SN

中华人民共和国出入境检验检疫行业标准

SN/T 1958—2007

进出口食品中伏马毒素 B_1 残留量检测方法 酶联免疫吸附法

Screening method of fumonisin B_1 residues in foodstuff for import and export—Enzyme linked immunosorbent assay method

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中华人民共和国
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前　言

本标准附录A、附录B和附录C均为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国天津出入境检验检疫局、天津科技大学。

本标准主要起草人：郑文杰、王硕、张宏伟、姚霞、魏亚东、刘烜、张燕、张骏。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中伏马毒素 B_1 残留量检测方法 酶联免疫吸附法

1 范围

本标准规定了食品中伏马毒素 B_1 残留量的酶联免疫吸附测定方法。

本标准适用于玉米、大米、黄米、燕麦、大麦及花生中伏马毒素 B_1 残留量的筛选检测。

2 规范性引用文件

下列文件中的条款通过本标准的引用而成为本标准的条款。凡是注日期的引用文件，其随后所有的修改单(不包括勘误的内容)或修订版均不适用于本标准，然而，鼓励根据本标准达成协议的各方研究是否可使用这些文件的最新版本。凡是不注明日期的引用文件，其最新版本适用于本标准。

GB/T 6682 分析实验室用水规格和试验方法

3 测定方法

3.1 方法提要

用甲醇/水提取样品中伏马毒素 B_1，用竞争性酶联免疫吸附法进行检测。

3.2 试剂和材料

除另有要求外，所有试剂均为分析纯，水为 GB/T 6682 规定的一级水。

3.2.1 伏马毒素 B_1 酶联免疫吸附测定试剂盒[1)](参见附录 A)。

3.2.2 甲醇。

3.2.3 磷酸氢二钠($Na_2HPO_4 \cdot 12H_2O$)。

3.2.4 磷酸二氢钠($NaH_2PO_4 \cdot 2H_2O$)。

3.2.5 氯化钠。

3.2.6 掩蔽剂稀释液：掩蔽剂：磷酸盐缓冲液(1∶20，体积比)稀释，于使用时配制。

3.2.7 溶液Ⅰ：甲醇：掩蔽剂稀释液(1∶10，体积比)。

3.2.8 伏马毒素 B_1 标准品(英文名称：fumonisin B_1，CAS 代码：116355-83-0)：纯度大于等于 99.5%。

3.2.9 标准品贮备液：配制成 10 mg/L 甲醇贮备液，−20℃保存，有效期 3 个月。

低温保存试剂，放置于室温不要超过 4 h，使用时，各溶液应达到室温。

3.3 仪器和设备

3.3.1 粉碎机。

3.3.2 天平(精确到 0.001 g)。

3.3.3 旋涡混合器。

3.3.4 气浴摇床。

3.3.5 酶标仪(测定波长 450 nm)。

3.3.6 洗板机。

3.3.7 单道微量加样器：20 μL，100 μL，200 μL。

1) 伏马毒素 B_1 酶联免疫吸附测定试剂盒是由天津生物芯片技术有限责任公司提供的产品的商品名称。给出这一信息是为了方便本标准的使用者，并不表示对该产品的认可。如果其他等效产品具有相同的效果，则可使用这些等效产品。

3.3.8 多通道微量加样器:200 μL。

3.4 试样制备

取样品约 500 g 混匀,均分成两份,装入洁净容器内作为试样,密封,并标明标记。

3.5 试样保存

将试样于 4℃冷藏保存。在制样的操作过程中,应防止样品受到污染或发生残留物含量的变化。

3.6 提取

将玉米等谷物样品各取 25 g 充分粉碎,从中取样品各 5 g,加入 20 mL 甲醇/水(3∶1,体积比),振荡萃取 15 min,静置 5 min,取部分上清液用掩蔽剂稀释 15 倍后进行 ELISA 检测。

3.7 酶联免疫法检测

3.7.1 标准曲线的制备

用溶液Ⅰ稀释 10 mg/L 的伏马毒素 B_1 标准溶液储备液至 100 μg/L,用溶液Ⅰ对 100 μg/L 的伏马毒素 B_1 标准溶液进行 1∶3 梯度稀释,得到一系列不同浓度的伏马毒素 B_1 标准溶液(100 μg/L,33.3 μg/L,11.1 μg/L,3.7 μg/L,1.2 μg/L,0.4 μg/L)。

