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中华人民共和国出入境检验检疫行业标准

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进出口蜂蜜中咖啡因含量检验 方法 液相色谱法

Determination of caffeine in honey for import and export —Liquid chromatography

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前　言

本标准的附录 A 为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准由中华人民共和国湖南出入境检验检疫局负责起草。

本标准主要起草人：戴华、李拥军、熊芳、李献坤、黄志强、袁智能。

本标准系首次发布的检验检疫行业标准。

进出口蜂蜜中咖啡因含量检验 方法 液相色谱法

1 范围

本标准规定了进出口蜂蜜中咖啡因含量检验的抽样、制样和液相色谱测定方法。

本标准适用于进出口蜂蜜中咖啡因含量的检验。

2 抽样和制样

2.1 检验批

以不超过1 000件(或25 t)为一检验批。

同一检验批的商品应具有相同的特性,如包装、标记、产地、规格和等级等。

2.2 抽样数量

抽样数量见表1。

表1

单位为件

批量	最低抽样数
1～10	1
11～50	5
51～100	10
101～500	每增加100,增取5
501以上	每增加100,增取2

2.3 抽样工具

2.3.1 取样管:不锈钢管,长约115 cm,内径约2.5 cm;玻璃管,长约115 cm,内径约2.5 cm。

2.3.2 混样器:搪瓷桶(或杯)。

2.3.3 样品瓶:500 mL具塞广口玻璃瓶。

2.4 抽样方法

按2.2规定的抽样件数随机抽取,逐件开启。将玻璃管缓缓从开口处放入直至桶底,吸取样品。如遇蜂蜜结晶,用不锈钢管缓缓插入,直至桶底,吸取样品。每件抽取样品不少于100 g作为原始样品。将所取样品倾入混样器内,混合均匀,分出约1 kg,装入清洁干燥的样品瓶内,加封后,标明标记,及时送交实验室。

如属瓶装蜂蜜,按应开件数,从每件中随机取一瓶,标明标记,及时送交实验室。

2.5 试样制备

将取回样品充分搅匀,均分成两份,装入试样瓶中作为试样,密封,标明标记。

如取回样品中有结晶析出,则将样品瓶加盖后,置于不超过40℃的水浴中温热。待结晶全部融化后,启盖,充分搅匀,均分成两份,密封,并标明标记。

如属瓶装蜂蜜,将取回的全部蜂蜜,倒在一起,充分搅匀,分取出约1 kg代表性样品。再将1 kg代表性样品经搅匀后,均分成两份,装入试样瓶中作为试样,密封,标明标记。在抽样和制样的操作过程

中，应防止样品受到污染或发生含量的变化。

2.6 试样保存

将试样于室温下保存。

3 测定方法

3.1 方法提要

样品中咖啡因在碱性条件下，用三氯甲烷提取，提取液经净化、浓缩后，用配有紫外检测器的液相色谱仪测定，外标法定量。

3.2 试剂和材料

所有试剂除特殊注明外，均为分析纯，水为重蒸馏水。

3.2.1 乙腈：液相色谱纯或相当者。

3.2.2 冰乙酸：99.9%。

3.2.3 三氯甲烷。

3.2.4 氢氧化钠水溶液：称取 5.0 g 氢氧化钠用 100 mL 水溶解。

3.2.5 咖啡因：纯度≥99.5%。

3.2.6 标准溶液(1 mg/mL)：准确称取 0.1 g(准确至 0.000 1 g)咖啡因标准品，用水溶解并定容至 100 mL，作为标准储备液。再根据需要用水将标准储备液稀释至适当浓度。

3.3 仪器和设备

3.3.1 高效液相色谱仪配紫外检测器。

3.3.2 旋涡混匀器。

3.3.3 离心机：4 000 r/min。

3.3.4 多功能微量化样品处理仪或类似者。

3.4 测定步骤

3.4.1 提取、净化

准确称取 20 g(准确至 0.01 g)试样于 50 mL 具塞离心管中，加入 10 mL 氢氧化钠水溶液混匀，然后定量加入 20.0 mL 三氯甲烷，加塞，在旋涡混匀器上混匀 5 min，于 2 000 r/min 离心 5 min，将上层水相用尖嘴吸管吸取弃去，有机相再用 2×5 mL 氢氧化钠水溶液洗涤两次，弃去水相；将有机相在旋涡混合器上混匀，用干燥滤纸过滤，弃去初液 3 mL，收集滤液，定量取滤液 10.0 mL，于 45℃空气流下浓缩至近干，定量加入 2.0 mL 水溶解残渣，过 0.45 μm 微孔滤膜后，上液相色谱仪测定，外标法定量。

3.4.2 测定

3.4.2.1 色谱条件

a) 色谱柱：ODS C18 柱，25 cm×4.6 mm(内径)×5 μm 或类似柱；

b) 流动相：乙腈：水：乙酸(16+83+1)；

c) 流速：0.50 mL/min；

d) 检测波长：273 nm；

e) 进样量：20 μL；

f) 柱温：40℃。

3.4.2.2 色谱测定

根据样液中咖啡因的含量情况，选定峰面积相近的标准工作溶液。标准工作溶液和样液中的咖啡因的响应值均应在仪器的检测线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述色谱条件下，咖啡因的保留时间约为 8.8 min，标准品色谱图参见附录 A。

