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中华人民共和国出入境检验检疫行业标准

SN/T 4002—2014

出口保健食品中水飞蓟宾的测定 高效液相色谱法

Determination of silybin in health foods for export—HPLC method

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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国湖南出入境检验检疫局、湖南省检验检疫科学技术研究院、中华人民共和国厦门出入境检验检疫局。

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出口保健食品中水飞蓟宾的测定 高效液相色谱法

1 范围

本标准规定了保健食品中水飞蓟宾的高效液相色谱测定方法。

本标准适用于胶囊和片剂类保健食品中水飞蓟宾的测定。

2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本文件。凡是不注日期的引用文件，其最新版本(包括所有的修改单)适用于本文件。

GB/T 6682 分析实验室用水规格和试验方法

3 原理

样品中的水飞蓟宾采用甲醇超声提取，高效液相色谱法测定，外标法定量。

4 试剂和材料

除特殊注明外，所有试剂均为分析纯，水为符合 GB/T 6682 规定的一级水。

4.1 甲醇：液相色谱级。

4.2 磷酸。

4.3 磷酸二氢钠。

4.4 磷酸溶液(0.2 mol/L)：移取 1.33 mL 磷酸于 100 mL 容量瓶中，用水稀释并定容至刻度。

4.5 磷酸二氢钠溶液(0.5 mol/L)：称取 6.0 g 磷酸二氢钠于 100 mL 容量瓶中，用水溶解并定容至刻度。

4.6 水飞蓟宾标准品(CAS 号：22888-70-6，分子式：$C_{25}H_{22}O_{10}$)：水飞蓟宾 A 和水飞蓟宾 B 混合物，纯度大于等于 98%。

4.7 水飞蓟宾标准储备液：称取水飞蓟宾标准品(4.6)适量，用甲醇溶解，配制成浓度为 1.0 mg/mL 的标准储备液，4 ℃下避光保存。

4.8 水飞蓟宾标准工作溶液：吸取适量的水飞蓟宾标准储备液(4.7)，用甲醇稀释成适当浓度的系列标准工作溶液，使用前配制。

5 仪器

5.1 高效液相色谱仪-配二极管阵列检测器。

5.2 超声波清洗器。

5.3 分析天平：感量为 0.000 1 g 和 0.01 g。

6 试样制备与保存

6.1 试样制备

6.1.1 片剂

研细，混匀，均分成两份，分别装入洁净容器内，加封后作出标记：一份作为试样；一份作为备测样。

6.1.2 硬胶囊

倾出所有内容物，研细，混匀，均分成两份，装入清洁容器内，加封后作出标记：一份作为试样；一份作为备测样。

6.1.3 软胶囊

倾出所有内容物，混匀，均分成两份，装入清洁容器内，加封后作出标记：一份作为试样；一份作为备测样。

6.2 试样的保存

将试样于 0 ℃～4 ℃保存。在取样、制样过程中，应防止样品受到污染或发生目标物含量的变化。

7 测定步骤

7.1 提取

称取 0.5 g(精确至 0.01 g)试样于 100 mL 容量瓶中，加入 90 mL 甲醇超声提取 25 min，间隔 10 min取出混匀 1 次，放冷，加甲醇稀释至刻度，摇匀，静置 2 min，取上清液过 0.45 μm 微孔有机滤膜后，供液相色谱仪测定。

7.2 测定

7.2.1 液相色谱条件

试验所用液相色谱条件如下：

a) 色谱柱：C_{18}柱，250 mm×4.6 mm(内径)，5 μm 或相当者；

b) 流动相：流动相 A：甲醇-水-0.2 mol/L 磷酸-0.5 mol/L 磷酸二氢钠(80＋120＋1＋8，体积比)，流动相 B：甲醇，梯度洗脱程序见表 1；

c) 流速：1.0 mL/min；

d) 柱温：40 ℃；

e) 进样量：10 μL；

f) 检测波长：288 nm。

表 1　流动相梯度洗脱程序

时间 min	流动相 A %	流动相 B %
0.0	100	0
5.0	80	20
24.0	75	25
24.1	100	0
30.0	100	0

7.2.2　定量测定

按照 7.2.1 色谱条件测定样液和标准工作溶液，外标法定量。待测样液中水飞蓟宾的量应在标准曲线范围之内，如果超出标准曲线范围，应稀释到合适浓度后分析。在上述仪器条件下，水飞蓟宾 A 和水飞蓟宾 B 保留时间分别约为 18.01 min、19.29 min。标准溶液的色谱图参见附录 A 中图 A.1。

