make exactly 100 mL. Take exactly a 5 mL aliquot of the solution and add 1 mL of water.

2) Clean-up

Inject 5 mL of methanol and 5 mL of water and methanol (1:9) mixed solution into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL of water and methanol (1:9) mixed solution and discard the effluent. Then, inject 10 mL of water and methanol (1:19) mixed solution, collect the eluate, concentrate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile to make exactly 1 mL and use this solution as the test solution.

6. Calibration curve

Prepare narasin A standard solutions (acetonitrile) of several concentrations, inject each solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of narasin A in the test solution corresponding to 0.005 mg/kg in the sample is 0.0025 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of narasin A from the calibration curve prepared in **6.**

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octylsilanized silica gel 2.1 mm inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of 0.1 vol% formic acid-acetonitrile solution and 0.1 vol% formic acid (4:1) to (19:1) in 5 min.

Ionization mode: ESI (−)

Major monitoring ions (*m/z*): Precursor ion 764, product ions 255, 87

Injection volume: 5 μL

Expected retention time: 3 min

10. Limit of quantification

0.005 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting narasin A from sample with methanol, cleaning up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

i) When the analytical method for narasin A using LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions (*m/z*): precursor ion 764, product ion 87

for qualitative ions (*m/z*): precursor ion 764, product ion 255

ii) Food items used to develop the analytical method: cattle muscle, cattle fat and cattle liver.

iii) As a high-purity reference standard for narasin A was not available at the time of developing the analytical method, it was stated in **4. Reagents** that "Reference standard of narasin A: Contains not less than 85% of narasin A". However, it is recommended to use a reference standard with a purity of 95% or higher for analysis if available.

12. Reference

None

13. Type

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