3.4.3 空白试验

除不加试样外，按上述测定步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中咖啡因的含量，计算结果需扣除空白值。

$$X = \frac{A \cdot c_s \cdot V}{A_s \cdot m} \quad \cdots\cdots (1)$$

式中：

X——试样中咖啡因的含量，单位为毫克每千克(mg/kg)；

A——样液中咖啡因的峰面积，单位为平方毫米(mm^2)；

c_s——标准工作液中咖啡因的浓度，单位为微克每毫升(μg/mL)；

A_s——标准工作液中咖啡因的峰面积，单位为平方毫米(mm^2)；

V——样液最终定容体积，单位为毫升(mL)；

m——最终样液所代表的试样量，单位为克(g)。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.025 mg/kg。

4.2 回收率

蜂蜜中咖啡因的添加浓度及其回收率的实验数据：

在 0.025 mg/kg 时，回收率为 93.2%；

在 0.050 mg/kg 时，回收率为 96.0%；

在 1.0 mg/kg 时，回收率为 98.4%。

附 录 A
（资料性附录）
标准品色谱图

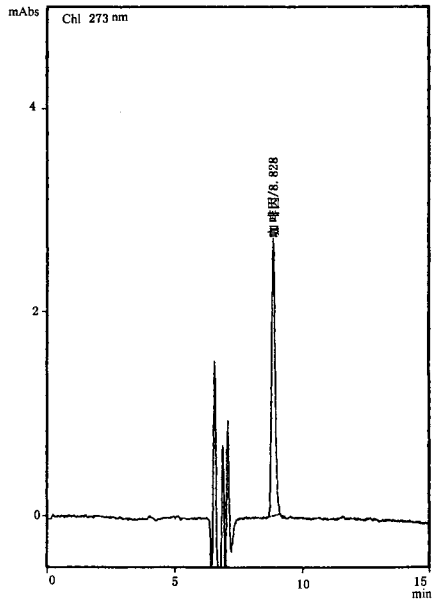


图 A.1 咖啡因标准品的液相色谱图

Foreword

Annex A of this standard are informative annex.

This standard was proposed by and is under the charge of National Regulatory Commission for Certification and Accreditation.

This standard was drafted by Hunan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Dai Hua, Li Yongjun, Xiong Fang, Li Xiankun, Huang Zhiqiang and Yuan Zhineng.

This standard is a professional standard promulgated for the first time.

Note: This English version, a translation from the Chinese text, is solely for guidance.

Determination of caffeine in honey for import and export—Liquid chromatography

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of caffeine contents in honey for export by liquid chromatography.

This standard is applicable to the determination of caffeine contents in honey for export by liquid chromatography.

2 Sampling and sample preparation

2.1 Inspection lot

Each inspection lot should not exceed 1 000 packages(or 25 t).

The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification and grade, should be the same.

2.2 Quantity of sample taken

Quantity of sample taken see table 1.

Table 1

Number of packages in an inspection lot	Minimum number of packages to be taken
1～10	1
11～50	5
51～100	10
101～500	5 more packages for every increment of 100 packages
501～1 000	2 more package for every increment of 100 packages

2.3 Sampling tools

2.3.1 Sampling tube: Stainless steel tube, length: ca 115 cm, internal diameter: ca 2.5 cm; glass tube, length: ca 115 cm, internal diameter: ca 2.5 cm.

2.3.2 Mixing vessel: Enamel drum(or cup).

2.3.3 Sample bottle: 500 mL, wide-mouthed glass bottle, with ground stopper.

2.4 Sampling procedure

Draw a number of sample packages specified in 2.2 at random and open the packages one by one.

Take the sample from the package by inserting slowly the sampling tube to the bottom. If the honey is crystallized, penetrate the stainless steel tube to the bottom of the container. The sample taken from each package should not be less than 100 g as a primary sample. Pour the drawn sample into the mixing vessel, and mix thoroughly. Draw ca 1 kg of the mixed sample and place in a clean dry sample bottle, seal, label and sent to the laboratory in time.

If the honey was packed in bottles inside the package, draw one bottle from each of the packages. Label and send to the laboratory in time.

2.5 Preparation of test sample

Mix all the samples taken back thoroughly, and divide into two equal portions. Each portion is placed in a sample bottle as the test sample, which is sealed and labeled. If the sample taken back becomes crystallized, melt it by warming in water-bath below 40℃ with the sample bottle covered tightly. When the sample has completely melted, remove the cover and mix thoroughly. Divide into two equal portions, each portion is placed in a sample bottle as the test sample, sealed and labeled.

For bottle-packed honey, pour all the honey taken back into a vessel and mix thoroughly. Draw ca 1 kg of the representative sample, mix well and divide into two equal portions. Each portion is placed in a sample bottle as the test sample, sealed and labeled. In the course of sampling and sample preparation, precautions should be taken to avoid contamination or any factors which may cause the change of content.