3.7.2 样品测定

在酶标板中分别加入 100 μL 不同浓度的伏马毒素 B_1 标准溶液和样品待测液。在空白和对照孔中分别加入 100 μL 的水。每个标准溶液、样品待测液、空白和对照孔均做平行。空白孔中加入 100 μL 磷酸盐缓冲液。除空白孔外,在每个孔中则加入 100 μL 的酶标记物稀释液。轻拍混匀,用粘胶纸封住微孔以防溶液挥发,室温孵育 60 min。弃掉酶标板中的液体,用洗涤液洗板三次。加入混合好的底物液 150 μL,轻轻混匀,室温放置 30 min 后,每个孔加入 50 μL 的终止液,轻轻晃动酶标板,10 min 内在酶标仪上读出每孔 450 nm 吸光值。

3.8 空白试验

除不加试样外,均按上述测定步骤进行。

3.9 结果的计算

3.9.1 计算抑制率值

不同浓度伏马毒素 B_1 对抗原抗体结合反应的抑制率计算见式(1):

$$IC = \left[1 - \frac{A_{样品} - A_{空白}}{A_{对照} - A_{空白}}\right] \times 100\% \quad \cdots\cdots(1)$$

式中:

IC——伏马毒素 B_1 对抗原抗体结合反应的抑制率,%;

$A_{对照}$——不加入伏马毒素 B_1 标准液,仅加入酶标记稀释物和磷酸盐缓冲液,在 450 nm 下测得的平均吸光度值;

$A_{样品}$——伏马毒素 B_1 标准液或样液在 450 nm 下的平均吸光度值;

$A_{空白}$——不加入酶标记稀释物及伏马毒素 B_1 标准液,仅加入水和磷酸盐缓冲液,在 450 nm 下测得的平均吸光度值。

3.9.2 绘制标准曲线

以抑制率为纵坐标,伏马毒素 B_1 浓度为横坐标(横坐标取对数刻度)绘制标准曲线。每次试验均应重新绘制标准曲线,参见附录 C。

3.9.3 结果计算

从标准曲线上读取样液抑制率所对应的伏马毒素 B_1 浓度(c),按式(2)计算试样中的伏马毒素 B_1 残留量:

$$X = c \times R \quad \cdots\cdots(2)$$

式中:

X——试样中伏马毒素 B_1 残留量,单位为微克每千克(μg/kg);

c——根据样品孔的抑制率查得试样中伏马毒素 B_1 浓度，单位为微克每升(μg/L)；

R——样品换算系数，其数值为 60。

4 检测低限、回收率

4.1 检测低限

本方法检测低限为 12 μg/kg。

4.2 回收率

伏马毒素 B_1 添加浓度和回收率的实验数据参见附录 B。

实验结果为阳性应用仪器方法进行确证。

附 录 A
（资料性附录）
伏马毒素 B_1 酶联免疫测定试剂盒

本标准酶联免疫测定步骤中使用的试剂盒为天津生物芯片技术有限责任公司产品[2)]，试剂盒包括：

a) 96 孔酶标板；

b) 伏马毒素 B_1 酶标记物溶液；

c) 底物液 A：于 4℃保存，使用时需达到室温；

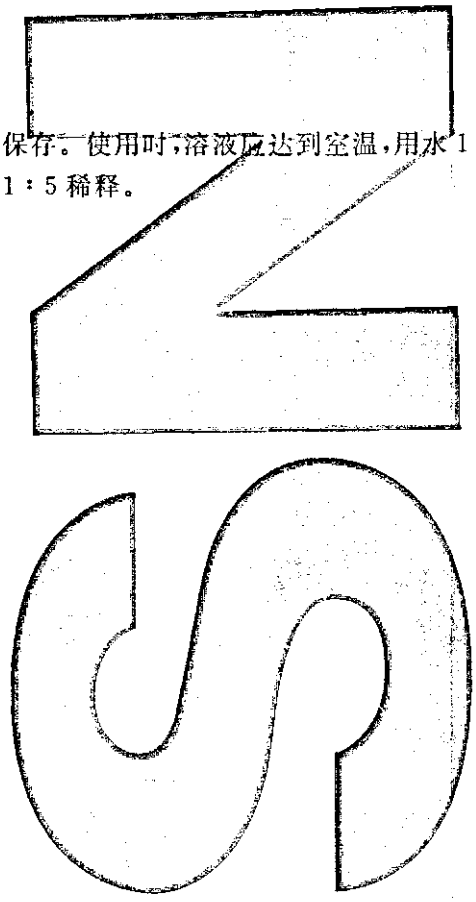
d) 底物液 B：使用前室温避光保存。使用时，按底物液 A：底物液 B 为 32：1（体积比）混合使用；

