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Method of Test for Veterinary Drug Residues in Foods - Test of 8-Hydroxyquinoline and Halquinol

1. Scope

This method is applicable to the determination of 8-hydroxyquinoline and halquinol (containing 5,7-dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline) in muscle, viscera, fat, eggs and milk of poultry and livestock products.

2. Method

After extraction and purification, analytes are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: Poroshell 120 Phenyl Hexyl, 2.7 μm, 4.6 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Homogenizer.

2.1.3. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder®, >1000 rpm, or an other mechanical shaker.

2.1.4. Water bath: capable of controlling temperature at ± 1°C.

2.1.5. Centrifuge: centrifugal force > 5000 ×g and temperature control < 4°C.

2.1.6. Shaker.

2.1.7. Vortex mixer.

2.1.8. Nitrogen evaporator.

2.2. Chemicals

Acetonitrile, HPLC grade;
Methanol, HPLC grade;
n-Hexane, HPLC grade;
Formic acid, reagent grade;
Dimethylsulfoxide, DMSO, reagent grade;
Magnesium sulfate anhydrous, reagent grade;
Sodium chloride, reagent grade;
Sodium citrate, reagent grade;
Sodium acetate, reagent grade;
Disodium hydrogen citrate, reagent grade;
Disodium ethylenediaminetetraacetate dihydrate, EDTA-Na_2•$2H_2O$, reagent

grade;
β-Glucuronidase solution (containing β-glucuronidase ≥ 85000 unit/mL and sulfatase ≤ 7500 unit/mL);
Deionized water, resistivity ≥ 18 MΩ•cm (at 25°C);
8-Hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline, reference standards.

2.3. Apparatus and materials

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Extraction powder(note): Containing 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate and 0.5 g of disodium hydrogen citrate, or an equivalent product.
Note: commercial extraction kit can be used as needed.

2.3.3. Ceramic homogenizer: Bond Elut QuEChERS P/N 5982-9313, or an equivalent product.

2.3.4. Volumetric flask: 1 mL and 10 mL, PP.

2.3.5. Membrane filter: 0.22 μm, PTFE.

2.4. Reagents

2.4.1. 50% methanol:
Mix methanol and deionized water at the ratio of 1:1 (v/v).

2.4.2. 25 mM sodium acetate buffer solution:
Dissolve and dilute 2.05 g of sodium acetate with deionized water to 1000 mL.

2.4.3. 25 mM sodium acetate buffer solution containing 9% disodium ethylene-diaminetetraacetate:
Stir to dissolve 90 g of disodium ethylenediaminetetraacetate with 900 mL of 25 mM sodium acetate buffer solution, and dilute with 25 mM sodium acetate buffer solution to 1000 mL.

2.4.4. *n*-Hexane saturated with acetonitrile
Add 50 mL of acetonitrile to 500 mL of *n*-hexane. Shake and then stand until complete layering. Take the *n*-hexane layer.

2.5. Mobile phase

2.5.1. Solvent A
Dilute 3 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B:

Dilute 3 mL of formic acid with methanol to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 10 mg of 8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute with methanol to volume as the standard stock solutions. Store in the freezer. When to use, mix appropriate volume of each standard stock solution, and dilute with 50% methanol to 10 μg/mL as the standard solution.

2.7. Sample solution preparation

Transfer about 2 g of the fine-cut and homogenized muscle, fat or visceral sample accurately weighed; remove eggs' shells, and transfer about 2 g of the mixed egg white and yolk sample accurately weighed; accurately transfer 2 mL of the milk sample into a centrifuge tube. Add one ceramic homogenizer, 10 mL of 25 mM sodium acetate buffer solution containing 9% disodium ethylenediaminetetraacetate and 250 μL of β-glucuronidase solution, vortex-mix, and hydrolyze in a water bath for 2 hr at 37°C. Add 10 mL of pre-cooled acetonitrile, cap the centrifuge tube, and vortex-mix for 1 min. Add the extraction powder, shake vigorously several times by hands to prevent coagulation of salts, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 3 min. Centrifuge at 5000 ×g for 5 min at 4°C, and collect the supernatant. Add 10 mL of acetonitrile to the residue, cap the centrifuge tube, vortex-mix for 1 min, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 3 min. Centrifuge at 5000 ×g for 5 min at 4°C, and collect the supernatant. Combine the supernatants, and transfer 5 mL of the supernatant into a centrifuge tube. Add 5 mL of *n*-hexane saturated with acetonitrile, and shake at 1000 rpm for 1 min by the high speed dispersing device. Centrifuge at 5000 ×g for 1 min, and collect the lower layer. Add 5 mL of *n*-hexane saturated with acetonitrile to the lower layer, and repeat the above procedure once. Take 1 mL of the lower layer, add 50 μL of DMSO(note), and evaporate to near dryness by gently flushing with a stream of nitrogen at room temperature. Dissolve the residue with 50% methanol to 1 mL, and filter with a membrane filter. Take the filtrate as the sample solution.

Note: Adding a small amount of DMSO is to avoid over dryness during nitrogen evaporation causing the loss of 8-hydroxyquinoline, 5,7-

dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline.

2.8. Calibration standard curve preparation

Take a blank sample, add 1 to 100 μL of the standard solutions separately, and follow the procedure described in section 2.7 to obtain the calibration standard solutions. Operate LC-MS/MS according to the following conditions. Establish the calibration standard curve of each drug by peak areas vs. the added concentrations in the range of 0.5 ~ 50 ng/mL.

