Original: Japanese
Provisional translation

Analytical Method for Diquat, Paraquat and Mepiquat chloride (Agricultural Products)

1. Analytes

Diquat

Paraquat

Mepiquat chloride

2. Application

Agricultural Products

3. Instruments

High performance liquid chromatograph-fluorometric detector (HPLC-FL)

Liquid chromatograph-mass spectrometer (LC-MS)

4. Reagents

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

Dipicrylamine sodium salt: Use a reagent with a purity of 98.0% or higher (after drying).

Propylsulphonylsilanized silica gel cartridge (500 mg): A polyethylene tube of 12–13 mm in inside diameter packed with 500 mg of propylsulphonylsilanized silica gel, or other cartridges with equal separation characteristics.

Heptafluoro-n-butyric acid: Use a reagent with a purity of 99.0% or higher.

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

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

Reference standard of mepiquat chloride: Contains not less than 95% of mepiquat chloride.

5. Procedure

1) Extraction

i) Grains, legumes, nuts, seeds, fruits, vegetables, herbs, powdered tea and hops

For grains, legumes, nuts and seeds, weigh 10.0 g of the sample. For powdered tea and hops, weigh 5.00 g of the sample. For fruits, vegetables and herbs, weigh 20.0 g of the sample. Add 60 mL of water and 30 mL of sulfuric acid to the sample and mix. Add 2–3 grains of boiling stones and approximately 1 mL of antifoaming agent, attach a reflux condenser and heat under reflux for 5 hrs. Allow to cool, filter with suction using a glass fiber filter, add 50 mL of water to the residue on the filter paper and filter as described above. Combine the resulting filtrates and add water to make exactly 200 mL. Take 20 mL of the solution accurately (equivalent to 1 g of the

sample, 40 mL for powdered tea and hops, and 10 mL for fruits, vegetables and herbs), and add 12 mol/L sodium hydroxide solution to adjust the pH to 7.

ii) Tea leaves except powdered tea

Immerse 9.00 g of the sample in 540 mL of water at 100ºC, let stand for 5 min at room temperature, and filter. After cooling, take 60 mL of the filtrate, add 30 mL of sulfuric acid, and mix. Add 2–3 grains of boiling stones and approximately 1 mL of antifoaming agent, attach a reflux condenser and heat under reflux for 5 hrs. Allow to cool, filter with suction using a glass fiber filter, add 50 mL of water to the residue on the filter paper and filter as described above. Combine the resulting filtrates, and add water to make exactly 200 mL. Take 20 mL of the solution accurately (equivalent to 0.1 g of the sample), and add 12 mol/L sodium hydroxide solution to adjust the pH to 7.

2) Clean-up

Inject 10 mL each of methanol and water into a propylsulphonylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. After transferring the solution obtained in 1) to the cartridge, discard the effluent. Additionally, add 10 mL each of water and methanol sequentially, and discard each effluent. Then, add 20 mL of saturated ammonium chloride-methanol solution, concentrate the eluate at below 40°C, and flow nitrogen gas to dry (I, mepiquat chloride fraction). Furthermore, inject 20 mL of 5 mol/L ammonium chloride solution into the cartridge, and add 5 mol/L ammonium chloride solution to the eluate to make exactly 20 mL (II, diquat and paraquat fractions).

3) Liquid-liquid extraction with dichloromethane and washing with dichloromethane (mepiquat chloride fraction)

Dissolve the residue obtained in (I) in 20 mL of 0.05% dipicrylamine sodium salt solution, and add 1 mL of 12 mol/L sodium hydroxide solution to adjust the pH to 13. Extract the solution with shaking three times with 30 mL each of dichloromethane. Combine the dichloromethane layers, filter through a cotton plug, concentrate at below 40°C, and flow nitrogen gas to dry. Dissolve the residue in 50 mL of 2 mol/L hydrochloric acid. Wash the solution twice with 10 mL each of dichloromethane, concentrate at below 60°C, and flow nitrogen gas to dry. Dissolve the residue in 5 mmol/L heptafluoro-n-butyric acid solution to make exactly 2 mL, and use this solution as the test solution for mepiquat chloride.