2.6 Storage of test sample

The test samples should be stored at room temperature.

3 Method of determination

3.1 Principle

The caffeine is extracted from the test sample with chloroform under alkaline condition, then the extract is cleaned up and concentrated before determination by HPLC with UV detector. External standard method is used for quantification.

3.2 Reagents and materials

Unless otherwise specified, all reagents are should be analytical pure, "water" is redistilled water.

3.2.1 Acetonitrile: HPLC grade or equivalent.

3.2.2 Acetic acid glacial, 99.9%.

3.2.3 Chloroform.

3.2.4 Sodium hydroxide solution: Dissolved 5.0 g of NaOH in 100 mL water.

3.2.5 Caffeine: purity≥99.5%.

3.2.6 Standard solution: Weigh accurately about 0.1 g(accurate to 0.000 1 g)caffeine standard into a 100 mL volumetric flask. Dissolve and make up to mark with water as the standard stock solution. Dilute the standard stock solution with water to the required concentration as the standard working solution.

3.3 Apparatus and equipment

3.3.1 HPLC equipped with UV detector.

3.3.2 Mixer.

3.3.3 Centrifuge: 4 000 r/min.

3.3.4 Multifunction sample treatment unit microchemical method or equivalent.

3.4 Procedure

3.4.1 Extraction and clean-up

Weigh 20 g(accurate to 0.01 g)sample into 50 mL centrifuge tube with stopper, add 10 mL sodium hydroxide solution, mix for 1 min. Then added 20.0 mL chloroform of the extract into the centrifuge tube sealed, mix on a mixer for 5 min, centrifugalize at 2 000 r/min for 5 min, draw out the water layer with a capillarytipped pipette and discard, then treated the organic layer with 2×5 mL sodium hydroxide solution two times, draw out the water layer and discard. The extract was filter by dry filter paper and discard the previous 3 mL filtrate, collect the filtrate and pipet 10.0 mL filtrate and evaporate to near dryness under a stream of air at 45℃. Dissolve the residue in 2.0 mL water, and the filter through 0.45 μm membrane filter. The solution is used for HPLC determination.

3.4.2 Determination

3.4.2.1 HPLC operating condition

a) Column: ODS C18 column 25 cm×4.6 mm(i.d.)×5 μm particle size or equivalent;
b) Mobile phase: Acetonitrile: water: acetic acid(16+83+1);
c) Flow rate: 0.50 mL/min;
d) Detector wavelength: 273 nm;
e) Injection volume: 20 μL.
f) Column temperature: 40℃

3.4.2.2 LC determination

According to the approximate concentration of caffeine in the sample solution, select the standard working solution with similar peak area to that of the sample solution. The responses of caffeine in

the standard working solution and sample solution should be within the linear range of the instrumental detection. The standard working solution should be randomly injected in-between the injections of the sample solution of equal volume. Under the above operating condition, the retention time of caffeine is about 8.8 min. For chromatogram of the standard, see FigA.1 in annex A.

3.4.3 Blank test

The operation of the blank test is the same as that describe in the method of determination but without addition of sample.

3.5 Calculation and expression of the result

The calculation of the content of caffeine in the test sample is carried out by LC data processor or according to the formula(1), The blank value should be subtracted from the result of calculation.:

$$X = \frac{A \cdot c_s \cdot V}{A_s \cdot m} \quad \cdots\cdots(1)$$

Where:

X——the residue content of caffeine in the test sample, mg/kg;

A——the peak area of caffeine in the sample solution, mm^2;

c_s——the concentration of caffeine in the standard working solution, $\mu g/mL$;

A_s——the peak area of caffeine in the standard working solution, mm^2;

V——the last volumn of the sample solution, mL;

m——the corresponding mass of the sample in the final sample solution, g.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.025 mg/kg.

4.2 Recovery

According to the experiment data, the fortifying concentrations of caffeine in honey and its corresponding recoveries are:

0.025 mg/kg, the recovery is 93.2%;

0.050 mg/kg, the recovery is 96.0%;

1.0 mg/kg, the recovery is 98.4%.

Annex A
(informative)
Chromatogram of the standard

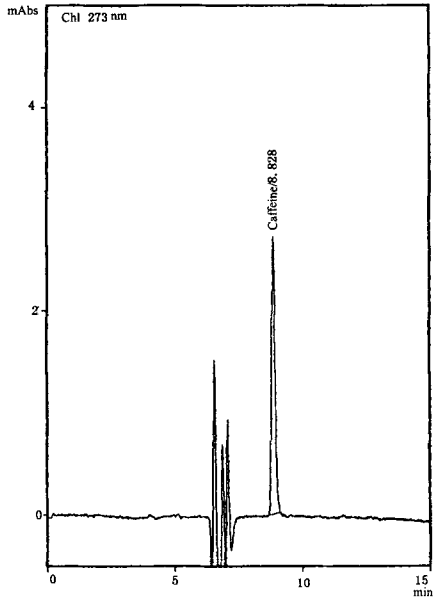


Fig A.1 Liquid chromatogram of caffeine standard