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**Method for the determination of metronidazole,
ronidazole and dimetridazole residues in honey -
Liquid chromatographic method**

蜂蜜中甲硝哒唑、洛硝哒唑、二甲硝咪唑残留量的测定方法
液相色谱法

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Foreword

Annex A and Annex B in this Part of GB/T 18932 are informative.

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

The Part shall be under the jurisdiction of China Supply and Marketing Cooperatives.

Drafting organization of this Part: Qinhuangdao Entry-exit Inspection and Quarantine Bureau of the People's Republic of China.

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The Part is first-time released national standard.

Method for the determination of metronidazole, ronidazole and dimetridazole residues in honey - Liquid chromatographic method

1 Scope

This Part of GB/T 18932 specifies the liquid chromatographic determination method for the residues of metronidazole, ronidazole and dimetridazole in honey.

This Part applies to the determination of the residues of metronidazole, ronidazole and dimetridazole in honey.

The detection limit of the method in this Part: 0.0010mg/kg for metronidazole, ronidazole and dimetridazole.

2 Normative references

The provisions in following documents become the provisions of this Part through reference in this Part of GB/T 18932. For dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Part, however, parties who reach an agreement based on this Part are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6379 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by interlaboratory tests (GB/T 6379-1986, neq ISO 5725:1981)

GB/T 6682 Water for analytical laboratory use - Specification and test methods (GB/T 6682-1992, neq ISO 3696:1987)

3 Principle

The nitroimidazole residues in the sample are extracted by ethyl acetate; after the extracted solution is evaporated to dryness, dissolve with water, purified with Oasis HLB solid phase extraction column and BAKERBOND Carboxylic Acid solid phase extraction column; determined by liquid chromatography ultraviolet detector; quantified by external standard method.

5.2 Analytical balances: 2 balances with sensitivity of 0.1mg and 0.01g respectively.

5.3 Liquid mixer.

5.4 Solid phase extraction vacuum device.

5.5 Oscillator.

5.6 Glass centrifuge tube with stoppers: 50mL.

5.7 Vacuum pump: the vacuum shall reach 80kPa.

5.8 Centrifuge.

5.9 Rotary evaporator.

5.10 Sample tube: 5mL.

5.11 pH meter: measurement accuracy is ± 0.02 .

5.12 Pear-shaped flask: 200mL.

6 Sample preparation and preservation

6.1 Sample preparation

For non-crystallized laboratory samples, stir them evenly. For samples with crystallization, under hermetic conditions, PLACE them in the water bath not more than 60°C to warm; SHAKE; STIR evenly until it is fully melt; rapidly cool to room temperature; TAKE 0.5kg as the sample. The prepared sample is placed in flask, sealed and marked.

6.2 Sample preservation

The test sample shall be preserved under room temperature.

7 Determination steps

7.1 Extraction

WEIGH 6g of sample (accurate to 0.01g); PLACE in a 50mL glass centrifuge tube; ADD 6mL of water; MIX in liquid mixer; ADD 20mL of ethyl acetate; OSCILLATE in the oscillator for 20min; CENTRIFUGE; TAKE the supernatant to a pear-shaped flask. Again, EXTRACT with 20mL of ethyl acetate; MERGE the supernatant; USE a rotary evaporator, on water bath of 45°C, to reduce the pressure and evaporate to dryness; DISSOLVE with 5mL of water; WAIT to be purified.

9.1 Repeatability

Under repeatability conditions, the absolute difference between two independent test results obtained shall not exceed the repeatability limit (r). The content range and repeatability equation of metronidazole, ronidazole and dimetridazole in honey are shown in Table 2.

Table 2 Content range and repeatability and reproducibility equations

Names	Content range/ (mg/kg)	Repeatability limit r	Reproducibility R
Metronidazole	0.010~0.100	$r = 0.0303m + 2.1673$	$R = 0.0807m - 1.0408$
Ronidazole	0.010~0.100	$r = 0.0608m + 1.8727$	$R = 0.0620m + 1.7190$
Dimetridazole	0.010~0.100	$r = 0.0627m + 1.4411$	$\lg R = 0.7099\lg m - 0.4377$
Note: m is the arithmetic mean of two determination results.			

If the difference exceeds the repeatability limit, it shall abandon the test results and re-complete the determination of two single tests.

9.2 Reproducibility

Under reproducibility conditions, the absolute difference between two independent test results obtained shall not exceed reproducibility limit (R). The content range and reproducibility equation of metronidazole, ronidazole and dimetridazole in honey are shown in Table 2.

Annex B**(Informative)****Recovery rate**

Test data of the added concentration and the average recovery rate of metronidazole, ronidazole and dimetridazole in this method is shown in Table B.1.

Table B.1 Test data of the added concentration and the average recovery rate of metronidazole, ronidazole and dimetridazole

Name of chemicals	Added concentration/($\mu\text{g/kg}$)	Average recovery rate/(%)
Metronidazole	0.010	86.27
	0.020	87.13
	0.050	87.35
	0.100	86.33
Ronidazole	0.010	84.64
	0.020	89.38
	0.050	82.87
	0.100	87.99
Dimetridazole	0.010	85.96
	0.020	84.86
	0.050	90.70
	0.100	88.61

END

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