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# Methods for the determination of sixteen sulfonamides residues in honey - LC-MS-MS method

蜂蜜中 16 种磺胺残留量的测定方法

液相色谱—串联质谱法



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# Table of Contents

# Foreword

This Part of GB/T 18932 modifies and adopts Canadian standard ACC-056-V2.0 “Methods for the determination of sixteen sulfonamides residues in honey - LC-MS-MS method”. The main changes are as follow:

- ADJUST the test varieties;
- ADD the purified steps of benzene sulfonic acid cation exchange column and Oasis HLB column;
- The liquid chromatogram’s column and mobile phase are different;
- REPLACE single quadrupole mass detector with tandem quadrupole mass detector.

Appendix A and Appendix B of this Part are informative.

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

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

Drafting organization of this Part: Qinhuangdao Entry-Exit Inspection and Quarantine Bureau of People’s Republic of China.

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This Part is the first-time released national standard.

# Methods for the determination of sixteen sulfonamides residues in honey - LC-MS-MS method

## 1 Scope

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

This Part applies to the determination of sixteen sulfonamides residues.

Sulfamethizole is 1.0μg/kg; N-((4-Aminophenyl)sulfonyl)acetamide, sulfadiazine, sulfapyridine, sulfisoxazole, sulfamerazine, sulfachloropyridazine, sulfamonomethoxine, sulfadoxine, sulfamethoxazole are 2.0 μg/kg respectively; sulfathiazole, sulfamethoxypyridazine, sulfadimethoxypyrimidine are 4.0 μg/kg respectively; sulfameter, sulfamethazine are 8.0 μg/kg respectively; sulfaphenazole is 12.0 μg/kg.

## 2 Normative references

The following documents contain provisions which, through reference in this Part, become the provisions of this Part. For dated references, their 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 documents 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 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

Use phosphoric acid solution (pH=2) to extract the sulfonamides residues in honey; leach the residues; purify through cation exchange column and Oasis HLB or equivalent solid phase extraction column; use methanol to elute; evaporate and dry it; use acetonitrile + ammonium acetate solution (0.1mol/L) to dissolve the residues. Use

# 7 Determination steps

### 7.1 Extraction

Weigh 5g of samples, accurate to 0.01g. Place them into a 150mL triangular flask; add 25mL of phosphoric acid solution (4.8); mix the samples uniformly and quickly at the liquid mixers for 1min, so as to make the sample dissolved entirely.

### 7.2 Purification

CONNECT the glass liquid reservoir with glass wool plug TO benzene sulfonic acid cation exchange column (4.11); pour sample solution (7.1) into glass liquid reservoir; under the decompression situation, the sample flows through the benzene sulfonic acid cation exchange column at the speed of less or equal to 3mL/min. After the sample solution flows out completely, use 5mL of phosphoric acid solution and 5mL of water to wash the column; discard all the effluent solution. Finally, use 40mL of phosphate buffered solution (4.9) to elute; collect the eluent in a 100mL flat-bottom flask. Add 1.5mL of sodium heptanesulfonate solution (4.10) into eluent; then use phosphoric acid (4.3) to adjust pH=6.

According to above methods, the eluent, of which the pH has been adjusted, flows through Oasis HLB or equivalent solid phase extraction column (4.12); adjust the flow rate to be less than or equal to 3mL/min; After the sample solution flows out completely, use 3mL of water to wash the column again; discard all the effluent solution. At the 65kPa negative pressure, depressurize and drain to dry for 5min; finally, use 10mL of methanol (4.1) to elute; collect the eluent in a 150mL heart-shaped bottle. Use rotary evaporator at 45°C water bath to depressurize and evaporate to dry. Accurately add 1.0mL of mobile phase to dissolve residues; use liquid chromatography - tandem mass spectrometry to determine.

### 7.3 Determination of chromatogram

7.3.1 Conditions of liquid Chromatography

a) Chromatographic column: Lichrospher®100 RP-18 5μm 250mm×4.6mm (i. d) or the equivalent;

b) Mobile phase: Acetonitrile + ammonium acetate solution (0.01mol/L)(12+88);

c) Flow rate: 0.8mL/min;

d) Column temperature: 35°C;

e) Injection volume: 40μL;

f) Split ratio: 1:3.

