Analytical Method for Iodosulfuron methyl, Ethametsulfuron-methyl, Ethoxysulfuron, Cinosulfuron, Sulfosulfuron, Triasulfuron, Nicosulfuron, Pyrazosulfuron-ethyl, Primisulfuron-methyl, Prosulfuron and Rimsulfuron (Agricultural Products)

1. Analyte

Compositional substances of agricultural chemicals	Analyte
Iodosulfuron methyl	Iodosulfuron methyl and Iodosulfuron methyl sodium salt
Ethametsulfuron-methyl	Ethametsulfuron-methyl
Ethoxysulfuron	Ethoxysulfuron
Cinosulfuron	Cinosulfuron
Sulfosulfuron	Sulfosulfuron
Triasulfuron	Triasulfuron
Nicosulfuron	Nicosulfuron
Pyrazosulfuron-ethyl	Pyrazosulfuron-ethyl
Primisulfuron-methyl	Primisulfuron-methyl
Prosulfuron	Prosulfuron
Rimsulfuron	Rimsulfuron

2. Instrument

Liquid chromatograph-mass spectrometry (LC-MS)

3. Reagents

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

Acid alumina cartridge (1,710 mg): A polyethylene tube with inside diameter 9–10 mm packed with 1,710 mg of acid alumina, or the other cartridge with equal separation characteristics.

Divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg): A polyethylene tube with inside diameter 12–13 mm packed with 500 mg of divinylbenzene-*N*-vinylpyrrolidone copolymer, or the other cartridge with equal separation characteristics.

Reference standard of iodosulfuron methyl: Contains not less than 92% of iodosulfuron methyl sodium. Melting point of the standard is 152℃.

Reference standard of ethametsulfuron-methyl: Contains not less than 98% of ethametsulfuron-methyl. Melting point of the standard is 194℃.

Reference standard of ethoxysulfuron: Contains not less than 97% of ethoxysulfuron. Melting point of the standard is 144-147℃.

Reference standard of cinosulfuron: Contains not less than 97% of cinosulfuron. Melting point of the standard is 127-135℃.

Reference standard of sulfosulfuron: Contains not less than 98% of sulfosulfuron. Melting point of the standard is 201-202℃.

Reference standard of triasulfuron: Contains not less than 97% of triasulfuron. Melting point of the standard is 178℃.

Reference standard of nicosulfuron: Contains not less than 98% of nicosulfuron. Melting point of the standard is 169-172℃.

Reference standard of pyrazosulfuron-ethyl: Contains not less than 98% of pyrazosulfuron-ethyl. Melting point of the standard is 178-180℃.

Reference standard of primisulfuron-methyl: Contains not less than 99% of primisulfuron-methyl. Melting point of the standard is 195-197℃.

Reference standard of prosulfuron: Contains not less than 98% of prosulfuron. Melting point of the standard is 155℃.

Reference standard of rimsulfuron: Contains not less than 99% of rimsulfuron. Melting point of the standard is 172-173℃.

4. Procedure

1) Extraction

For grains, legumes, nuts and seeds, weigh 10.0 g of sample, add 30 mL of water, and let stand for 2 hours. For fruits, vegetables and herbs, weigh 20.0 g of sample.

Add 100 mL of acetone, shake for 60 minutes (30 minutes for fruits, vegetables and herbs), and filter under reduced pressure. Add 50 mL of acetone to the residue on the filter paper, wash the residue, and filter as described above. Combine the resulting filtrates, and concentrate to approximately 30 mL at 40℃ or below.

Add 100 mL of 10% sodium chloride solution and 10 mL of 1 mol/L hydrochloric acid, and extract with shaking twice with 50 mL each of ethyl acetate. Combine the resulting extracts, add 100 mL of *n*-hexane, and extract with shaking twice with 50 mL each of 2% dipotassium hydrogen phosphate. Add 10 g of sodium chloride and 3 mL of 6 mol/L hydrochloric acid to the extract, and extract with shaking twice with 50 mL each of ethyl acetate. Combine the extracts, dehydrate the extracts with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at 40℃ or below and remove the solvent. Dissolve the residue in acetone to make exactly 10 mL (20 mL for fruits, vegetables and herbs).

