Original: Japanese
Provisional translation

# Analytical Method for Metazosulfuron (Agricultural Products)

**1. Analyte**

Metazosulfuron

**2. Application**

Agricultural 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.

Graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg): A polypropylene tube of 12–13 mm inside diameter packed with 500 mg each of graphite carbon in the upper layer and ethylenediamine-*N*-propylsilanized silica gel in the lower layer or other cartridges with equal separation characteristics.

Reference standard of metazosulfuron: Contains not less than 98% of metazosulfuron.

**5. Procedure**

1) Extraction

For fruits and vegetables, weigh 20.0 g of sample. For grains, legumes, nuts and seeds, add 20 mL of water to 10.0 g (5.00 g for tea leaves) of sample and let stand for 30 min. Then, add 100 mL of acetone to the sample, homogenize and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize and filter as described above. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take exactly a 10 mL aliquot of the solution, add 0.2 mL of 2 mol/L hydrochloric acid and 10 mL of saturated sodium chloride solution, extract with shaking with 10 mL of *n*-hexane, centrifuge at 3,000 rpm for 5 min, and collect the organic layer. Extract with shaking by adding 10 mL of *n*-hexane to the aqueous layer, centrifuge as described above, collect the organic layer and combine the resulting organic layer with the previous organic layer.

2) Clean-up

Inject 10 mL each of acetone and *n*-hexane into a graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg) sequentially and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetone and discard the effluent. Then, add 10 mL of 5 vol% formic acid-acetone solution, concentrate

the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile and water (1:1) mixed solution to make exactly 10 mL for fruits and vegetables, exactly 5 mL for grains, legumes, nuts and seeds, or exactly 2.5 mL for tea leaves, and use these solutions as test solutions.

**6. Calibration curve**

Prepare metazosulfuron standard solutions (acetonitrile and water [1:1]) 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 metazosulfuron 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 metazosulfuron 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, 100 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially a mixed solution of acetonitrile, 2 vol% acetic acid and water (10:1:9) for 0.5 min, followed by a linear gradient to (18:1:1) in 9.5 min and hold for 3 min. Alternatively, initially a mixed solution of 0.1 vol% acetic acid and 0.1 vol% acetic acid-acetonitrile solution (1:1) for 0.5 min, followed by a linear gradient to (1:9) in 9.5 min and hold for 3 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*):

Precursor ion 476, product ion 182

Precursor ion 478, product ion 182

Injection volume: 5 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 metazosulfuron from sample with acetone, transferring

it into *n*-hexane for re-dissolution under acidity using hydrochloric acid, cleaning up with a graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

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

for quantitative ions (*m/z*): precursor ion 476, product ion 182

for qualitative ions (*m/z*): precursor ion 478, product ion 182

ii) Food items used to develop the analytical method: brown rice, soybean, spinach, cabbage, potato, orange, apple, and tea.

**12. Reference**

None

**13. Type**

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