Original: Japanese
Provisional translation

Analytical Method for Asulam (Animal Products)

1. Analyte

Asulam

2. Applicable food

Animal Products

3. Instrument

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

4. Reagents

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

Reference standard of asulam: Contains not less than 95% of asulam.

5. Procedure

1) Extraction

Add 10 mL of water to 10.0 g of the sample, homogenize, add 50 mL of acetone, and homogenize again. Centrifuge at 3,000 rpm for 5 min and collect the supernatant. Add 25 mL of acetone to the residue, homogenize, centrifuge as described above, and collect the supernatant. Combine the resulting supernatant and add acetone to make exactly 100 mL. Take exactly a 5 mL aliquot of the solution, concentrate at below 40℃, and remove the solvent. Add 5 mL of *n*-hexane to the residue and extract by shaking twice with 5 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts and concentrate to approximately 1 mL at below 40℃.

2) Clean-up

Inject 10 mL of 0.1 mol/L hydrochloric acid and 20 mL of 0.1 vol% formic acid-acetonitrile solution into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. Inject 10 mL of 0.1 mol/L hydrochloric acid and 20 mL of 0.1 vol% formic acid -acetonitrile solution into an octadecylsilanized silica gel cartridge (1,000 mg) sequentially and discard each effluent. Connect the octadecylsilanized silica gel cartridge to the bottom of the ethylenediamine-*N*-propylsilanized silica gel cartridge. Transfer the solution obtained in 1) to this cartridge, wash the container with a 2 mL mixture of 0.1 vol% formic acid-acetonitrile solution and water (1:4, v/v), and transfer the washings to the cartridge. Then, add 7 mL of 0.1 vol% formic acid-acetonitrile solution, collect the total eluate including transferred solutions and washings, add 0.1 vol% formic acid-acetonitrile solution to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare asulam standard solutions (0.1 vol% formic acid-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 asulam in the test solution corresponding to 0.01 mg/kg in the sample results in 0.0005 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of asulam from the calibration curve made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 100 mm in length, and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 0.1 vol% formic acid and 0.1 vol% formic acid-acetonitrile solution (49:1, v/v) to (7:3, v/v) in 4 min, further linear gradient to (1:49, v/v) in 4 min, and hold for 3 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): Precursor ion 231, product ions 156, 92

Injection volume: 5 μL

Expected retention time: 5 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of asulam from the sample with acetone, defatting by acetonitrile/hexane partitioning, clean-up using a cartridge with an octadecylsilanized silica gel cartridge connected to the bottom of an ethylenediamine-*N*-propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical methods for asulam using LC-MS/MS were developed, the following monitoring ions were used:

for quantitative ions *(m/z)*: precursor ion 231, product ion 156

for qualitative ions (*m/z*): precursor ion 231, product ion 92

ii) Sonication is recommended when dissolving residues after concentration.

iii) Since ashram adheres readily to container walls along with food ingredients, it is recommended not to dry out when concentrating the extract after acetonitrile/hexane partitioning.

iv) Residues after concentrating the extract caused by acetonitrile/hexane partitioning are

insufficiently dissolved in 0.1 vol% formic acid-acetonitrile solution used as elution solvents in column purification. Therefore, it is necessary to wash the container with 0.1 vol% formic acid-acetonitrile solution and water (1:4, v/v) to dissolve any residues thoroughly, and then inject the washing into the column.

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

12. References

None

13. Type