Original: Japanese
Provisional Translation

Analytical Method for Iminoctadine (Agricultural Products)

1. Analytes

Iminoctadine
Iminoctadine triacetate
Iminoctadine albesilate

2. Instrument

High performance liquid chromatograph-fluorometric detector with post-column reactor (HPLC-FL (post-column))

3. Reagents

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

0.1 mol/L Hydrochloric acid-methanol solution: Add methanol to 10 mL of hydrochloric acid to make 1,000 mL.

Triethylamine solution: Add water to 40.0 g of sodium hydroxide and 0.75 mL of triethylamine to make 1,000 mL.

Fluorescence reagent: Add water to 3 g of ninhydrin to make 1,000 mL.

Phosphate buffer (pH 6)

Solution 1: Dissolve 2.713 g of potassium dihydrogen phosphate in water to make 1,000 mL.

Solution 2: 0.1 mol/L sodium hydroxide solution

Mix 400 mL of solution 1 and 7 mL of solution 2, and adjust pH to 6 with both solutions.

Reference standard of iminoctadine triacetate: Contains not less than 98% of iminoctadine triacetate

4. Procedure

1) Extraction

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

For grains, legumes, nuts and seeds, weigh 20.0 g of sample, add 5 g of guanidine hydrochloride, 20 mL of triethylamine solution and 20 mL of water, and let stand for 30 minutes.

For fruits and vegetables, weigh about 100 g of sample accurately, add 25 g of guanidine hydrochloride and 100 mL of triethylamine solution, homogenize, and then take the sample equivalent to 20.0 g.

For powered tea, weigh 2.00 g of sample, add 5 g of guanidine hydrochloride, 20 mL of triethylamine solution and 20 mL of water, and let stand for 30 minutes.

Add 5 g of sodium chloride and 100 mL of *n*-butanol/*n*-hexane (1:1, v/v), homogenize, and centrifuge at 3,000 rpm for 10 minutes. Transfer the supernatant to a separating funnel containing 50 mL of triethylamine solution. Add 50 mL of *n*-butanol/*n*-hexane (1:1, v/v) to the residue, homogenize, and centrifuge as described above. Collect the supernatants, shake

gently, let stand, and discard the aqueous layer.

Add 30 mL of water and 2 mL of 1 mol/L sulfuric acid, shake, let stand, and collect the aqueous layer. Add 20 mL of water and 0.5 mL of 1 mol/L sulfuric acid to the remaining *n*-butanol/*n*-hexane (1:1, v/v) layer and treat as described above. Combine the aqueous layers and concentrate to about 2 mL at below 50°C. Add 5 mL of phosphate buffer (pH 6) and adjust the pH to 6 using 0.1 mol/L sodium hydroxide solution.

ii) Tea leaves, except powdered tea

Immerse 9.00 g of sample in 540 mL of water at 100°C, let stand for 5 minutes at room temperature, filter, cool, and transfer 120 mL of the filtrate to a separating funnel containing 100 mL of triethylamine solution. Add 20 g of sodium chloride, 1 g of guanidine hydrochloride and 100 mL of *n*-butanol/*n*-hexane (1:1, v/v), shake, let stand, and transfer the *n*-butanol/*n*-hexane (1:1, v/v) layer to a separating funnel containing 50 mL of triethylamine solution. Add 50 mL of *n*-butanol/*n*-hexane (1:1, v/v) to the aqueous layer and treat as described above. Combine the *n*-butanol/*n*-hexane (1:1, v/v) layers, shake gently, let stand, and discard the aqueous layer.

Add 30 mL of water and 2 mL of 1 mol/L sulfuric acid, shake, let stand, and collect the aqueous layer. Add 20 mL of water and 0.5 mL of 1 mol/L sulfuric acid to the remaining *n*-butanol/*n*-hexane (1:1, v/v) layer and treat as described above. Combine the aqueous layers and concentrate to about 2 mL at below 50°C. Add 5 mL of phosphate buffer (pH 6) and adjust the pH to 6 using 0.1 mol/L sodium hydroxide solution.

2) Clean-up

Add 10 mL each of methanol and water to a carboxyethylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Transfer the solution obtained in 1) to the cartridge, and discard the effluent. Add 5 mL of phosphate buffer (pH 6) and discard the effluent. Elute with 10 mL of 0.1 mol/L hydrochloric acid-methanol solution, concentrate the eluate at below 40°C, and remove hydrochloric acid and methanol. Dissolve the residue in the mobile phase described in **8** to make exactly 5 mL, and use this solution as the test solution.

5. Calibration curve

Prepare iminoctadine triacetate standard solutions (mobile phase described in **8**) of several concentrations. Inject each standard solution to HPLC-FL with post-column reactor, and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample of grains, legumes, nuts, seeds, fruits, and vegetables containing 0.02 mg/kg of iminoctadine gives the test solution of 0.08 mg/L in concentration, and the sample of tea leaves containing 0.2 mg/kg of iminoctadine gives the test solution of 0.08 mg/L in concentration.

6. Quantification

Inject the test solution to HPLC-FL (post-column), and calculate the concentration of iminoctadine triacetate from the calibration curve made in **5**. Use the following equation to calculate the concentration of iminoctadine.

Concentration of iminoctadine (ppm)

= concentration of iminoctadine triacetate (ppm) × 0.6637

7. Confirmation

Confirm using HPLC-FL (post-column).

8. Measurement conditions

Example

Detector: FL (Excitation wavelength 395 nm, emission wavelength 500 nm)

Column: Octadecylsilanized silica gel, 4.6 mm in inside diameter, 150 mm in length, and 5 μm in particle diameter

Column temperature: 50°C

Mobile phase: Dissolve 14.1 g of sodium perchlorate, 400 mg of sodium hydroxide and 1.8 mL of lactic acid in water to make 1,000 mL. Add 250 mL of acetonitrile to 850 mL of this solution.

Fluorescence reactor: Flow 0.5 mol/L sodium hydroxide solution and fluorescence reagent into mobile phase. Maintain flow rate constant.

Fluorescence reactor temperature: 60°C

Injection volume: 20 μL

Expected retention time: 11 min

9. Limit of quantification

0.02 mg/kg (0.2 mg/kg for tea leaves)

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of iminoctadine from sample with *n*-butanol/*n*-hexane (1:1, v/v) under basic condition using triethylamine, clean-up with a carboxyethylsilanized silica gel cartridge, quantification and confirmation using HPLC-FL with post-column reactor.

2) Notes

i) Quantify iminoctadine triacetate, and convert the concentration of iminoctadine triacetate to the concentration of iminoctadine by multiplying by the conversion factor. The analytical result for iminoctadine includes iminoctadine, iminoctadine triacetate and iminoctadine albesilate.

ii) Because iminoctadine adsorbs onto glass under basic conditions, add triethylamine solution to glassware before use to cover the glassware's silanol groups during the extraction and washing steps. During the homogenization procedure, add guanidine hydrochloride to prevent the adsorption of iminoctadine onto the components of plants.

iii) Homogenize for more than 3 minutes because the viscosity of *n*-butanol/*n*-hexane (1:1, v/v) is relatively high. Fruits are especially difficult to mix; treat them with precaution.

iv) The fluorescence intensity varies depending on the flow rate of the fluorescence reagent. Thus, optimize and maintain the flow rate.

11. References

None

12. Type

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