e) 反应终止液；

f) 掩蔽剂：于 4℃保存；

g) 磷酸盐缓冲液：于 4℃保存。使用时，溶液应达到室温，用水 1：5 稀释；

h) 洗涤液：使用时，用水 1：5 稀释。



2) 给出该信息是为了方便本标准的使用者，并不表示对某一产品的认可。如果其他产品具有相同的效果，需经实验评估后使用这些等效产品。

附 录 B
（资料性附录）
伏马毒素 B_1 添加浓度和平均回收率

表 B.1 伏马毒素 B_1 添加浓度和平均回收率

样品	添加浓度/(μg/kg)	平均回收率/%
玉米	12	100
	250	104
	500	86
大米	12	100
	250	114
	500	110
黄米	12	100
	250	82
	500	93
大麦	12	100
	250	102
	500	104
燕麦	12	100
	250	88
	500	87
花生	12	100
	250	94
	500	91

附 录 C
（资料性附录）
伏马毒素 B_1 标准曲线

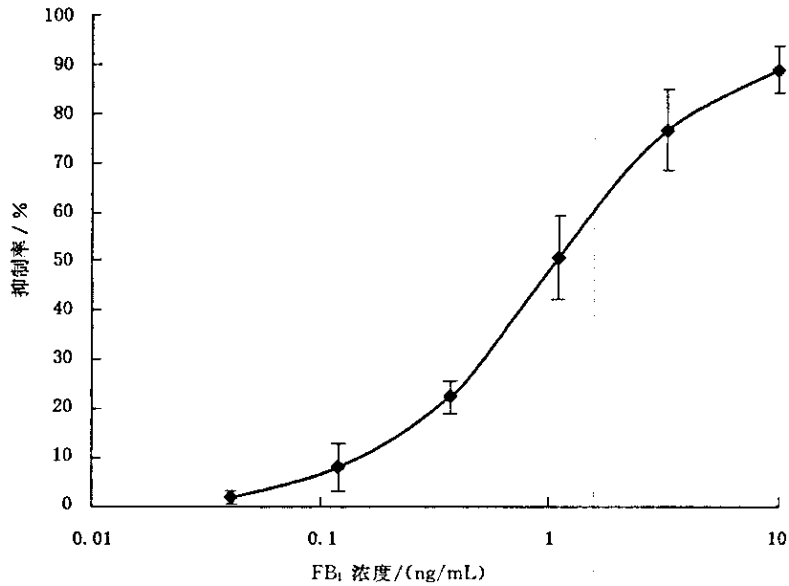


图 C.1 伏马毒素 B_1 标准曲线

Foreword

Annex A, B and C of this standard are all informative annexes.

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

This standard was drafted by Tianjin Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China. , Tianjin University of Science and Technologuy.

This standard was mainly drafted by Wenjie Zheng, Shuo Wang, Hongwei Zhang, Xia Yao, Yadong Wei, Xuan Liu, Yan Zhang, Jun Zhang.

This standard is a professional standard for entry-exit inspection and quarantine promulgated for the first time.

Screening method of fumonisin B_1 residues in foodstuff for import and export —Enzyme linked immunosorbent assay method

1 Scope

This standard specifies the method of determination by ELISA method of fumonisin B_1 residues in foodstuff.

This standard is applicable to the screen of fumonisin B_1 residues in corn, barley, rice, oat, peanut and sorghum. Positive results should be confirmed by another method.

2 Normative references

The following standard contain provisions which, through reference in this text, constitute provisions of this standard. Parties to agreements based on this standard are encouraged to inveatigate the possibility of applying the most recent editions of the standards indicated below.