2) Clean-up

i) Acid alumina cartridge chromatography

Add sequentially 10 mL each of acetonitrile and acetone to an acid alumina cartridge (1,710 mg), and discard the effluents. Transfer 2 mL of the solution obtained in 1) to the cartridge, and discard the effluent. Transfer sequentially 8 mL of acetone and 20 mL of acetonitrile to the cartridge, and discard the effluent. Elute with 10 mL of acetonitrile/water (9:1, v/v). Concentrate the eluate at 40℃ or below, and remove the solvent. Dissolve the residue in 10 mL of water.

ii) Divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge chromatography

Add sequentially 10mL each of methanol and water to a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg), and discard the effluent. Transfer the solution obtained in i) to the cartridge, and discard the effluent. Transfer 10 mL of water/methanol (4:1, v/v) to the cartridge, and discard the effluent. Elute with 15 mL of methanol, concentrate the eluate at 40°C or below, and remove the solvent. Dissolve the residue in acetonitrile/water (1:1, v/v) to make exactly 1 mL, and use this solution as the test solution.

5. Calibration curve

Prepare standard solutions of several concentrations (0.02-0.4 mg/L) in each of iodosulfuron methyl, ethametsulfuron-methyl, ethoxysulfuron, cinosulfuron, sulfosulfuron, triasulfuron, nicosulfuron, pyrazosulfuron-ethyl, primisulfuron-methyl, prosulfuron or rimsulfuron standard solutions (acetonitrile/water (1:1, v/v)). Inject 4 μL of each standard solution to LC-MS, and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 4μL of the test solution to LC-MS, and calculate the concentrations of iodosulfuron methyl, ethametsulfuron-methyl, ethoxysulfuron, cinosulfuron, sulfosulfuron, triasulfuron, nicosulfuron, pyrazosulfuron-ethyl, primisulfuron-methyl, prosulfuron and rimsulfuron using the calibration curves made in **5**.

For iodosulfuron methyl, concentration of iodosulfuron methyl sodium salt is converted to iodosulfuron methyl by multiplying the conversion factor 0.96.

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

LC-MS

1) For ethametsulfuron-methyl, ethoxysulfuron, cinosulfuron, sulfosulfuron, triasulfuron, nicosulfuron, pyrazosulfuron-ethyl, primisulfuron-methyl and rimsulfuron

   Column: Octadecylsilanized silica gel, inside diameter 2-3 mm, 150 mm in length and 5 μL in particle diameter

   Colum temperature: 40°C

   Mobile phase: Linear gradient from 1% formic acid to acetonitrile/1% formic acid (19:1, v/v) in 20 min

   Ionization mode: ESI (+)

   Major monitoring ions (*m/z*): Ethametsulfuron-methyl and Nicosulfuron; 411, Ethoxysulfuron; 399, Cinosulfuron; 414, Sulfosulfuron; 471, Triasulfuron; 402, Pyrazosulfuron-ethyl; 415, Primisulfuron-methyl; 469, Rimsulfuron; 432

   Expected retention time: Primisulfuron-methyl 20 min

2) For iodosulfuron methyl and prosulfuron

Column: Octadecylsilanized silica gel, inside diameter 2-3 mm, 150 mm in length and 5 μL in particle diameter

Colum temperature: 40℃

Mobile phase: Linear gradient from 1% formic acid to acetonitrile/1% formic acid (19:1, v/v) in 20 min

Ionization mode: ESI (-)

Major monitoring ions (m/z): Iodosulfuron-methyl; 506, Prosulfuron; 418

Expected retention time: Prosulfuron 19.5 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of the extraction of iodosulfuron methyl, ethametsulfuron-methyl, ethoxysulfuron, cinosulfuron, sulfosulfuron, triasulfuron, nicosulfuron, pyrazosulfuron-ethyl, primisulfuron-methyl, prosulfuron and rimsulfuron from samples with acetone, and the transferring of the extract into ethyl acetate under acidic condition, the back extraction with dipotassium hydrogen phosphate, the transferring into ethyl acetate under acidic condition, the clean-up with an acid alumina cartridge and a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and the quantification and confirmation using LC-MS.

2) Notes

i) For primisulfuron-methyl, take care in the inverse extraction with dipotassium hydrogen phosphate, because the extraction rate is slightly lower than other substances.

ii) Retention time of each agricultural chemical is 16-21 min under the measurement conditions.

iii) Some samples may have interference peaks at the same retention time. In such a case, use a HPLC column with length 250 mm.

iv)

11. References

MHLW Notification No. 94, Analytical method for azimsulfuron, halosulfuron methyl and flazasulfuron (March 13, 2002)

MOE Notification No. 13, Analytical method for ethoxysulfuron (April 24, 1998)

MOE Notification No. 35, Analytical method for cinosulfuron (April 10, 1990)

MOE Notification No. 88, Analytical method for pyrazosulfuron-ethyl (November 16, 1989)

12. Type

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