7.2.3　空白实验

除不加试样外，均按上述测定步骤进行样品空白试验。

7.3　结果计算和表述

用色谱数据处理软件或按式(1)计算试样中水飞蓟宾含量，计算结果应扣除空白值：

$$X = \frac{c_s \times V \times A}{m \times A_s \times 1\,000} \qquad \cdots\cdots(1)$$

式中：

X ——试样中水飞蓟宾的含量，单位为毫克每克(mg/g)；

c_s ——标准工作液中水飞蓟宾的浓度，单位为微克每毫升(μg/mL)；

A_s——标准工作液中水飞蓟宾 A 和水飞蓟宾 B 的峰面积之和；

A ——样液中水飞蓟宾 A 和水飞蓟宾 B 的峰面积之和；

V ——试样定容体积，单位为毫升(mL)；

m ——称样量，单位为克(g)。

8　测定低限、回收率

8.1　定量限

本方法的定量限为 0.5 mg/g。

8.2　回收率

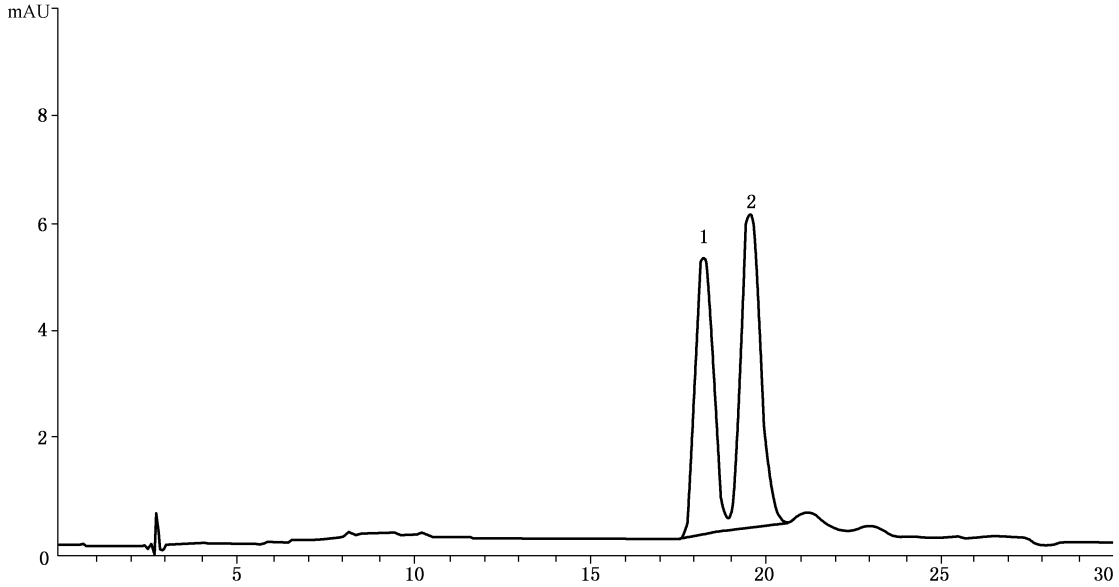
保健食品胶囊和片剂中水飞蓟宾的添加水平及回收率数据见表 2。

表 2　胶囊和片剂中水飞蓟宾的添加水平及回收率

样品基质	添加水平 mg/g	回收率范围 %
胶囊	0.5	96.8～108.0
	5.0	96.0～102.9
	50	95.0～102.5
	250	96.2～101.5
	800	97.8～100.2
片剂	0.5	93.2～107.6
	5.0	96.0～104.7
	50	93.3～100.9
	250	97.2～99.9
	800	95.7～100.5

附 录 A
（资料性附录）
水飞蓟宾标准溶液的色谱图

水飞蓟宾标准溶液的色谱图见图 A.1。



说明：

1——水飞蓟宾 A；

2——水飞蓟宾 B。

图 A.1 水飞蓟宾标准溶液的色谱图

Foreword

This standard was drafted according with GB/T 1.1—2009.

