

ICS 67.050

CCS X 04



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 30636-2014

**Determination of sialic acid in swiftlets nest and swiftlets nest
products -- Liquid chromatographic method**

燕窝及其制品中唾液酸的测定 液相色谱法

(English Translation)

Issued on: December 31, 2014

Implemented on: May 1, 2015

**Issued by: General Administration of Quality Supervision Inspection and
Quarantine of PRC**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed by the National Food Industry Standardization Technical Committee (SAC/TC 64).

This standard shall be under the jurisdiction of the National Food Industry Standardization Technical Committee Food General Testing Technical Subcommittee (SAC/TC 64/SC 8).

Drafting organizations of this standard. National Agricultural Deep-Processing Product Quality Supervision and Inspection Center, Jilin Province Product Quality Supervision and Inspection Institute.

1 Scope

This standard specifies the method for the determination of sialic acid in swiflets nest and its products by liquid chromatography.

This standard applies to the determination of sialic acid in swiflets nest and its products.

The detection limit of this standard is 0.3 g/kg for swiflets nest and 0.003 g/kg

for swiftlets nest products.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this standard.

GB/T 6379.2 Accuracy (trueness and precision) of measurement methods and results - Part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method

GB/T 6682 Water for analytical laboratory use - Specification and test methods

3 Method summary

Swiftlets nest and swiftlets nest products are heated and hydrolyzed in acetic acid solution to release sialic acid.

4.3 Glacial acetic acid. Analytically pure.

4.7 0.1% phosphoric acid solution. Take 1 mL of phosphoric acid; add water to make the volume to 1000 mL; mix well; use 0.45 µm microporous membrane to filter it.

4.8 50% acetic acid solution. Take 100 mL of glacial acetic acid; add 100 mL of water; mix it uniformly.

4.9 10% polyethylene glycol solution. Weigh 50 g of polyethylene glycol; add

4.10 Standard stock solution of sialic acid (1.00 mg/mL). Weigh 100.0 mg of

sialic acid; add 20 mL of water to dissolve it; add acetonitrile to make the volume reach to 100 mL. Store in the dark at 4 °C; the shelf life is one month.

4.11 Dialysis bag. It allows the penetration of compounds which have a

molecular weight of less than 10000; soak in water for 1 h before use.

4.12 0.45 μm microporous membrane. Organic phase.

4.13 0.45 μm syringe filter. Organic phase.

6.1.1 Dried swiftlets nest

Take an appropriate amount of specimen and dry it at 105 °C for 60 minutes.

Cool it in a desiccator.

6.1.2 Swiftlets nest products with rock sugar

Take an appropriate amount of specimen in a beaker. Homogenize at a speed of 10000 r/min for 3 minutes.

6.2 Preparation of sialic acid series standard working solution

Measure 1.00 mL, 2.00 mL, 4.00 mL, 6.00 mL, 8.00 mL, 10.0 mL of the standard stock solution of sialic acid, respectively. Place them in a 100 mL volumetric flask. Add the mobile phase to the mark.

7.1.1 Chromatographic column. 300SCX cation exchange chromatographic column, 4.6 mm x 250 mm, particle size 5 μm , or equivalent.

7.1.2 Mobile phase. Acetonitrile-0.1% phosphoric acid aqueous solution (90 + 10, volume ratio).

7.2 Liquid chromatography determination

Take the sialic acid series standard solution and test solution, respectively. Use the peak area of the standard series solution as the ordinate and the concentration as the abscissa, draw the standard curve.

8 Result calculation

The measurement results can be automatically calculated by the instrument workstation, or calculated according to formula (1).

9.1 General provisions

The precision of this standard is determined in accordance with the provisions of GB/T 6379.2. The repeatability and reproducibility values are calculated with

95% confidence.

9.2 Repeatability

Under repeatability conditions, the absolute difference between two independent test results as obtained does not exceed the repeatability limit (r). The content range and repeatability equation of different samples are as shown in Table 1.

9.3 Reproducibility

Under the condition of reproducibility, the absolute difference between the two independent test results as obtained does not exceed the reproducibility limit (R). The content range and reproducibility equation of different samples are as shown in Table 1.

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