4) Fluorescence derivatization (oxidation) (diquat and paraquat fractions)

i) Diquat

Take 5 mL of the solution obtained in (II) accurately, add 25 mL of 12 mol/L sodium hydroxide solution and 1 mL of 1% potassium ferricyanide solution, shake to mix

uniformly, and extract with shaking twice with 20 mL each of ether. Combine the ether layers, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, concentrate the filtrate at below 40°C, and flow nitrogen gas to dry. Dissolve the residue in a mixed solution of acetonitrile and water (1:9) to make exactly 2 mL, and use this solution as the test solution for diquat.

ii) Paraquat

Take 10 mL of the solution obtained in (II) accurately, add 10 mL of 12 mol/L sodium hydroxide solution and 1 mL of 1% potassium ferricyanide solution, shake to mix uniformly, and extract with shaking twice with 20 mL each of dichloromethane. Combine the dichloromethane layers, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, concentrate the filtrate under reduced pressure at below 40°C, and flow nitrogen gas to dry. Dissolve the residue in a mixed solution of acetonitrile and water (1:9) to make exactly 2 mL, and use this solution as the test solution for paraquat.

6. Calibration curve

1) Diquat

Prepare a 4 mg/L diquat standard solution with 0.01 mol/L hydrochloric acid add 5 mL of 5 mol/L ammonium chloride solution to 0.5 mL of the solution, and then follow the procedure described in "**5**, 4) Fluorescence derivatization (oxidation), i)". Dissolve the concentrated residue in a mixed solution of acetonitrile and water (1:9) to make exactly 20 mL (0.1 mg/L). Dilute the solution appropriately to prepare 0.00125–0.05 mg/L solutions. Inject 40 μL of each solution into HPLC-FL, and make a calibration curve by peak-height or peak-area method.

2) Paraquat

Prepare a 4 mg/L paraquat standard solution with 0.01 mol/L hydrochloric acid, add 10 mL of 5 mol/L ammonium chloride solution to 0.5 mL of the solution, and then follow the procedure described in "**5**, 4) Fluorescence derivatization (oxidation), ii)". Dissolve the concentrated residue in a mixed solution of acetonitrile and water (1:9) to make exactly 20 mL (0.1 mg/L). Dilute the solution appropriately to prepare 0.0025–0.1 mg/L solutions. Inject 20 μL of each solution into HPLC-FL and make a calibration curve by peak-height or peak-area method.

3) Mepiquat chloride

Prepare 0.005–0.2 mg/L mepiquat chloride standard solutions with 5 mmol/L heptafluoro-n-butyric acid of several concentrations. Inject 10 μL of each solution into LC-MS and make a calibration curve by peak-height or peak-area method.

7. Quantification

1) Diquat

Inject 40 μL of the test solution into HPLC-FL, and calculate the concentration of diquat using the calibration curves prepared in **6**. 1). Use the following equation to calculate the concentration of diquat (diquat ion equivalent).

Concentration (ppm) of diquat ion = A × 0.5355

A: Concentration (ppm) of diquat

2) Paraquat

Inject 20 μL of the test solution into HPLC, and calculate the concentrations of paraquat using the calibration curves prepared in **6**. 2). Use the following equation to calculate the concentration of paraquat (paraquat ion equivalent).

Concentration (ppm) of paraquat ion = A × 0.7243

A: Concentration (ppm) of paraquat

3) Mepiquat chloride

Inject 10 μL of the test solution into LC-MS, and calculate the concentrations of mepiquat chloride using the calibration curves prepared in **6**. 3).

8. Confirmation

Confirm using LC-MS.