LC-MS/MS operating conditions(note):

Column: Poroshell 120 Phenyl Hexyl, 2.7 μm, 4.6 mm i.d. × 10 cm.

Column temperature: 40°C.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0.0 → 3.0	95 → 0	5 → 100
3.0 → 6.0	0 → 0	100 → 100
6.0 → 7.0	0 → 95	100 → 5
7.0 → 10.0	95 → 95	5 → 5

Flow rate: 0.5 mL/min.

Injection volume: 2 μL.

Interface voltage: 4 kV.

Ionization mode: ESI^+

Interface temperature: 300°C.

Nebulizing gas flow: 3.0 L/min.

Heating gas flow: 15.0 L/min.

Desolvent line temperature: 250°C.

Heat block temperature: 400°C.

Drying gas flow: 5.0 L/min.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage (Q1/Q3 Pre Bias) and collision voltage are as follows:

Analyte	Ion pair: Precursor ion (*m/z*) > product ion (*m/z*)	Q1/Q3 cone voltage (V)	Collision voltage (V)
8-Hydroxyquinoline	146 > 101*	17/17	31
	146 > 128	28/22	27

5,7-Dichloro-8-hydroxyquinoline	214 > 150*	15/27	28
	214 > 123	15/12	36
5-Chloro-8-hydroxyquinoline	180 > 117*	24/21	30
	180 > 145	23/21	31

* Quantitative ion pair.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification:

Accurately inject 2 μL of the sample solution and the calibration standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.8. Identify each drug based on the retention time and the relative ion intensities(note 1). Calculate the amount of each drug in the sample(note 2) by the following formula:

$$\text{The amount of each drug in the sample (ppm)} = \frac{C \times V}{M \times 1000}$$

Where,

C：the concentration of each drug in the sample solution calculated by the calibration standard curve (ng/mL)

V：the final make-up volume of the sample (20 mL)

M：the weight of sample (g or mL)

Note: 1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions. Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

2. The amount of halquinol in the sample is expressed as sum of 5,7-dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline.

Remark

1. Limits of quantification (LOQs) for 8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline are all 0.005 ppm.
2. Further validation shall be performed when interfering compounds appear in samples.

References

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3. Hall, A. L., Chicoine, A. and Boison, J. 2017. Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 85th Meeting 2017.

Reference chromatogram

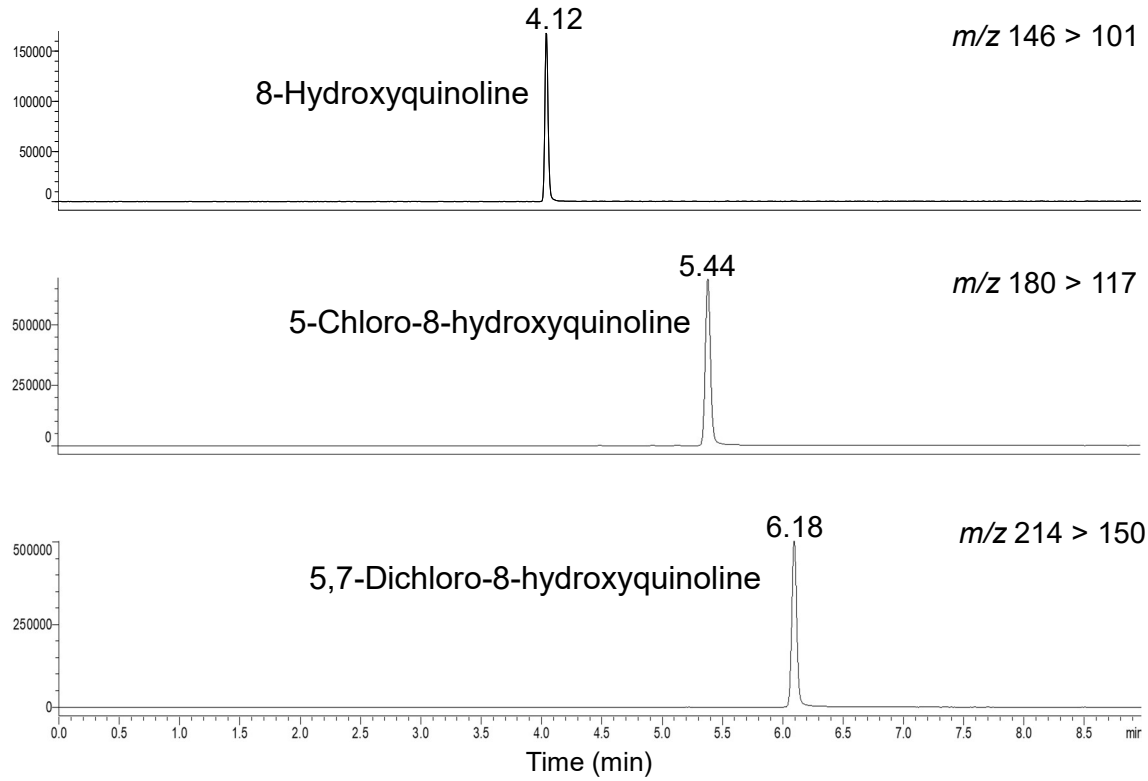

Figure. MRM chromatograms of 8-hydroxyquinoline、5,7-dichloro-8-hydroxyquinoline and 5-chloro-8-hydroxyquinoline standards analyzed by LC-MS/MS.