Analytical Method for Amisulbrom (Agricultural Products)

1. Analyte

Amisulbrom

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

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

4. Procedure

1) Extraction

For fruits and vegetables, weigh 20.0 g of sample. For grains, legumes, nuts and seeds, weigh 10.0 g of sample, for tea leaves, weigh 5.00 g of sample, add 20 mL of water and let stand for 30 minutes.

Add 100 mL of acetonitrile/water (4:1, v/v), homogenize, and filter with suction. Add 50 mL of acetonitrile/water (4:1, v/v) to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 200 mL. Take a 10 mL aliquot of the extract for fruits and vegetables, a 20 mL aliquot for grains, legumes, nuts and seeds, and a 40 mL aliquot for tea leaves. Add 10 mL of water, and concentrate to about 10 mL (about 15 mL for tea leaves) at below 40°C.

2) Clean-up

i) Octadecylsilanized silica gel column chromatography

Add 5 mL each of acetonitrile and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Transfer the extract obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (1:1, v/v), and discard the effluent. Elute with 10 mL of acetonitrile/water (7:3, v/v), and collect the eluate.

ii) Graphitized carbon black column chromatography

Add 5 mL each of acetonitrile and water to a graphitized carbon black cartridge (500 mg) sequentially, and discard the effluent. Transfer the solution obtained in i) to the cartridge, add 10 mL of acetonitrile, and discard the effluent. Elute with 30 mL of acetonitrile/toluene (3:1, v/v), concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in 5 mL of ethyl acetate/*n*-hexane (1:19, v/v).

iii) Synthetic magnesium silicate column chromatography

Add 5 mL of ethyl acetate/*n*-hexane (1:19, v/v) to a synthetic magnesium silicate cartridge (900 mg) and discard the effluent. Transfer the solution obtained in ii) to the cartridge, add 5 mL of ethyl acetate/ *n*-hexane (1:19, v/v), and discard the effluent. Elute

with 10 mL of ethyl acetate/ *n*-hexane (1:4, v/v), concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in water/methanol (1:3, v/v) to make exactly 2 mL, and use this solution as the test solution.

5. Calibration curve

Prepare amisulbrom standard solutions (water/methanol (1:3, v/v)) of several concentrations. Inject 10 μL of each standard solution to LC-MS, and make a calibration curve by peak-height method or peak-area method. When the test solution is prepared following the above procedure, amisulbrom concentration in the test solution corresponding to the limit of quantification is 0.005 mg/L.

6. Quantification

Inject 10 μL of the test solution to LC-MS and calculate the concentration of amisulbrom from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 0.1 vol% acetic acid/0.1 vol% acetic acid-methanol (1:3, v/v) for 15 min, followed by a linear gradient to (1:19, v/v) in 0.5 min, and hold for 8 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): 468

Expected retention time: 11 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of amisulbrom from sample with acetonitrile/water (4:1, v/v), clean-up with an octadecylsilanized silica gel cartridge, a graphitized carbon black cartridge and a synthetic magnesium silicate cartridge, and quantification and confirmation using LC-MS.

2) Notes

i) It has been reported that “Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)” is not applicable to amisulbrom in soybeans.

ii) For samples containing a large amount of matrix components such as soybeans, ion

suppression may occur with column washing time of 8 min in the mobile phase. In such cases, it is recommended to prolong the washing time to 15 min.

11. References

None

12. Type

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