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中华人民共和国进出口商品检验行业标准

SN 0339－95

出口茶叶中黄曲霉毒素 B_1 检验方法

Method for the determination of aflatoxin B_1 in tea for export

1995-05-29发布　　1995-11-01实施

中华人民共和国国家进出口商品检验局　发布

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1 主题内容与适用范围

本标准规定了出口茶叶中黄曲霉毒素 B_1 含量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口茶叶中黄曲霉毒素 B_1 含量的检验。

2 抽样和制样

2.1 检验批

以不超过 2 000 件为一检验批。

同一检验批的商品应具有相同的特征，如包装、标记、产地、规格、等级等。

2.2 抽样数量

批量，件	最低抽样数，件
1～5	1
6～50	2
51～500	11
501～1 000	16
1 001～1 500	19
1 501～2 000	20

2.3 抽样方法

从整批产品堆垛的上下不同部位随机抽取 2.2 规定的件数，逐件开启。分别倒出全部茶叶于塑料布上，用取样铲从每件中各取出有代表性的样品约 500 g。将所取样品充分混匀，用四分法或分样器逐步缩分出 500 g，装入洁净密封的样品筒内，加封后，标明标记，及时送实验室。

2.4 试样制备

将所取回样品全部磨碎，通过 20 目筛，混匀，均分成两份试样，装入洁净容器内，密封，标明标记。

2.5 试样保存

将试样于室温下保存。

注：在抽样和制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

样品用三氯甲烷提取，提取液经硅胶柱净化，净化后的提取液用三氟乙酸衍生，用配有荧光检测器的液相色谱仪测定，外标法定量。

3.2 试剂和材料

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所用试剂，除特殊注明外，均为分析纯，水为重蒸馏水。

3.2.1 三氯甲烷。

3.2.2 正己烷。

3.2.3 苯。

3.2.4 甲醇：紫外光谱级。

3.2.5 乙腈：紫外光谱级。

3.2.6 三氟乙酸。

3.2.7 乙腈-水溶液(1+1)。

3.2.8 三氯甲烷-甲醇溶液(95+5)。

3.2.9 苯-乙腈溶液(98+2)。

3.2.10 黄曲霉毒素 B_1 标准品：纯度≥99%。

3.2.11 黄曲霉毒素 B_1 标准溶液：准确称取适量的黄曲霉毒素 B_1 标准品，以苯-乙腈溶液(3.2.9)于棕色容量瓶中，配成浓度为 10 μg/mL 标准贮备液。根据需要，再配成适当浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 液相色谱仪，配有荧光检测器。

3.3.2 硅胶小柱：Waters Sep-pak Silica 前处理小柱或相当的硅胶前处理小柱。

3.3.3 振荡器。

3.3.4 旋转蒸发器，配有 100 mL 具尾管的圆底烧瓶。

3.3.5 微量注射器。

3.3.6 离心管：5 mL 具塞磨口。

3.3.7 粉碎机。

3.3.8 滤膜：有机系用，0.45 μm。

3.3.9 微孔滤膜过滤器：有机系用，0.5 μm。

3.4 测定步骤

3.4.1 提取

称取试样 5.0 g(精确到 0.1 g)置于 100 mL 具塞锥形烧瓶中，加入 15 mL 三氯甲烷，于振荡器上提取 30 min，然后经垫有玻璃纤维的漏斗过滤。收集滤液于旋转蒸发器的具尾管圆底烧瓶内，并用三氯甲烷洗涤滤渣，收集滤液至约 20 mL。

3.4.2 净化

用旋转蒸发器将上述滤液在 50℃水浴中浓缩至约 1 mL，经 0.45 μm 滤膜过滤后，注入硅胶小柱中。用 2～4 mL 正己烷洗涤烧瓶后淋洗小柱，弃去流出液。然后用 3～4 mL 三氯甲烷-甲醇溶液(3.2.8)以每秒钟 2 滴的流速洗脱，收集洗脱液于离心管中。用氮气缓缓吹干，供衍生用。

3.4.3 衍生

3.4.3.1 试样

加 200 μL 正己烷和 50 μL 三氟乙酸于上述离心管中，盖紧磨口塞，超声振荡 1 min，静置 10 min，打开磨口塞，缓缓通入氮气至干。用乙腈-水溶液(1+1)(3.2.7)定容至 1.0 mL，超声 1 min，用 0.5 μm 滤膜过滤，滤液供液相色谱用。

