Original: Japanese
Provisional Translation

Analytical Method for Spiromesifen (Agricultural Products)

1. Analytes

Spiromesifen

4-Hydroxy-3-mesityl-1-oxaspiro[4.4]nona-3-en-2-one (hereafter referred to as the enol compound)

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 spiromesifen: Contains not less than 98% of spiromesifen. Melting point of the standard is 96–101°C.

Reference standard of the enol compound: Contains not less than 99% of the enol compound. Melting point of the standard is 256–258°C.

4. Procedure

1) Extraction

For fruits and vegetables, weigh 20.0 g of sample. For grains and legumes, weigh 10.0 g of sample, and for tea leaves, weigh 5.00 g of sample, add 20 mL of water, and let stand for 2 hours.

Add 100 mL of acetonitrile/formic acid/water (80:1:20, v/v/v), homogenize, and filter through a filter paper, covered with a glass fiber filter, with suction. Add 50 mL of acetonitrile/formic acid/water (80:1:20, v/v/v) to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetonitrile/formic acid/water (80:1:20, v/v/v) to make exactly 200 mL. Take a 50 mL (20 mL for grains and legumes, and 40 mL for tea leaves) aliquot of the solution, and concentrate to about 10 mL (4 mL for grains and legumes, and 8 mL for tea leaves) at below 40°C. Add water to the concentrated solution to make 50 mL, add 0.1 mL of formic acid, and extract with shaking twice with 50 mL of ethyl acetate/*n*-hexane (1:19, v/v). Dehydrate the extract with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, concentrate the filtrate at below 40°C, and remove the solvent. Dissolve the residue in 10 mL of *n*-hexane.

2) Clean-up

i) Silica gel column chromatography

Add 10 mL of *n*-hexane to a silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, wash the container with 10 mL of ethyl acetate/*n*-hexane (1:19, v/v), add the washing to the cartridge, and discard the effluent. Add 10 mL of ethyl acetate/*n*-hexane (1:19, v/v), and discard the effluent. Elute with 15 mL of

ethyl acetate/*n*-hexane (1:19, v/v) and collect the eluate (I: spiromesifen fraction). Then, elute with 10 mL of formic acid/ethyl acetate/*n*-hexane (0.1:25:75, v/v/v) and collect the eluate (II: the enol compound fraction). Concentrate eluate (I) at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/0.01% formic acid (1:1, v/v) to make exactly 10 mL for fruits and vegetables (2 mL for grains, legumes, and tea leaves), and use this solution as the test solution of spiromesifen.

ii) Graphitized carbon black column chromatography

Add 5 mL of formic acid/ethyl acetate/*n*-hexane (0.1:25:75, v/v/v) to a graphitized carbon black cartridge (500 mg) and discard the effluent. Transfer the eluate (II) to the cartridge. Elute with 5 mL of formic acid/ethyl acetate/*n*-hexane (0.1:25:75, v/v/v) and 10 mL of acetonitrile/formic acid (99:1, v/v) sequentially, combine the eluates, concentrate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/0.01% formic acid (1:1, v/v) to make exactly 10 mL for fruits and vegetables (2 mL for grains, legumes, and tea leaves), and use this solution as the test solution of the enol compound.

5. Calibration curve

Prepare 0.005–0.1 mg/L spiromesifen and 0.0025–0.05 mg/L the enol compound standard solutions (acetonitrile/0.01% formic acid (1:1, v/v)). Inject 2 µL of each standard solution to LC-MS, and make calibration curves by peak-height or peak-area method.

6. Quantification

Inject 2 µL of the test solution to LC-MS, and calculate the concentration of spiromesifen and the enol compound from the calibration curves made in **5**. Use the following equation to calculate the concentration of spiromesifen including that of the enol compound.

Concentration (ppm) of spiromesifen (including that of the enol compound)

$= A + B \times 1.36$

A: Concentration (ppm) of spiromesifen

B: Concentration (ppm) of the enol compound

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

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

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.01% formic acid (3:7, v/v) to (4:1, v/v) in 15 min and from (4:1, v/v) to (9:1, v/v) for 7 min

Ionization mode: Spiromesifen ESI (+), the enol compound ESI (-)

Major monitoring ion (*m/z*): Spiromesifen 273, the enol compound 271

Expected retention time: Spiromesifen 20 min, the enol compound 11 min

9. Limit of quantification

0.01 mg/kg for each analyte (The concentration of the enol compound is calculated as

spiromesifen)

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of spiromesifen and the enol compound with acetonitrile/formic acid/water (80:1:20, v/v/v), transfer of the extract to ethyl acetate/*n*-hexane (1:19, v/v), fractionation of spiromesifen and the enol compound with a silica gel cartridge, clean-up of the enol compound with a graphitized carbon black cartridge, quantification and confirmation using LC-MS. Spiromesifen and the enol compound are quantified individually.

Concentration of the enol compound is converted to concentration of spiromesifen by multiplying the conversion factor, and the sum of the concentration of spiromesifen and the enol compound is regarded as the analytical result of spiromesifen.

2) Notes

i) Extraction is performed under acidic condition with formic acid to prevent the transformation of spiromesifen to the corresponding enol compound.

ii) Filtration of the extract through a glass fiber filter on filter paper prevents clogging and makes the filtration easier.

iii) For samples containing small amounts of chlorophyll, an ethylenediamine triacetate-*N*-propylsilanized silica gel cartridge (1000 mg) can be used for clean-up.

Outline of the procedure is as follows. Add 5 mL each of methanol and water to an ethylenediamine triacetate-*N*-propylsilanized silica gel cartridge (1000 mg) sequentially, and discard the effluents. Transfer the eluate (II) obtained after silica gel cartridge clean-up to the ethylenediamine triacetate-*N*-propylsilanized silica gel cartridge, and discard the effluent. Add 5 mL of formic acid/ethyl acetate/*n*-hexane (0.1:25:75, v/v/v), and discard the effluent. Elute with 10 mL of methanol, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/0.01% formic acid (1:1, v/v) to make exactly 10 mL for fruits and vegetables (2 mL for grains, legumes, and tea leaves), and use this solution as the test solution of the enol compound.

11. References

None

12. Type

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