Original: Japanese

Provisional Translation

Analytical Method for Acequinocyl (Animal and Fishery Products)

1. Analytes

Acequinocyl

3-Dodecyl-2-hydroxy-1,4-naphthoquinone (hereafter referred to as "acequinocyl-hydroxy")

2. Instrument

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

3. Reagents

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

Styrene-divinylbenzene copolymer cartridge (500 mg): Polyethylene tube of 10–12 mm in inside diameter packed with 500 mg of styrene-divinylbenzene copolymer, or other cartridge with equal separation characteristics.

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

Reference standard of acequinocyl-hydroxy: Contains not less than 95% of acequinocyl-hydroxy.

4. Procedure

1) Extraction

i) Muscle, liver, kidney, milk, egg and fish/shellfish

Add 5 mL of 0.4 mol/L hydrochloric acid and 100 mL of acetone to 10.0 g of 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 20 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Add 20 mL of *n*-hexane to the residue, and extract with shaking three times with 40 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of *n*-hexane.

ii) Honey

Dissolve 10.0 g of sample in 20 mL of water. Add 5 mL of 0.4 mol/L hydrochloric acid and 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 20 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and

remove the solvent. Dissolve the residue in 2 mL of *n*-hexane.

iii) Fat

Add 5 mL of 0.4 mol/L hydrochloric acid and 100 mL of acetone to 5.00 g of 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 40 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Add 20 mL of *n*-hexane to the residue, and extract with shaking three times with 40 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of *n*-hexane.

2) Clean-up

i) Silica gel column chromatography

Add 10 mL of *n*-hexane to a silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of *n*-hexane, and discard the effluent. Elute with 10 mL of ethyl acetate/*n*-hexane (1:4, v/v), concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile/water (1:1, v/v).

ii) Styrene-divinylbenzene copolymer column chromatography

Add 10 mL each of acetonitrile and acetonitrile/water (1:1, v/v) to a styrene-divinylbenzene copolymer cartridge (500 mg) sequentially, and discard the effluents. Transfer the solution obtained in i) to the cartridge, add 10 mL of acetonitrile/water (1:1, v/v), and discard the effluent. Elute with 20 mL of acetonitrile, concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile to make exactly 1 mL, and use this solution as the test solution.

5. Calibration curve

Dissolve reference standards of acequinocyl and acequinocyl-hydroxy in acetone to make 500 mg/L respectively, and use these solutions as stock standard solutions. Mix these stock standard solutions appropriately, dilute with acetonitrile, and prepare standard solutions of several concentrations. Inject each standard solution to 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 sample containing 0.01 mg/kg of each analyte gives the test solution of 0.01 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of acequinocyl and acequinocyl-hydroxy from the calibration curves made in **5**. Use the following equation to calculate the concentration of acequinocyl including acequinocyl-hydroxy:

Concentration (ppm) of acequinocyl (including acequinocyl-hydroxy)

$= A + B \times 1.123$

A: Concentration (ppm) of acequinocyl

B: Concentration (ppm) of acequinocyl-hydroxy

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 3 µm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.1 vol% formic acid (1:1, v/v) to (19:1, v/v) in 10 min and hold for 10 min

Ionization mode: APCI (-)

Major monitoring ions (*m/z*):

Acequinocyl: precursor ion 384, product ion 342, 187

Acequinocyl-hydroxy: precursor ion 341, product ion 313, 200, 186

Injection volume: 10 µL

Expected retention time:

Acequinocyl: 18 min

Acequinocyl-hydroxy: 17 min

9. Limit of quantification

0.01 mg/kg for each analyte

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of acequinocyl and acequinocyl-hydroxy from sample with 0.4 mol/L hydrochloric acid and acetone, transferring into *n*-hexane, defatting by acetonitrile/hexane partitioning (omitted for honey), clean-up with a silica gel cartridge and a styrene-divinylbenzene copolymer cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Acequinocyl and acequinocyl-hydroxy are quantified individually. The concentration of acequinocyl-hydroxy is converted to the concentration of acequinocyl by multiplying by a conversion factor, and the sum of the concentrations of acequinocyl and acequinocyl-hydroxy is regarded as the analytical result of acequinocyl.

ii) Acequinocyl is unstable under light. Therefore, analytical testing should be performed with light shielding. Brown glassware was used in the development of this analytical method.

iii) When the analytical method for acequinocyl and acequinocyl-hydroxy using LC-MS/MS was developed, the following monitoring ions were used:

Acequinocyl

for quantification (*m/z*): precursor ion 384, product ion 342

for confirmation (*m/z*): precursor ion 384, product ion 187

Acequinocyl-hydroxy

for quantification (*m/z*): precursor ion 341, product ion 186

for confirmation (*m/z*): precursor ion 341, product ion 200

for confirmation (*m/z*): precursor ion 341, product ion 313

11. References

None

12. Type

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