9. Measurement conditions

(Example)

1) HPLC-FL (diquat and paraquat)

Column: Triacontylsilanized silica gel, 4.6 mm inside diameter, 250 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: A mixed solution of acetonitrile and water (2:23)

Wavelength

Diquat: excitation wavelength: 368 nm, fluorescence wavelength: 430 nm

Paraquat: excitation wavelength: 330 nm, fluorescence wavelength: 436 nm

Expected retention time

Diquat: 18.5 min

Paraquat: 20 min

2) LC-MS (Mepiquat chloride)

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

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of 5 mmol/L heptafluoro-n-butyric acid and methanol solution (19:1) to (1:1) in 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): 114

Expected retention time: 9 min

3) LC-MS (diquat and paraquat)

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

Column temperature: 40°C

Mobile phase: A mixed solution of acetonitrile and 0.1% acetic acid (1:9)

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Diquat: 215

Paraquat: 217

Injection volume

Diquat: 10 μL

Paraquat: 5 μL

Expected retention time

Diquat: 5.7 min

Paraquat: 5.4–5.6 min

10. Limit of quantification

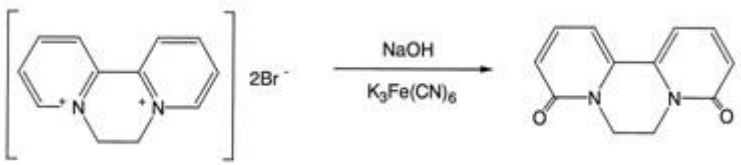
0.01 mg/kg for each analyte (0.1 mg/kg for tea except powdered tea)

11. Explanatory note

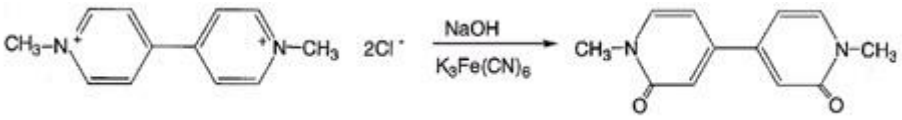
1) Outline of analytical method

The method consists of extracting diquat, paraquat and mepiquat chloride from sample by heating under reflux with 6 mol/L sulfuric acid, cleaning up using a propylsulphonylsilanized silica gel cartridge to separate into mepiquat chloride fraction (I) and diquat and paraquat fraction (II), for mepiquat chloride fraction (I), liquid-liquid extraction with dichloromethane, washing with dichloromethane under acidity using hydrochloric acid, quantifying and confirming using LC-MS, for diquat and paraquat fraction (II), fluorescence derivatization (oxidation), quantifying using HPLC-FL, and confirming using LC-MS.

Fluorescence derivatization of diquat



Fluorescence derivatization of paraquat



2) Notes

i) In this analytical method, diquat refers to diquat dibromide [$C_{12}H_{12}Br_2N_2$] and paraquat refers to paraquat dichloride [$C_{12}H_{14}Cl_2N_2$].

ii) For 0.05% dipicrylamine sodium salt solution, the rate of liquid-liquid extraction for mepiquat chloride decreases as time passes after preparation of the solution.

iii) The sensitivity of fluorescent derivatives of diquat and paraquat in LC-MS is 1/4 that of HPLC-FL method. If the limit of quantification exceeds the standard value, lower it by increasing the amount of solution used for fluorescence derivatization.

12. References

1) MOE Notification No. 20, Analytical method for diquat dibromide (April 14, 1986).

2) MOE Notification No. 40, Analytical method for paraquat dichloride (June 11, 1976).

3) MOE Notification No. 21, Analytical method for mepiquat chloride (April 1, 1991).

4) Association of Feed Analysis Methods. *Methods of Analysis in Feeds and Feed Additives* p.6-148–6-152. Japan Scientific Feeds Association.

5) Kouichi Akaki. (2004). *Food Hygiene and Safety Science*, **45**, 197–200.

6) Ohkura *et al*. (1990). *Annual Report of Ehime Prefectural Institute of Public Health,* **51**, 27–32.

13. Type

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