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

3 Method of inspection

3.1 Principle of determination

Fumonisin B_1 is distilled by methanol/H_2O. The basis of the test is the competitive enzyme-linked immunoreaction.

3.2 Reagents and materials

In this standard, all the chemical reagents should be analytically pure and "water" is the first-degree water as GB/T 6682 described.

3.2.1 Fumonisin B_1 detection kit[1] (see Annex A).

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1) Fumonisin B_1 detection kit is the commercial name which was provided by the Tianjin Biochip Technologies Co., Ltd. This information being given is convenient for the user of the standard and is not the confirm of some products' protocol. Other products' protocol should be confirmed by experiments if any difference.

3.2.2 Methanol.

3.2.3 $Na_2HPO_4 \cdot 12H_2O$.

3.2.4 $NaH_2PO_4 \cdot 2H_2O$.

3.2.5 NaCl.

3.2.6 0.5% FG-PBS dilute.

3.2.7 Solution I.

3.2.8 Fumonisin B_1 standard.

3.2.9 Prepared standard solution: 10 μg/mL in methanol, storage at −20℃.

All solution must be stored at low temperature and never be placed at room temperature longer than 4 hours; reach room temperature before use.

3.3 Apparatus and equipment

3.3.1 Muller.

3.3.2 Balance.

3.3.3 Vortex mixer.

3.3.4 Vibrator.

3.3.5 Microwell system.

3.3.6 Plate-washing machine.

3.3.7 Pipettes: 20 μL, 100 μL, 200 μL.

3.3.8 Multichannel pipette: 200 μL.

3.4 Preparation of test sample

The combined primary sample is reduced to 500 g, which is blended, homogenized thoroughly, and then divided into two equal portions. Each portion is placed in a clean container as the test sample, which is sealed and labeled.

3.5 Storage of test sample

The test sample should be stored at 4℃. In the course of sampling and sample preparation, precaution must be taken to avoid the contamination or any factors which may cause the change of residue content.

3.6 Sample extraction

Samples were finely ground with a laboratory blender and add 20 mL methanol/H_2O(3 : 1, v/v) to 5 g dried sample, shaking extraction for 15 min, standing for 5 min, upper clear solution samples were diluted 15 fold with 0.5% FG-PBS.

3.7 ELISA determination

3.7.1 Manufacture of calibration curve

Dilute the prepared standard solution with solution I into 100 μg/L, 33.3 μg/L, 11.1 μg/L, 3.7 μg/L, 1.2 μg/L, 0.4 μg/L.

3.7.2 Detection of sample

Insert a sufficient amount of microtitre wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions. add 100 μL of six kinds of prepared fumonisin B_1 standard solution and sample solution to separate duplicate wells. Add 100 μL double water as blank. Add 100 μL of diluted fumonisin B_1 enzyme conjugate (used within 8 h) to the bottom of each well and add 100 μL PBS in blank. flap lightly and mix thoroughly. Sealing all wells with adhesive paper to avoid solution evaporating, incubate for 60 min at room temperature. Pour the liquid out of the wells and wash the wells three times with PBST. Add 150 μL of substrate and chromogen to each well (Take care the substrate solution in the dark) flap lightly and mix thoroughly and incubate for 30 min at room temperature. Add 50 μL of stop solution to each well, flap lightly and mix thoroughly. Measure and record the absorbance value of each well solution at 450 nm within 10 min.

3.8 Blank test

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

3.9 Calculating the result and express

3.9.1 Calculating inhibition value in percentages

Calculate the inhibition value of the combination of each fumonisin B_1 standard and antigen-antibody, the fumonisin B_1 standard and sample inhibition value are calculated in percentages according to the

formula(1):

$$IC = \left[1 - \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}}\right] \times 100\% \quad \cdots\cdots(1)$$

where

IC—the inhibition value of fumonisin B_1 to the response of antigen and antibody, %;

$A_{control}$—0 μg/L fumonisin B_1 standard mean absorbance value in percentages;

A_{sample}—fumonisin B_1 standard or sample solution mean absorbance value;

A_{blank}—0 μg/L fumonisin B_1 standard solution and enzyme conjugate mean absorbance value.

