

Analytical Method for Chlormequat (Animal Products)

1. Analyte

Chlormequat chloride

2. Applicable food

Animal products

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.

Graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg): A polyethylene tube of 12–13 mm inside diameter packed with 500 mg each of graphitized carbon black in the upper layer and ethylenediamine-*N*-propylsilanized silica gel in the lower layer, or a cartridge equivalent to the specified one in separation capability.

Reference standard of chlormequat chloride: Contains not less than 98% of chlormequat chloride.

5. Procedure

1) Extraction

Add 1 g of sodium chloride and 10 mL of 1 vol% formic acid to 10.0 g of the sample, homogenize, add 50 mL of methanol, and homogenize again. Centrifuge at 3,000 rpm for 5 min and collect the supernatant. Add 25 mL of methanol to the residue, homogenize, centrifuge as described above, and collect the supernatant. Combine the resulting supernatants and add methanol to make exactly 100 mL. Take exactly a 5 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Add 5 mL of *n*-hexane to the residue and extract with shaking twice using 5 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts and concentrate to approximately 1 mL at below 40°C.

2) Clean-up

Inject 10 mL of 0.1 mol/L hydrochloric acid and 20 mL of acetonitrile into a graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg) sequentially and discard the effluents. Transfer the solution obtained in 1) to the cartridge, add another 8 mL of acetonitrile, and collect the total eluate including the transferred solutions. Add acetonitrile to the eluate to make exactly 10 mL and use this solution as the test solution.

6. Calibration curve

Prepare chlormequat chloride standard solutions (acetonitrile) of several concentrations, inject each

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 chlormequat chloride in the test solution corresponding to 0.01 mg/kg in the sample results in 0.0005 mg/L.

7. Quantification

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

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Silica gel modified with octadecylsilyl groups, cation-exchange groups, and anion-exchange groups, 2.0 mm inside diameter, 100 mm in length, and 3 µm in particle diameter

Column temperature: 40°C

Mobile phase: Initially, 2 mmol/L ammonium acetate solution and methanol (4:1, v/v) for 1 min, followed by a linear gradient to (1:1, v/v) in 2 min, then another linear gradient to (1:49, v/v) in 2 min, and hold for 3 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): Precursor ion 122, product ions 58, 59

Injection volume: 5 µL

Expected retention time: 3 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of chlormequat chloride from the sample with methanol containing sodium chloride and formic acid, defatting by acetonitrile/hexane partitioning, clean-up with a graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge, and quantification and confirmation using LC-MS/MS. Note that chlormequat chloride is the generic name for the active ingredient, while chlormequat is used as the common name for the pesticide.

2) Notes

- i) Since chlormequat chloride adheres easily to glass walls, polypropylene equipment is recommended.
- ii) When the analytical methods for chlormequat chloride using LC-MS/MS were developed, the following monitoring ions were used:
 - for quantitative ions (*m/z*): precursor ion 122, product ion 58
 - for qualitative ions (*m/z*): precursor ion 122, product ion 59
- iii) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, milk,

and chicken egg

12. References

None

13. Type

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