Original: Japanese

Provisional translation

Analytical Method for Glufosinate (Agricultural Products)

1. Analytes

Glufosinate (D and L-isomers) (including metabolite Z [*N*-acetyl-glufosinate])

Metabolite B [3-methylphosphinicopropionic acid]

2. Application

Agricultural Products

3. Instruments

For grains, legumes, nuts, seeds and sugar beets, use a gas chromatograph-flame photometric detector (with interference filter for phosphorus) (GC-FPD (P)) and a gas chromatograph-mass spectrometer (GC-MS).

For fruits, vegetables (except sugar beets) and tea leaves, use a gas chromatograph-flame thermionic detector (GC-FTD), GC-FPD (P) or a gas chromatograph-nitrogen phosphorus detector (GC-NPD) and GC-MS.

4. Reagents

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

Silica gel for column chromatography: Heat silica gel manufactured for column chromatography (63–200 μm in particle size) at 130°C for at least 12 hrs, and allow to cool in a desiccator. Add 5% water to this.

Reference standard of glufosinate-ammonium: Contains not less than 95% of glufosinate-ammonium.

Reference standard of metabolite B: Contains not less than 95% of metabolite B.

5. Procedure

1) Grains, legumes, nuts, seeds and sugar beets

i) Extraction

For grains, legumes, nuts and seeds, grind the sample to pass through a standard sieve (420 μm) and weigh 10.0 g of the sample. Add 70 mL of water to the sample, homogenize for 3 min, and add water to make exactly 150 mL. Then, shake well, let stand, and transfer 20 mL of the aqueous layer to a 100 mL separating funnel.

For sugar beets, weigh approximately 1 kg of the sample accurately, weigh and add an appropriate amount of water as needed, homogenize, and weigh an amount

equivalent to 20.0 g of the sample. Add 70 mL of water to the sample, homogenize for 3 min, and add water to make exactly 150 mL. Then, shake well, let stand, and transfer 20 mL of the aqueous layer to a 100 mL separating funnel.

Add 30 mL of ether to the separating funnel, shake gently for 1 min, let stand, and discard the ether layer. Add 30 mL of ether to the aqueous layer, shake well for 1 min, let stand, and take a 15 mL aliquot of the aqueous layer. Add 1 mL of saturated lead acetate solution to the aqueous layer, let stand for 5 min with occasional shaking, centrifuge at 3,000 rpm for about 5 min, and collect the supernatant.

ii) Clean-up

Connect a benzenesulfonylpropylsilanized silica gel cartridge (500 mg) to the lower part of a styrene-divinylbenzene copolymer cartridge (265 mg) followed by connecting a trimethylaminopropylsilanized silica gel cartridge (1,000 mg) to the bottom of it. Add 10 mL of acetonitrile to the cartridge and discard the effluent. Then, add 10 mL of water and discard the effluent. Inject the supernatant obtained in i) Extraction into the cartridge, add 10 mL of water, and discard the effluent. Then, detach and discard the styrene-divinylbenzene copolymer cartridge (265 mg) and the benzenesulfonylpropylsilanized silica gel cartridge (500 mg). Inject 10 mL of acetic acid and water (1:1) mixed solution into the trimethylaminopropylsilanized silica gel cartridge (1,000 mg), collect the eluate in a vacuum rotary evaporator flask, and remove acetic acid and water at below 50°C.

iii) Derivatization

Dissolve the residue obtained in ii) Clean-up in 0.2 mL of acetic acid and 0.8 mL of trimethyl orthoacetate, seal tightly, heat at 100°C for 2 hrs, allow to cool, and dry under a nitrogen stream. Dissolve the residue in acetone to make exactly 0.5 mL, and use this solution as the test solution.

2) Fruits, vegetables (except sugar beets) and tea leaves

i) Glufosinate test solution

a) Extraction

For fruits and vegetables (except sugar beets), weigh approximately 1 kg of the sample accurately, weigh and add an appropriate amount of water as needed, homogenize, and weigh an amount equivalent to 20.0 g of the sample. Add 50 mL of dichloromethane and 150 mL of water to the sample, shake vigorously for 30 min with a shaker, centrifuge at 3,000 rpm for about 5 min, and transfer the supernatant to a 300 mL conical flask. Add 50 mL of water to the precipitate, shake well, centrifuge under the same conditions as above and combine the

supernatant in the conical flask above. Filter the supernatant and add water to the filtrate to make 500 mL.

For powdered tea, weigh 5.00 g of the sample. Add 100 mL of water to the sample, shake vigorously for 30 min with a shaker, centrifuge at 3,000 rpm for about 5 min, and transfer the supernatant to a 300 mL conical flask. Add 50 mL of water to the precipitate, shake well, centrifuge under the same conditions as above, and combine the supernatant in the conical flask above. Add 4 mL of saturated lead acetate solution to the supernatant, let stand for 5 min with occasional shaking, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a 1,000 mL recovery flask. Next, wash the conical flask with 50 mL of water, and use the washing to wash the residue on the filter paper. Combine the washing in the recovery flask above. Transfer the solution to a 1,000 mL separating funnel, add 100 mL of dichloromethane, shake gently for 1 min, let stand and discard the dichloromethane layer. Add 100 mL of dichloromethane to the aqueous layer, treat as described above, and discard the dichloromethane layer. Add 100 mL of ethyl acetate to the aqueous layer, shake gently for 1 min, let stand, collect the aqueous layer, and add water to make 500 mL.

