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**Method for the determination of metronidazole,
ronidazole and dimetridazole residues in honey –
LC-MS-MS method**

蜂蜜中甲硝唑、洛硝哒唑、二甲硝咪唑残留量的测定

液相色谱-串联质谱法

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Foreword

The Appendix A and Appendix B of this Standard are informative.

This Standard was proposed by Qinhuangdao Entry - Exit Inspection and Quarantine Bureau of the People's Republic of China.

This Standard shall be under the jurisdiction of the General Administration of Quality Supervision, Inspection and Quarantine of People's Republic of China.

Drafting organizations of this Standard: Qinhuangdao Entry - Exit Inspection and Quarantine Bureau of the People's Republic of China, Shandong Agricultural University.

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This Standard is the first-time issued national standard.

6.2 Sample preservation

PRESERVE the sample under room temperature.

7 Measurement step

7.1 Extraction

WEIGH 10g of sample (accurate to 0.01g); PLACE it in a 50mL plugged glass centrifuge tube; ADD 10 mL of water; MIX it uniformly on the liquid mixer; ADD 20mL of ethyl acetate; USE oscillator to vibrate it for 20min; MAKE it subject to 3000 r/min centrifugation for 5 min; TAKE the supernatant, MAKE it pass through a cylindrical funnel containing 25 g of anhydrous sodium sulfate AND reach into pear-shaped flask. USE 20 mL of ethyl acetate to extract it again, MAKE it pass through the anhydrous sodium cylindrical funnel AND COMBINE with the supernatant; USE a rotary evaporator to evaporate it at 45°C water bath under reduced pressure to about 2mL; PREPARE for purification.

7.2 Purification

TRANSFER the aforementioned concentrate into the Carboxylic Acid solid-phase extraction column; respectively USE 4 mL of ethyl acetate and 4mL of acetonitrile to wash pear-shaped flask and extraction column; DISCARD all of the effluent. Under the 65kPa negative pressure, REDUCE pressure and EMPTY the extraction column for 2 min; USE 2 mL of eluent to conduct elution at the flow rate ≤ 3 mL/min; COLLECT the elution solution into a 5mL scale sample tube; USE eluent to make the volume reach to 2 mL; MAKE it pass through 0.20 μ m filter membrane; PREPARE for the measurement by liquid chromatography - tandem mass spectrometer.

7.3 Measurement

7.3.1 Liquid chromatography conditions

- a) Chromatography column: Atlantis dC₁₈, 3 μ m, 150 mm X 2.1 mm (inner diameter) or the equivalent;
- b) Mobile phase: acetonitrile + 0.1% formic acid solution (30 + 70);
- c) Flow rate: 200 μ L/min;
- d) Column temperature: 30°C;
- e) Sample injection volume: 20 μ L.

7.3.2 MS conditions

Appendix B
(Informative)
Recovery

As for the adding concentration and average recovery of the metronidazole, ronidazole, and dimetridazole in this method, see Table B.1.

**Table B.1 Test data of metronidazole, ronidazole, and dimetridazole
adding concentration and average recovery**

Drug name	Adding concentration/($\mu\text{g/kg}$)	Mean recovery /(%)
Metronidazole	0.05	76.0
	0.10	79.0
	0.20	77.0
	1.00	82.4
Ronidazole	0.10	69.5
	0.20	78.5
	0.40	73.9
	2.00	71.7
Dimetridazole	0.10	75.6
	0.20	74.9
	0.40	75.4
	2.00	81.7

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