3.9.2 Plot calibration curve

Make the inhibition value in percentages(arithmetic grade)as ordinate, and fumonisin B_1 concentration as abscissa, plot the calibration curve of the inhibition value in percentages and fumonisin B_1 standard concentration(see Annex C). A new calibration curve should be prepared for each determination.

3.9.3 Calculating the result

The fumonisin B_1 concentration of the test sample solutin can be read corresponding to the percentage inhibition value of each sample from calibration curve and namely equal to the residue content of fumonisin B_1 in the test sample(μg/L).

$$X = c \times R \quad \cdots\cdots(2)$$

where

X—the residue of fumonisin B_1(μg/kg);

c—the residue of fumonisin B_1 read from inhibition value(μg/L);

R—the sample convertion factor;60.

4 Limit of determination recovery and precision

4.1 Limit of determination

The limit of determination of this method is 12 μg/kg.

4.2 Recovery

The experimental data of these fortifying concentrations of fumonisin B_1 and average recoveries are as Annex B.

The positive result should be confirmed by the equipment method.

Annex A
(Informative Annex)
Fumonisin B_1 detection kit

Fumonisin B_1 detection kit is provided by the Tianjin Biochip Technologies Co. ,Ltd[2)]. It includes:

a) 96-microwell plate;

b) Fumonisin B_1 enzyme conjugate;

c) Substrate solution A;

d) Substrate solution B:Store the solution at room temperature in dark place. Substrate A : Substrate B : 32 : 1;

e) Stop solution;

f) 0.5% FG-PBS:Store at 4℃;

g) Phosphate Buffered Saline(PBS):Store at 4℃,dilute five times with distilled water;

h) Washing buffer(PBST):Dilute five times with distilled water.

2) The information supplying herein is just for the convenience usage for the users of this standard and not for some certain product's authorization. The testing process of another equivalent kits maybe differ,so it should be used aftertesting,evaluation andverification.

Annex B
(Informative Annex)
Addition and average recovery of fumonisin B_1

Table B. 1—Addition and average recovery of fumonisin B_1

Sample	Addition/(μg/kg)	Average recovery/%
corn	12	100
	250	104
	500	86
rice	12	100
	250	114
	500	110
sorghum	12	100
	250	82
	500	93
barley	12	100
	250	102
	500	104
oat	12	100
	250	88
	500	87
peanut	12	100
	250	94
	500	91

Annex C
(Informative Annex)
Standard curve of fumonisin B_1

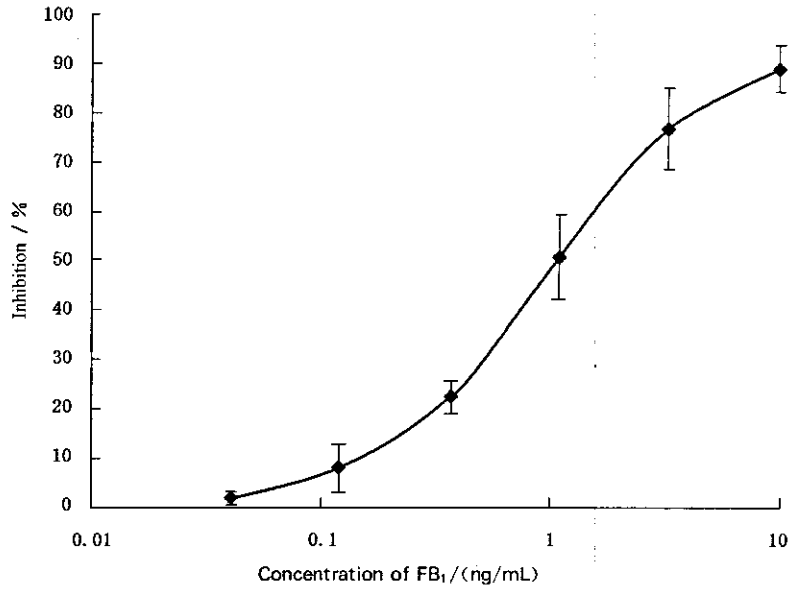


Figure C. 1—Standard curve of fumonisin B_1

中华人民共和国出入境检验检疫
行 业 标 准
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