For tea leaves other than powdered tea, immerse 10.0 g of the sample in 600 mL of water at 100ºC, let stand for 5 min at room temperature, and filter. After cooling, transfer 300 mL of the filtrate to a 500 mL conical flask. Add 4 mL of saturated lead acetate solution to the conical flask, let stand for 5 min with occasional shaking, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a 1,000 mL recovery flask. Then, wash the conical flask with 50 mL of water, and use the washing to wash the residue on the filter paper. Combine the washing in the recovery flask above. Transfer the solution to a 1,000 mL separating funnel, add 100 mL of dichloromethane, shake gently for 1 min, let stand, and discard the dichloromethane layer. Add 100 mL of dichloromethane to the aqueous layer, treat as described above, and discard the dichloromethane layer. Add 100 mL of ethyl acetate, shake gently for 1 min, let stand, collect the aqueous layer, and add water to make 500 mL.

b) Derivatization

Place 20 mL of strongly basic anion-exchange resins (149–297 μm in particle size) suspended in water in a chromatographic tube of 15 mm inside diameter and 300 mm in length and let water flow out until a small amount of water

remains at the top of the column. Inject 40 mL of 1 mol/L sodium hydroxide solution into the column and add water until the pH of the effluent reaches 8–9. Discard the effluent. Then, inject 40 mL of acetic acid and water (1:9) mixed solution, and add water until the pH of the effluent reaches 5. Discard the effluent. Then, inject 80 mL of acetic acid and water (1:1) mixed solution, and add another 100 mL of water. Discard the effluent. Inject the solution obtained in a) Extraction into the column and discard the effluent. Next, inject 200 mL of acetic acid and water (1:9) mixed solution, discard 50 mL of the first effluent, take the next 150 mL of the eluate in a vacuum rotary evaporator flask, and remove acetic acid and water at below 50°C. Add 1 mL of acetic acid to the residue, followed by 4 mL of trimethyl orthoacetate, and heat at 100°C for 2 hrs. After cooling, transfer the solution to the vacuum rotary evaporator flask, concentrate to approximately 1 mL at below 40°C, and dry under a nitrogen stream at room temperature. Dissolve the residue in 10 mL of acetone.

c) Clean-up

Place 3 g of silica gel for column chromatography suspended in acetone in a chromatographic tube of 10 mm in inside diameter and 300 mm in length and let acetone flow out until a small amount of acetone remains at the top of the column. Inject the solution obtained in b) Derivatization into the column, add 70 mL of acetone and discard the effluent. Then, add 80 mL of acetone and water (19:1) mixed solution, collect the eluate in a vacuum rotary evaporator flask, and remove acetone and water at below 40°C. Dissolve the residue in ethyl acetate to make exactly 2 mL, and use this solution as the test solution for glufosinate.

ii) Metabolite B test solution

a) Extraction

"i) Glufosinate test solution, a) Extraction" shall apply mutatis mutandis.

b) Methylation

Place 20 mL of strongly basic anion-exchange resins (149–297 μm in particle size) suspended in water in a chromatographic tube of 15 mm in inside diameter and 300 mm in length and let water flow out until a small amount of water remains at the top of the column. Inject 40 mL of 1 mol/L sodium hydroxide solution into the column and add water until the pH of the effluent reaches 8–9. Discard the effluent. Then, inject 40 mL of acetic acid and water (1:9) mixed solution, and add water until the pH of the effluent reaches 5. Discard the effluent. Next, inject 80 mL of acetic acid and water (1:1) mixed solution,

followed by 100 mL of water. Discard the effluent. Inject the solution obtained in a) Extraction into the column and discard the effluent. Then, inject 200 mL of acetic acid and water (1:9) mixed solution, add another 50 mL of acetic acid and water (3:7) mixed solution, and discard the effluent. Next, inject 150 ml of acetic acid and water (1:1) mixed solution, collect the eluate in a vacuum rotary evaporator flask, and remove the acetic acid and water at below 50°C. Add 1 mL of acetic acid to the residue, followed by 4 mL of trimethyl orthoacetate, and heat at 100°C for 2 hrs. After cooling, transfer the solution to the vacuum rotary evaporator flask, concentrate to approximately 1 mL at below 40°C, and dry under a nitrogen stream at room temperature. Dissolve the residue in 10 mL of acetone.

c) Clean-up

Place 3 g of silica gel for column chromatography suspended in acetone in a chromatographic tube of 10 mm in inside diameter and 300 mm in length, and let acetone flow out until a small amount of acetone remains at the top of the column. After injecting the solution obtained in b) Methylation into the column, add 100 mL of acetone, discard the first 20 mL of eluate, take the next 80 mL of eluate into a vacuum rotary evaporator flask, and remove acetone at below 40°C. Dissolve the residue in ethyl acetate to make exactly 4 mL, and use this solution as the test solution for metabolite B.

