Original: Japanese
Provisional translation

Analytical Method for Oxine-copper (Agricultural Products)

1. Analytes

Oxine-copper

2. Applicable foods

Agricultural products (grains, fruits and vegetables)

3. Instrument

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

4. Reagents

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

Styrene-divinylbenzene copolymer cartridge (500 mg): A polyethylene column with inside diameter 8-13 mm packed with 500 mg of styrene-divinylbenzene copolymer, or a equivalent cartridge in separation capability.

Ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate: Use a reagent with a purity of not less than 98%.

Reference standard of oxine-copper: Contains not less than 98% of oxine-copper

5. Procedure

1) Extraction

i) Grains

Add 20 mL of 0.1 mol/L hydrochloric acid to 10.0 g of sample, and let stand for 30 minutes. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the solution accurately, add 10 mL of 30 mmol/L ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate, 10 mL of ethyl acetate and 2 g of sodium chloride, and shake. Centrifuge at 3,000 rpm for 5 minutes, and collect the organic layer. Wash the container after collecting the organic layer with 2-3 mL of ethyl acetate, and transfer the washing to the previously obtained organic layer. Add 10 mL of 30 mmol/L ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate, 2 g of sodium chloride and 3 mL of 2 mol/L hydrochloric acid to the organic layer, and shake. Centrifuge at 3,000 rpm for 5 minutes, discard the organic layer. Add 3 mL of 2 mol/L sodium hydroxide and 10 mL of water to the resultant aqueous layer, and mix.

ii) Fruits and vegetables

Weigh the sample accurately, add 2 mol/L of hydrochloric acid at 1/10 amount in weight ratio to the sample, grind and homogenize, and weigh the sample equivalent to 20.0 g. Add 100 mL of acetone to the sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the

resulting filtrates, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the solution accurately, add 10 mL of 30 mmol/L ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate, 10 mL of ethyl acetate and 2 g of sodium chloride, and shake. Centrifuge at 3,000 rpm for 5 minutes, and collect the organic layer. Wash the container after collecting the organic layer with 2-3 mL of ethyl acetate, and transfer the washing to the previously obtained organic layer. Add 10 mL of 30 mmol/L ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate, 2 g of sodium chloride and 3 mL of 2 mol/L hydrochloric acid to the organic layer, and shake. Centrifuge at 3,000 rpm for 5 minutes, and discard the organic layer. Add 3 mL of 2 mol/L sodium hydroxide and 10 mL of water to the resultant aqueous layer, and mix.

2) Clean-up

Add 10 mL each of methanol, water and 30 mmol/L ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate into a styrene-divinylbenzene copolymer cartridge (500 mg) sequentially, and discard the effluents. Transfer the solution obtained in **1)** to the cartridge, add 10 mL of water and water/methanol (2:3, v/v) sequentially, and discard the effluents. Add 10 mL of methanol, add water to the eluate to make exactly 20 mL, and use this solution as the test solution. For grains, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in water/methanol (1:1, v/v) to exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare oxine-copper standard solutions (water/methanol [1:1, v/v]) of several concentrations, inject each standard solution to LC-MS/MS, and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of each analyte gives the test solution of 0.0005 mg/L in concentration.

7. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of oxine-copper 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 in inside diameter, 100 mm in length, 3 μm in particle diameter)

Column temperature: 40°C

Mobile phase: Initially 0.05 vol% acetic acid/0.05 vol% acetic acid-acetonitrile (19:1, v/v) for 0.5 minutes, followed by a linear gradient to (1:9, v/v) in 4.5 minutes, and hold for 5 minutes.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 146, product ions 128, 118

Injection volume: 5 μL

Expected retention time: 5 minutes

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of oxine-copper from sample with acetone under acidic condition of hydrochloric acid, adding ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate, ethyl acetate and sodium chloride, transferring into the organic layer, then transferring into aqueous layer as acidic condition of hydrochloric acid, adding sodium hydroxide to adjust pH, clean-up with a styrene-divinylbenzene copolymer cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Styrene-divinylbenzene copolymer cartridge (500 mg) with 22 mm inside diameter can also be used.

ii) When the analytical method for oxine-copper using LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions (*m*/*z*): precursor ion 146, product ion 118

for qualitative ions (*m*/*z*): precursor ion 146, product ion 128

iii) Since oxine-copper tends to degrade in neutral samples such as potatoes, add hydrochloric acid when preparing the sample to prevent degradation. It can be tested without adding hydrochloric acid in samples such as oranges.

iv) Oxine-copper dissociates under weak acidity and becomes water-soluble. After adding 2 mol/L sodium hydroxide, confirm that the pH is about 5.

v) Since oxine-copper is a coordinating compound, it may coordinate with metal impurities in octadecylsilanized silica gel of a column packing and cause tailing, therefore use a column packing with high-purity silica gel, and select a column which has been fully end-capped.

vi) To prevent adherence of oxine-copper which is a coordinating compound to a container, use a centrifuge tube made by polypropylene.

vii) The food items used to develop the analytical method: Wheat, parsley, cabbages, potatoes, oranges, apples

12. Reference

None

13. Type

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