GB

ICS 67.180.10

X 31

National Standard

of the People's Republic of China

GB 18392.19-2003

Method for the determination of chloramphenicol residues in honey - LC-MS-MS method

蜂蜜中氯霉素残留量的测定方法

液相色谱-串联质谱法

Issued on: December 26, 2003 **Implemented on: June 1, 2004**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China

Table of Contents

Foreword 3

1 Scope 4

2 Normative References 4

3 Principles 4

4 Reagents & Materials 5

5 Instruments 5

6 Sample preparation and storage 6

7 Determination steps 6

8 Calculation of results 8

9 Precision 9

Foreword

This Part of GB/T18932 is modified on the basis of adopting the Canadian Standard ACC-062-V1.0 "Method for the determination of chloramphenicol residues in honey - LC-MS method". The main modified contents are as follows:

- Purification column is modified from C18 solid phase extraction column to Oasis HLB solid phase extraction column;
- Eluent is modified from acetonitrile + water (3+7) to ethyl acetate;
- Single quadrupole mass-spectrometry detector is modified to tandem quadrupole mass-spectrometry detector;
- Internal standard method is modified to the external standard method.

Appendix A and Appendix B of this Part are informative.

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

This Part shall be under the jurisdiction of National Federation of Supply and Marketing Cooperatives.

The drafting organization of this Part: Qinhuangdao Entry - Exit Inspection and Quarantine Bureau of the People's Republic of China.

Main drafters of this Part: Pang Guofang, Cao Yanzhong, Zhang Jinjie, Jia Guangqun, Fan Chunlin, Li Xuemin, Liu Yongming and Shi Yuqiu.

This Part was issued for the first time.

Method for the determination of chloramphenicol residues in honey - LC-MS-MS method

1 Scope

This Part of GB/T 18932 specifies the method for the determination of chloramphenicol residues in honey - LC-MS-MS method.

This Part is applicable to the determination of chloramphenicol residues in honey.

The method detection limit of this Part: chloramphenicol is 0.10 μg/kg.

2 Normative References

The following standards contain the provisions which, through reference in this Part of the GB/T18932, constitute the provisions of this Part. For dated references, all subsequent amendments (excluding corrections) or revisions do not apply to this Part. However, the parties who enter into agreement based on this Part are encouraged to investigate whether the latest versions of these standards are applicable. For undated reference documents, the latest versions apply to this Part.

GB/T 6379 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests (GB/T 6379-1986,neq IS05725:1981)

GB/T 6682 Water for analytical laboratory use - Specification and test methods (GB/T 6682-1992, neq IS03696,1987)

3 Principles

Use ethyl acetate to extract the sample. After the extract is concentrated, dissolve it in water. Purify the Oasis HLB solid phase extraction column. Use liquid chromatography - tandem mass spectrometer to determine. And adopt the external standard method to quantify.

5.7 Solid phase extraction device.

5.8 Liquid reservoir: 50 mL.

5.9 Vacuum pump: the vacuum degree shall be 80 kPa.

5.10 Centrifuge.

5.11 Graduated centrifuge tube: 10 mL, the accuracy is 0.1 mL.

5.12 Pipette: 10 mL.

5.13 Centrifuge tube: 50 mL with stopper.

6 Sample preparation and storage

6.1 Sample preparation

Evenly stir the non-crystallized laboratory sample. For crystallized sample, in a enclosed circumstance, place it in the water bath at not more than 60°C; warm it; oscillate it till it is totally melted; stir it and cool it to room temperature. Take 0.5 kg as the sample. Place the prepared sample in the sample flask, seal and mark it.

6.2 Sample storage

Store the sample at room temperature.

7 Determination steps

7.1 Extraction

Weigh 5 g of sample, accurate to 0.01 g. Place it into a 50 mL stoppered centrifuge tube; add 5 mL of water. Quickly mix it in the liquid mixer for 1 min till it is completely dissolved. Accurately add 15 mL of ethyl acetate; oscillate it in an oscillator at 3000 r/min for 10 min. Accurately extract 12 mL of upper-layer's ethyl acetate into an evaporation tube of the automatic concentrator. Use the automatic concentrator to relieve pressure and distill to dry at 55°C. Add 5 mL of water to dissolve the residue; wait to be purified.

7.2 Purification

Pour the extract (7.1) into a liquid reservoir that is connected to Oasis HLB column (4.6) in the lower. The solution flows through the Oasis HLB solid phase extraction column at the flow rate ≤ 3 mL/min. After the solution completely flows out, use 2×5 mL of water to wash the evaporation tube and the liquid

321/152		-23
321/194		-20

7.3.3 LC-MS-MS determination

Respectively inject the chloramphenicol standard working solutions (4.9) under LC-MS-MS conditions. Take the peak area as ordinates, and take working solution concentration (ng/mL) as abscissa; draw the seven-point standard working curve. Use standard working curve to conduct quantification of the sample. The response value of chloramphenicol in the sample solution shall all be within the linear measurement range of the instrument. In the aforementioned chromatographic conditions, the reference retention time of chloramphenicol 12.31 min. For the total ion chromatogram and the mass spectrogram of chloramphenicol's reference material, refer to Figure A.1 and Figure A.2 of Appendix A.

7.4 Parallel test

Follow the previous steps to carry out the parallel test for the same sample.

7.5 Blank test

Follow the previous steps, except weighing the sample.

8 Calculation of results

The result is calculated according to equation (1):

$$X = c \cdot \frac{V}{m} \cdot \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

Where,

X - residues of determined components in the sample, in micrograms per kilogram (μg/kg);

c - solution concentration of determined components, obtained from the standard working curve, in nanograms per milliliter (ng/mL);

V - constant volume of sample solution, in milliliters (mL);

m - sample mass represented by sample solution, in grams (g).

Note: The blank value shall be deducted from the results.

Appendix B

(Informative)

Recovery Rate

Test data of adding concentration and average recovery of chloramphenicol in honey:

When the added amount is 0.1 μg/kg, the average recovery is 103.3%;

When the added amount is 0.3 μg/kg, the average recovery is 98.2%;

When the added amount is 1.0 μg/kg, the average recovery is 96.4%;

When the added amount is 5.0 μg/kg, the average recovery is 97.2%.

END

This is an excerpt of the PDF (Some pages are marked off intentionally)

------ The End ------