6. Calibration curve

1) Grains, legumes, nuts, seeds and sugar beets

For each reference standard of glufosinate-ammonium and metabolite B, the standard solutions for the calibration curve are prepared by the same procedure as in "**5. Procedure**, 1) Grains, legumes, nuts, seeds and sugar beets, ii) Clean-up and iii) Derivatization". Inject each standard solution into GC-FPD or GC-MS, and make calibration curves by peak-height or peak-area method.

2) Fruits, vegetables (except sugar beets) and tea leaves

The standard solutions for the calibration curve are prepared by the same procedure as in "**5. Procedure**, 2) Fruits, vegetables (except sugar beets) and tea leaves, i) Glufosinate test solution, b) Derivatization and c) Clean-up" for the reference standard of glufosinate-ammonium, and by the same procedure as in "**5. Procedure**, 2) Fruits, vegetables (except sugar beets) and tea leaves, ii) Metabolite B test solution, b) Methylation and c) Clean-up" for the reference standard of metabolite B. Inject each standard solution into GC-FTD, GC-FPD, GC-NPD or GC-MS, and make calibration curves by peak-height or peak-area method.

7. Quantification

Inject the test solution into GC-FPD or GC-MS for grains, legumes, nuts, seeds and sugar beets, and inject the test solution into GC-FTD, GC-FPD, GC-NPD or GC-MS for fruits, vegetables (except sugar beets) and tea leaves, followed by calculating the concentration of glufosinate-ammonium (including metabolite Z) and metabolite B from the calibration curves prepared in **6**. Use the following equation to calculate the concentration of glufosinate (D and L-isomers) including metabolite Z and metabolite B.

Concentration of glufosinate (D and L-isomers) (including metabolite Z and metabolite B) (ppm) = A × 0.9141 + B × 1.191

A: Concentration of glufosinate-ammonium (including metabolite Z) (ppm).

B: Concentration of metabolite B (ppm).

8. Confirmation

Confirm using GC-MS.

9. Measurement conditions

(Example)

Column: Silicate glass capillary 0.25 mm in inside diameter, 30 m in length coated with 50% phenyl-methyl silicone for gas chromatography 0.25 μm in film thickness.

Column temperature: 100°C (1 min) → 10°C/min heating → 260°C (3 min)

Inlet temperature: 250°C

Injection method: splitless

Detector temperature: 270°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute 2-acetylamino-4-[methoxy (methyl)phosphinoyl]propionate in about 14 min. Optimize the flow rate of air and hydrogen.

10. Limit of quantification

0.01 mg/kg (0.05 mg/kg for grains, legumes, nuts, seeds and sugar beets)

11. Explanatory note

1) Outline of analytical method

For grains, legumes, nuts, seeds and sugar beets, the method consists of extracting glufosinate (D and L-isomers) (including metabolite Z) and metabolite B from sample with water, washing with ether, cleaning up with a saturated lead acetate solution, then cleaning up using a styrene-divinylbenzene copolymer cartridge, a benzenesulfonylpropylsilanized silica gel cartridge and a trimethylaminopropylsilanized silica gel cartridge, derivatizing using trimethyl orthoacetate, quantifying using GC-FPD or GC-MS, and confirming using GC-MS.

For fruits and vegetables (except sugar beets), the method consists of extracting glufosinate (D and L-isomers) (including metabolite Z) and metabolite B from sample with water in

the presence of dichloromethane, for powdered tea, extracting from the sample with water, for tea leaves, leaching from the sample in water at 100ºC, cleaning up using saturated lead acetate solution, washing with dichloromethane, then ethyl acetate, followed by cleaning up with strongly basic anion-exchange resin, derivatizing using trimethyl orthoacetate, cleaning up with a silica gel column, quantifying using GC-FTD, GC-FPD, GC-NPD or GC-MS, and confirming using GC-MS.

2) Notes

i) Analytical value

Glufosinate-ammonium (including metabolite Z) and metabolite B are quantified respectively and each concentration of glufosinate-ammonium (including metabolite Z) and metabolite B is converted to the concentration of glufosinate (D and L-isomers) (including metabolite Z and metabolite B) by multiplying each conversion factor, respectively, and the sum of these values is the analytical value of glufosinate. Glufosinate includes glufosinate-ammonium salt.

ii) For the benzenesulfonylpropylsilanized silica gel cartridge (500 mg) used for testing grains, beans, nuts, seeds, and sugar beets, it is recommended to use the one with a particle size of 120 μm. In addition, a column with an inner diameter of 0.53 mm, a length of 30 m, and a film thickness of 1.0 μm may also be used when a flame photometric detector is used for gas chromatograph detection.

12. Reference

None

13. Type

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