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中华人民共和国出入境检验检疫行业标准

SN/T 4259—2015

出口水果蔬菜中链格孢菌毒素的测定 液相色谱-质谱/质谱法

Determination of alternaria toxins residues in fruit and
vegetables for export—LC-MS/MS 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：中华人民共和国陕西出入境检验检疫局。

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出口水果蔬菜中链格孢菌毒素的测定

液相色谱-质谱/质谱法

1 范围

本标准规定了水果和蔬菜中链格孢霉素、链格孢酚、腾毒素、链格孢酚甲醚四种链格孢菌毒素含量的液相色谱-串联质谱检测方法。

本标准适用于苹果、梨、葡萄、猕猴桃、柑桔、西红柿、黄瓜、白菜、辣椒等样品中链格孢霉素、链格孢酚、腾毒素、链格孢酚甲醚含量的测定和确证。

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 方法提要

试样中的链格孢菌毒素用乙腈提取后,经聚苯乙烯-二乙烯基苯共聚物固相萃取柱串联氨基固相萃取柱净化,或凝胶渗透色谱仪(GPC)结合氨基固相萃取柱净化,用液相色谱-串联质谱仪进行测定和确证,外标法定量。

4 试剂和材料

除另有规定外,所用试剂均为分析纯,水为符合 GB/T 6682 规定的一级水。

4.1 乙腈:HPLC 级。

4.2 甲醇:HPLC 级。

4.3 二氯甲烷。

4.4 乙酸乙酯。

4.5 环己烷。

4.6 甲酸。

4.7 氯化钠。

4.8 无水硫酸钠:650 °C 灼烧 4 h,贮于密封容器中备用。

4.9 乙酸乙酯-环己烷(1+1,体积比):量取 1 000 mL 乙酸乙酯与 1 000 mL 环己烷混合均匀。

4.10 甲醇-水溶液(1+4,体积比):量取 100 mL 甲醇与 400 mL 水混合均匀。

4.11 甲酸-甲醇-乙酸乙酯溶液(0.5+50+50,体积比):量取 100 mL 甲醇、100 mL 乙酸乙酯、1 mL 甲酸混合均匀。

4.12 乙腈-水溶液(1+1,体积比):量取 100 mL 乙腈与 100 mL 水混合均匀。

4.13 链格孢霉素标准物质(Altenuene, $C_{15}H_{16}O_6$, CAS:29752-43-0):纯度大于等于 99.0%。

4.14 链格孢酚标准物质(Alternariol, $C_{14}H_{10}O_5$, CAS:641-38-3):纯度大于等于 98.5%。

- 4.15 腾毒素标准物质(Tentoxin, $C_{22}H_{30}N_4O_4$, CAS:28540-82-1):纯度大于等于 99.5%。
- 4.16 链格孢酚甲醚标准物质(Alternariol monomethyl-ether, $C_{15}H_{12}O_5$, CAS:26894-49-5):纯度大于等于 99.0%。
- 4.17 标准储备溶液:准确称取适量的链格孢霉素、链格孢酚、腾毒素、链格孢酚甲醚,分别用乙腈制成 100 $\mu\text{g/mL}$ 的标准储备溶液,0 $^{\circ}\text{C}$ ~4 $^{\circ}\text{C}$ 保存。
- 4.18 混合标准工作溶液:分别取适量的链格孢霉素、链格孢酚、腾毒素、链格孢酚甲醚标准储备液,根据需要用乙腈-水溶液(4.12)逐级稀释,配制适当浓度的标准工作溶液。0 $^{\circ}\text{C}$ ~4 $^{\circ}\text{C}$ 保存。
- 4.19 聚苯乙烯-二乙烯基苯共聚物固相萃取柱:HLB 柱,60 mg,3 mL 或相当者。使用前依次用 3 mL 甲醇、5 mL 水活化,保持柱体湿润。
- 4.20 氨基固相萃取柱:500 mg,3 mL。使用前在柱上端装填约 2 cm 高的无水硫酸钠(4.8),然后用 5 mL 二氯甲烷活化,保持柱体湿润。
- 4.21 微孔滤膜:0.2 μm ,有机系。

5 仪器和设备

- 5.1 液相色谱-串联质谱仪:配有 ESI 电离源、串联四级杆质量分析器。
- 5.2 凝胶渗透净化系统。
- 5.3 分析天平:感量 0.01 mg、0.01 g。
- 5.4 组织捣碎机。
- 5.5 振荡器。
- 5.6 离心机:转速不低于 3 000 r/min。
- 5.7 固相萃取装置,带真空泵。
- 5.8 减压旋转蒸发器。
- 5.9 氮吹仪。
- 5.10 具塞离心管:50 mL。
- 5.11 浓缩瓶:50 mL、100 mL。

6 试样制备与保存

取代表性样品约 500 g,将其可食用部分切碎后,用组织捣碎机将样品加工成浆状,混匀,装入洁净的容器内,密闭并标明标记,于-18 $^{\circ}\text{C}$ 冷冻保存。

注: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。

7 测定步骤

7.1 提取

7.1.1 苹果、梨、葡萄、猕猴桃、柑桔、西红柿、黄瓜、白菜

称取 5 g(精确至 0.01 g)试样于 50 mL 离心管中,加入 4 g 氯化钠,10 mL 水,15 mL 乙腈,振荡提取 10 min,3 000 r/min 离心 5 min,将上层乙腈提取液转移到浓缩瓶中。再分别用 15 mL 乙腈重复提取两次,合并乙腈提取液,于 40 $^{\circ}\text{C}$ 水浴中减压浓缩至近干,氮气吹干,用 1.0 mL 甲醇溶解残渣,再用 9.0 mL 水稀释,过滤,待净化。

