Original: Japanese
Provisional translation

Analytical Method for Propoxycarbazone (Animal Products)

1. Analyte

Propoxycarbazone

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 propoxycarbazone sodium salt: Contains not less than 98% of propoxycarbazone sodium salt.

5. Procedure

1) Extraction

Add 100 mL of methanol to 10.0 g of the sample, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize and filter as described above. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution and concentrate to about 1 mL at below 40°C. Add 30 mL of *n*-hexane to the solution and extract with shaking twice with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetonitrile.

2) Clean-up

Inject 5 mL each of water and acetonitrile into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL of acetonitrile, and discard the effluent. Then, inject 10 mL of acetonitrile and water (7:3) mixed solution, collect the eluate, add a mixed solution of acetonitrile and water (7:3) to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Dissolve the reference standard of propoxycarbazone sodium salt in methanol to make 200 mg/L (equivalent to propoxycarbazone), and use the solution as a stock standard solution. Dilute the stock standard solution with a mixed solution of acetonitrile and water (7:3) appropriately, and prepare standard solutions 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 propoxycarbazone in the test solution corresponding to 0.01 mg/kg in the sample is 0.001 mg/L.

7. Quantification

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

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized 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 acetonitrile and 0.1 vol% formic acid (3:7) to (7:3) in 10 min and hold for 5 min at (9:1).

Ionization mode: ESI (−)

Major monitoring ions (*m/z*): Precursor ion 397, product ions 156, 113

Injection volume: 4 μL

Expected retention time: 9 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting propoxycarbazone from sample with methanol, defatting by acetonitrile/hexane partitioning, cleaning up with an ethylenediamine-*N*-propylsilanized silica gel cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

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

for quantitative ions (*m/z*): precursor ion 397, product ion 156

for qualitative ions (*m/z*): precursor ion 397, product ion 113

ii) Since propoxycarbazone is hard to dissolve in acetonitrile, care should be taken when preparing standard stock solutions depending on the concentration.

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

12. Reference

None

13. Type

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