3.4.3.2 标准工作溶液

取 1.0 mL 标准工作溶液，用氮气缓缓吹干，按 3.4.3.1 步骤操作。

3.4.4 测定

3.4.4.1 色谱条件

a. 色谱柱：NOVA PAK C_{18}，300 mm×3.9 mm(内径)。

b. 流动相：甲醇-水溶液(42+58)。

c. 流速：0.8 mL/min。

d. 荧光检测器：激发波长 375 nm，发射波长 425 nm。

e. 色谱柱温度：室温。

3.4.4.2 测定

根据样液中黄曲霉毒素 B_1 的含量情况，选定峰高相近的标准工作溶液。标准工作溶液和样液中黄曲霉毒素 B_1 衍生物的响应值均应在仪器检测线性范围内。对标准工作溶液和样液的衍生物溶液等体积参插进样测定。在上述色谱条件下，黄曲霉毒素 B_1 衍生物保留时间约为 8 min。

3.4.4.3 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下式计算试样中黄曲霉毒素 B_1 的含量：

$$X = \frac{A \cdot c_s}{A_s \cdot c}$$

式中：X——试样中黄曲霉毒素 B_1 的含量，mg/kg；

A——样液中黄曲霉毒素 B_1 衍生物的峰面积，mm^2；

c_s——标准工作溶液中黄曲霉毒素 B_1 的浓度，μg/mL；

A_s——标准工作溶液中黄曲霉毒素 B_1 衍生物的峰面积，mm^2；

c——最终样液所代表试样的浓度，g/mL。

注：计算结果需扣除空白值。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.001 mg/kg。

4.2 回收率

回收率的实验数据：黄曲霉毒素 B_1 的添加浓度在 0.001～0.5 mg/kg 范围内，回收率为 89.1%～104.9%。

附加说明：

本标准由中华人民共和国国家进出口商品检验局提出。

本标准由中华人民共和国江西进出口商品检验局负责起草。

本标准主要起草人徐文彦、黄小明、施小珊。

Professional Standard of the People's Republic of China for Import and Export Commodity Inspection

SN 0339—95

Method for the determination of aflatoxin B_1 in tea for export

1 Scope and field of application

This standard specifies the method of sampling, sample preparation and determination by high performance liquid chromatography of aflatoxin B_1 in tea for export.

This standard is applicable to the determination of aflatoxin B_1 in tea for export.

2 Sampling and sample preparation

2.1 Inspection lot

Each inspection lot should not exceed 2 000 packages.

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 in a lot, packages	Minimum quantity to be taken, packages
1—5	1
6—50	2
51—500	11
501—1 000	16
1 001—1 500	19
1 501—2 000	20

2.3 Sample procedure

Draw the number of packages specified in 2.2 at different parts of the pile at random. Open and empty out all contents of each package on a clean plastic sheet. Draw the representative sample from the respective packages, the quantity of the sample drawn from each package should be about 500 g. Mix and reduce the mixed representative sample to ca 500 g by quartering or by a riffle sampler, place in a clean container which is then sealed, labeled and sent to laboratory in time.

2.4 Preparation of test sample

Grind all the sample thoroughly and let pass through a 20-mesh sieve, divide into 2 equal portions. Place in clean containers, seal and label.

2.5 Storage of test sample

The test sample should be stored at room temperature.

Note: In the course of sampling and sample preparation, precaution must be taken to avoid contamination or any factors which may cause the change of residue content.

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on May. 29, 1995

Implemented from Nov. 1, 1995

3 Method of determination

3.1 Principle

The aflatoxin B_1 is extracted from the sample with trichloromethane, the extract is cleaned up by silica gel column, then the cleaned-up extract is derivatized by trifluoroacetic acid and determinated by HPLC with fluorescence detector, using external standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents are analytical grade, water is redistilled water.

3.2.1 Trichloromethane.

3.2.2 n-Hexane.

3.2.3 Benzene.

3.2.4 Methanol, UV grade.

3.2.5 Acetonitrile, UV grade.

3.2.6 Trifluoroacetic acid.

3.2.7 Acetonitrile-water solution((1+1).

3.2.8 Trichloromethane-methanol solution(95+5).

3.2.9 Benzene-acetonitrile solution(98+2).

3.2.10 Aflatoxin B_1 standard: Purity≥99%.

3.2.11 Aflatoxin B_1 standard solution: Weigh a suitable amount of aflatoxin B_1, and dissolve with benzene-acetonitrile solution(3.2.9)in brown volumetric flask and dilute to form a standard stock solution of 10 μg/mL. According to the requirement, prepare the standard working solution of appropriate concentration.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph, equipped with fluores-cence detector.

