

Analytical Method for Benzyladenine (Agricultural Products)

1. Analyte

Benzyladenine (Benzylaminopurine)

2. Applicable food

Vegetables and fruits

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

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

0.5 mol/L phosphate buffer solution (pH 7.0)

Solution 1: Weigh 68.0 g of potassium dihydrogen phosphate and dissolve in water to make 1,000 mL.

Solution 2: Weigh 87.1 g of dipotassium hydrogen phosphate and dissolve in water to make 1,000 mL.

Mix Solution 1 with twice its volume of Solution 2, and adjust the pH to 7.0 using both solutions.

Reference standard of benzyladenine: Contains not less than 98% of benzyladenine.

5. Procedure

1) Extraction

Add 100 mL of acetone to 20.0 g of the sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter with suction as described above. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take exactly a 10 mL aliquot of the solution, add 40 mL of 0.5 mol/L phosphate buffer solution (pH 7.0), and extract with shaking twice using 40 mL and 20 mL of ethyl acetate. Combine the extracts, dehydrate the extracts with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and concentrate the filtrates to approximately 2 mL at below 40°C.

2) Clean-up

Inject 5 mL each of methanol and ethyl acetate into a benzenesulfonyl propylsilylated silica gel cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL each of ethyl acetate and methanol sequentially, and discard each effluent. Then, add a 5 mL mixture of ammonia water, water and methanol (1:30:20, v/v/v), collect the eluates, add methanol to make exactly 5 mL, and use this solution as the test solution.

6. Calibration curve

Prepare several methanol solutions of benzyladenine standards, inject the solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of benzyladenine in the test solution corresponding to 0.005 mg/kg in the sample results in 0.001 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of benzyladenine from the calibration curve made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length, and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 5 mmol/L ammonium acetate solution and methanol (4:1, v/v) to (1:19, v/v) in 15 min.

Ionization mode: ESI (+)

Major monitoring ion (m/z): Precursor ion 226, product ions 91, 65

Injection volume: 2 μL

Expected retention time: 9 min

10. Limit of quantification

0.005 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of benzyladenine from the sample with acetone, transfer into ethyl acetate under neutral conditions for re-dissolution, clean-up with a benzenesulfonyl propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Pay attention to the solubility when preparing standard stock solutions of benzyladenine in acetone. Precipitates may be observed when a 1,000 mg/L benzyladenine solution using acetone is stored at 4°C. Using methanol, a 1,000 mg/L solution can be prepared.

ii) When the analytical methods for benzyladenine using LC-MS/MS were developed, the following monitoring ions were used:

for quantitative ions (m/z): precursor ion 226, product ion 91

for qualitative ions (m/z): precursor ion 226, product ion 65

iii) Food items used to develop the analytical method: asparagus, pumpkin, watermelon, apples

and grapes

12. References

Tada, Hiroyuki, *et al.* Determination of Benzylaminopurine in Agricultural Products. *Shokuhin Eiseigaku Zasshi*, **49**, 136-140 (2008).

13. Type

C