# 8 Calculation of results

The residues of each kind of sulfonamides in the samples are calculated by data processing system or calculated according to equation (1):

$$X = c \bullet \frac{V}{m} \bullet \frac{1000}{1000} \quad \text{..... (1)}$$

Where,

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

c – concentration of the tested component solution, obtained from standard working curve, in nanograms per milliliter (ng/mL);

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

m – mass of the representative samples in sample solution, in grams (g).

Note: the blank values shall be deducted from the calculation results.

# 9 Precision

The precision data in this Part are determined in accordance with the specifications of GB/T 6379. The repeatability and reproducibility values are calculated by 95% confidence.

## 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 of 16 sulfonamides in honey and the repeatability equation are shown in Table 3.

If the different value exceeds the repeatability limit (r), the test results shall be abandoned, and re-complete the two determinations of single-test.

## 9.2 Reproducibility

Under reproducibility conditions, the absolute difference between two independent test results obtained shall not exceed reproducibility limit (*R*). the content range of 16 sulfonamides in honey and the reproducibility equation are shown in Table 3.

| | | |
|---|---|---|
| | 20.0 | 84.2 |
| | 40.0 | 88.9 |
| | 200.0 | 76.4 |
| Sulfamethoxypyridazine | 4.0 | 89.8 |
| | 10.0 | 92.0 |
| | 20.0 | 80.2 |
| | 100.0 | 75.2 |
| Sulfaphenazole | 12.0 | 85.0 |
| | 30.0 | 83.8 |
| | 60.0 | 86.4 |
| | 300.0 | 77.6 |
| Sulfadimethoxypyrimidine | 4.0 | 90.4 |
| | 10.0 | 81.9 |
| | 20.0 | 89.8 |
| | 100.0 | 74.4 |
| sulfisoxazole | 2.0 | 90.2 |
| | 5.0 | 75.5 |
| | 10.0 | 76.9 |
| | 50.0 | 87.5 |
| Sulfamonomethoxine | 2.0 | 95.6 |
| | 5.0 | 88.9 |
| | 10.0 | 94.8 |
| | 50.0 | 102.5 |
| Sulfathiazole | 4.0 | 85.3 |
| | 10.0 | 70.9 |
| | 20.0 | 76.5 |
| | 100.0 | 86.7 |
| Sulfadoxine | 2.0 | 97.4 |
| | 5.0 | 96.5 |
| | 10.0 | 92.1 |
| | 50.0 | 95.4 |
| Sulfamethazine | 8.0 | 95.0 |
| | 20.0 | 82.4 |
| | 40.0 | 92.4 |
| | 200.0 | 94.8 |

## Translator's note

**[Additional information by the translator - This is not a part of this standard]**

### CAS number of the 16 sulfonamides

| S/N | Drug name | CAS No. |
|---|---|---|
| 1 | Sulfamethizole | 144-82-1 |
| 2 | N-((4-Aminophenyl)sulfonyl)acetamide | 144-80-9 |
| 3 | Sulfadiazine | 68-35-9 |
| 4 | Sulfapyridine | 144-83-2 |
| 5 | Sulfisoxazole | 127-69-5 |
| 6 | Sulfamerazine | 127-79-7 |
| 7 | Sulfachloropyridazine | 80-32-0 |
| 8 | Sulfamonomethoxine | 1220-83-3 |
| 9 | Sulfadoxine | 2447-57-6 |
| 10 | Sulfisoxazole | 127-69-5 |
| 11 | Sulfathiazole | 72-14-0 |
| 12 | Sulfamethoxypyridazine | 80-35-3 |
| 13 | Sulfadimethoxypyrimidine | 155-91-9 |
| 14 | Sulfamethazine | 57-68-1 |
| 15 | Sulfaphenazole | 526-08-9 |
| 16 | Sulfameter | 651-06-9 |

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