Please note that some of the content of this document may involve patents, the publisher of this document does not assume the responsibility to identify these patents.

This standard was proposed by and is under the charge of the Certification and Accreditation Administration of the People's Republic of China.

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

The standard was mainly drafted by Meiling Wang, Yamei Shi, Yanna Jiao, Jingtao Liang, Shanliang Fu, Hongfei Yan, Ying Zhang, Zhiqiang Huang.

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

Determination of silybin in health foods for export—HPLC method

1 Scope

This standard specifies the determination of silybin in health foods for export by HPLC method.

This standard is applicable to the determination of silybin in capsule and troche.

2 Cited normative documents

The following normative documents are indispensable to the application of this standard. For dated references, only the edition bearing such date applies to this standard. For undated references, the latest edition of the normative document referred to (including all the amendments) applies.

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

3 Abstract of method

Silybin in sample is extracted with methanol in an ultrasonic washer. The extract is determined by HPLC. External standards method is used for quantitative measurement.

4 Reagents and materials

Unless otherwise specified, all the reagents should be of analytical grade. "water" is the first grade water prescribed by GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Phosphoric acid.

4.3 Sodium dihydrgen phosphate.

4.4 0.2 mol/L phosphoric acid solution: Accurately transfer 1.33 mL phosphoric acid into 100 mL volumetric flask, then make up to graduation with water.

4.5 0.5 mol/L sodium dihydrgen phosphate solution: Accurately weight 6.0 g sodium dihydrgen phosphate solution into 100 mL volumetric flask, dissolve with water and make up to graduation.

4.6 Silibin standard (CAS: 22888-70-6, molecular formula: $C_{25}H_{22}O_{10}$): Mixture of silibin A and silibin B), purity≥98%.

4.7 Standard stock solution: Accurately weight an adequate amount of silybin (4.6) dissolve in methanol to make a standard stock solution of 1.0 mg/mL in concentration. The standard solution stored below 4 ℃ avoiding sunlight.

4.8 Standard working solution: Dilute the standard stock solution with methanol (4.7) to prepare a series of standard working solutions just before use.

5 Apparatus and equipment

5.1 High performance liquid chromatography equipped with diode-array detector (DAD).

5.2 Ultrasonic washer.

5.3 Electronic balance: Readability 0.000 1 g and 0.01 g.

6 Sample preparation and storage

6.1 Preparation of test sample

6.1.1 Tablet

The test sample are grinded and blended to produce homogeneous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled.

6.1.2 Capsule

The test sample are spilled out, ground into power and blended to produce homogeneous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled.

6.1.3 Soft capsule

The test sample are spilled out and blended to produce homogeneous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled.

6.2 Storage of test sample

The test sample should be stored at 0 ℃～4 ℃. While sampling and sample preparation, precaution

must be taken to avoid contamination or any factors that may cause the change of residue content.

7 procedure

7.1 Extraction

Weigh ca 0.5 g(accurate to 0.01 g)of the test sample into a 100 mL volumetric flask. Add 90 mL methanol and extracted in an ultrasonic washer for 25 min. Take it out and shake every ten minute. Cool down to room temperature, and dilute to mark with methanol. Mix the contents and allowed to stand for 2 min. The supernatant layer is filtered through the 0.45 μm membrane filter and ready for HPLC analysis.

7.2 Determination

7.2.1 LC operating condition

LC operating condition is as following:

a) Chromatographic column: Octadecyl silica(ODS) or equivalent column(4.6 mm i.d. × 250 mm, 5 μm);

b) Mobile phase: A Methanol-water-0.2 mol/L phosphoric acid solution-0.5 mol/L sodium dihydrgen phosphate solution (80 + 120 + 1 + 8, *V*/*V*); B: Methanol. Gradient program see Table 1;

c) Flow rate: 1.0 mL/min;

d) Column temperature: 40 ℃;

e) Injection volume: 10 μL;

f) Detection wavelength: 288 nm.