3.3.2 Silica gel column: Waters Sep-pak Silica column or equivalent.

3.3.3 Shaker.

3.3.4 Rotary evaporator, with round-bottom flask with 100 mL of protruded tip.

3.3.5 Micro-injector.

3.3.6 Centrifuge tube: 5 mL, with ground stopper.

3.3.7 Grinder.

3.3.8 Filtration membrane(for organic solvent), 0.45 μm.

3.3.9 Microporous membrane(for organic solvent), 0.5 μm.

3.4 Procedure

3.4.1 Extraction

Weigh 5.0 g(accurate to 0.1 g)of the test sample into a 100 mL Erlenmeyer flask with stopper, add 15 mL trichloromethane and extract for 30 min on the shaker. Filter through a funnel with some fibreglass and collect the clear filtrate in the round-bottom flask (with protruded tip)of the rotary evaporator. Wash the residue with trichloromethane, the total volume of the filtrate is about 20 mL.

3.4.2 Cleanup

Evaporate the above filtrate to about 1 mL in a water-bath at 50℃ with the rotary evaporator, filter through a 0.45 μm membrane and transfer into Sep-pak Silica column, rinse the flask then the column with 2—4 mL of n-hexane and discard the effluent. Finally, elute the column with 3—4 mL trichloromethane-methanol(3.2.8)(flow rate 2 drop each second), and the eluate is collected in a cen-

trifuge tube, evaporate gently to dryness with nitrogen current.

3.4.3 Derivatization

3.4.3.1 For sample

Add 200 μL of n-hexane and 50 μL of trifluoroacetic acid into the above centrifuge tube. Cover tightly and vibrate by ultrasonic means for 1 min. Let stand for 10 min, open the cover of the tube, and blow gently to dryness with nitrogen current. Dissolve the residue with 1.0 mL of acetonitrile-water solution(1+1), vibrate it by ultrasonic means for 1 min, and filter through the 0.5 μm membrane filter, and collect the filtrate in a sample vial for HPLC analysis.

3.4.3.2 For standard working solution

Take 1.0 mL of standard working solution, evaporate to dryness under nitrogen current, and proceed as in 3.4.3.1.

3.4.4 Determination

3.4.4.1 HPLC operating condition

a. Chromatographic column: NOVA PAK C_{18}, 300 mm×3.9 mm(id);

b. Mobile phase: methanol-water(42+58);

c. Flow rate: 0.8 mL/min;

d. Fluorescence Detector:

Excitation wavelength: 375 nm,

Emission wavelength: 425 nm;

e. Column temperature: Room temperature.

3.4.4.2 Determination

According to the content of aflatoxin B_1 in the sample solution, select the approximate standard working solution with similar peak height to that of sample solution. The responses of aflatoxin B_1 derivative in the standard solution and sample solution should be in the linear range of the instrumental detection. The derivative of standard working solution should be injected randomly in-between the injections of the derivative of sample solution of equal volume. Under above condition, the retention time of aflatoxin B_1 derivative is about 8 min.

3.4.4.3 Blank test

The operation of the blank test is the same as that described in the method of determination, but with omission of sample addition.

3.5 Calculation and expression of result

The calculation of content of aflatoxin B_1 can be carried out by a data processor or calculated by the following formula:

$$X = \frac{A \cdot c_s}{A_s \cdot c}$$

where

X—Content of aflatoxin B_1 in test sample, mg/kg;

A—Peak area of aflatoxin B_1 derivative in sample solution, mm^2;

c_s—Concentration of aflatoxin B_1 in standard working solution, μg/mL;

A_s—Peak area of aflatoxin B_1 derivative in standard working solution, mm^2;

c—Corresponding concentration of the test sample in its final solution, g/mL.

Note: The blank value should be subtracted from the calculated result.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.001 mg/kg.

4.2 Recovery

According to the experimental data, when the fortifying concentration of aflatoxin B_1 is in the range of 0.001—0.5 mg/kg, the recovery is 89.1%—104.9%.

Additional explanations:

This standard was proposed by the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by Jiangxi Import and Export Commodity Inspection Bureau of the People's Republic of China.

This standard was mainly drafted by Xu Wenyian, Huang Xiaoming, Shi Xiaoshan.

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