Table 1 gradient program of mobile phase

Time min	A %	B %
0.0	100	0
5.0	80	20
24.0	75	25
24.1	100	0
30.0	100	0

7.2.2 Qualitative determination

According to LC operating condition assigned 7.2.1 analyze the standards solution and the sample solution. The quality of the results obtained using external standards. The responses of silibin in the sample solution should be in the linear range of standards solution. If the response is above the linear range, dilute the sample solution. Under the above HPLC operating conditions, the retention time of silybin A and silybin B is ca 18.01 min, 19.29 min respectively. The chromatogram of standard solution is shown in Figure A.1 in annex A.

7.2.3 Blank test

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

7.3 Calculation and expression of result

Calculate the content of silybin in the test sample by LC data processor or using the followed formula (1), the blank value should be subtracted from the result of calculation:

$$X = \frac{c_s \times V \times A}{m \times A_s \times 1\,000} \qquad \cdots\cdots(1)$$

Where:

X —the content of silybin in the test samples, mg/g;

c_s —the concentration of silybin in the standard working solution, μg/mL;

A_s —the sum of peak areas of silybin A and silybin B in the working standard solution;

A —the sum of peak areas of silybin A and silybin B in the test sample solution;

V —the final volume of sample solution, mL;

m —the corresponding mass of test sample, g.

8 Limit of quantitation and recovery

8.1 The limit of quantitation

The limit of quantitation of silybin is 0.5 mg/g.

8.2 Recovery

According to the experiment data, the fortified concentration of silybin for each sample and the range of recovery are shown in table 2.

Table 2 The fortifying concentration and the range of recovery of silybin in capsule and tablet

Sample	Fortified level mg/g	Range of recovery %
Capsule	0.5	96.8～108.0
	5.0	96.0～102.9
	50	95.0～102.5
	250	96.2～101.5
	800	97.8～100.2
Tablet	0.5	93.2～107.6
	5.0	96.0～104.7
	50	93.3～100.9
	250	97.2～99.9
	800	95.7～100.5

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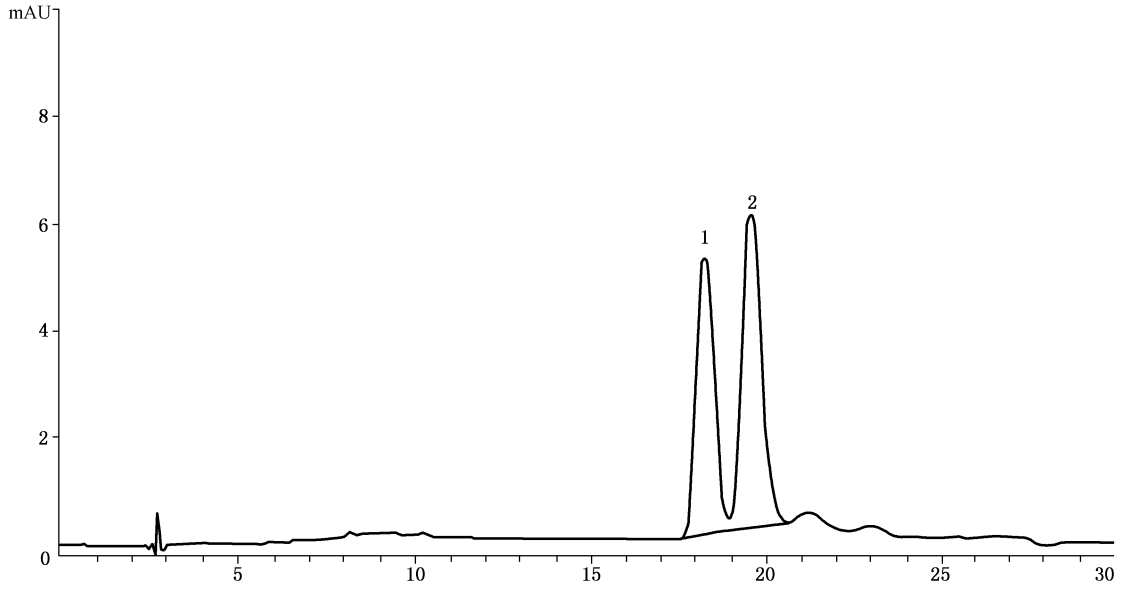
Annex A
(Informative)
The high performance liquid chromatogram of silybin standard solution

The high performance liquid chromatogram of silybin standard solution see figure A.1.



1—silybin A.
2—silybin B.

Figure A.1 The high performance liquid chromatogram of silybin standard solution