7.1.2 辣椒

称取 5 g(精确至 0.01 g)试样于 50 mL 离心管中,加入 4 g 氯化钠,10 mL 水,15 mL 乙腈,10 mL 正己烷,振荡提取 10 min,3 000 r/min 离心 5 min,用玻璃吸管移取中间的乙腈层于浓缩瓶中。再分别用 15 mL 乙腈重复提取两次,合并乙腈提取液,于 40 ℃水浴中减压浓缩至近干,氮气吹干,用 10.0 mL 乙酸乙酯-环己烷(4.9)溶解残渣,过滤,待净化。

7.2 净化

7.2.1 苹果、梨、葡萄、猕猴桃、柑桔、西红柿、黄瓜、白菜

取提取液(7.1.1)5.0 mL 加入 HLB 固相萃取柱(4.19),待样品溶液流完后,保持柱体湿润,用 5 mL 甲醇-水(4.10)淋洗,减压抽干 2 min,将氨基柱(4.20)串接在 HLB 固相萃取柱下方,用 5 mL 二氯甲烷淋洗,弃去淋洗液,再用 7 mL 甲酸-甲醇-乙酸乙酯溶液(4.11)洗脱,整个净化过程保持 2 mL/min~3 mL/min 的流速,收集洗脱液,40 ℃减压蒸至近干,氮气吹干,用 2.00 mL 乙腈-水(4.12)溶解定容,过 0.2 μm 滤膜,供液相色谱-串联质谱测定和确证。

7.2.2 辣椒

取提取液(7.1.2)5.0 mL 用凝胶渗透色谱净化,收集洗脱液,于 40 ℃减压蒸至近干,加入 2 mL 二氯甲烷,过氨基柱(4.20)净化,用 2 mL 二氯甲烷洗涤浓缩瓶,洗涤液一并过氨基柱,再用 5 mL 二氯甲烷淋洗,弃去淋洗液,用 7 mL 甲酸-甲醇-乙酸乙酯溶液(4.11)洗脱,整个净化过程保持 2 mL/min~3 mL/min 的流速,收集洗脱液,40 ℃减压蒸至近干,氮气吹干,用 2.00 mL 乙腈-水(4.12)溶解定容,过 0.2 μm 滤膜,供液相色谱-串联质谱测定和确证。

凝胶渗透色谱净化参考条件:

- a) 凝胶柱:内径 25 mm,柱长 400 mm,填料为 Bio-Beads-S-X3 Beads(200 mesh~400 mesh),或相当者;
- b) 流动相:乙酸乙酯-环己烷(4.9);
- c) 流速:5.0 mL/min;
- d) 进样量:5.0 mL;
- e) 收集方式:时间模式,收集 19.0 min~30.0 min 洗脱流出液。

7.3 测定

7.3.1 液相色谱参考条件

液相色谱参考条件如下:

- a) 色谱柱:C₁₈柱,长 50 mm,内径 2.1 mm,粒径 1.7 μm,或相当者;
- b) 流动相:乙腈-水梯度洗脱,参见表 1,梯度变化模式为线性递增或递减;

表 1 流动相条件

时间 min	流速 mL/min	乙腈 %	水 %
0.00	0.30	15	85
3.00	0.30	70	30

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表 1 (续)

时间 min	流速 mL/min	乙腈 %	水 %
3.50	0.30	70	30
3.60	0.30	15	85
5.00	0.30	15	85

- c) 柱温:35 ℃;
- d) 进样量:2.0 μL。

7.3.2 质谱参考条件

质谱检测参考条件参见附录 A。

7.3.3 液相色谱-串联质谱检测及确证

7.3.3.1 定量测定

根据样液中链格孢霉毒素含量情况,选定与样液浓度相近的标准工作溶液,标准工作溶液和样液中链格孢霉毒素的响应值均应在仪器检测线性范围内,如果样液中链格孢霉毒素含量超出检测的线性范围,则稀释后再进样。对标准工作溶液和样液等体积分时段参插进样测定,外标法定量,在 7.3 规定的色谱及质谱条件下,4 种链格孢霉毒素标准品的液相色谱-串联质谱选择性离子流图参见附录 B 中图 B.1。

7.3.3.2 定性测定

对标准工作液及样液按 7.3 规定的条件进行测定时,如果样液与标准工作液的选择离子图中,样液中目标物质的保留时间与相近浓度标准溶液的保留时间偏差在±2.5%以内;且定性离子对的相对丰度与相近浓度标准溶液对应定性离子对的相对丰度进行比较,若偏差不超过表 2 规定的范围,则可判定为样品中存在对应的待测物。

表 2 定性确证时相对离子丰度的最大允许偏差

相对离子丰度/%	>50	>20~50	>10~20	≤10
允许的相对偏差/%	±20	±25	±30	±50

7.4 空白试验

除不加试样外,均按上述检测步骤进行。

7.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中链格孢霉毒素含量。

$$X = \frac{A \times c_s \times V}{A_s \times m} \dots\dots\dots (1)$$

式中:
X —— 试样中链格孢霉毒素的含量,单位为毫克每千克(mg/kg);

A ——样液中链格孢霉毒素的色谱峰面积；

c_s ——标准工作液中链格孢霉毒素的浓度,单位为微克每毫升($\mu\text{g/mL}$)；

V ——样液最终定容体积,单位为毫升(mL)；

A_s ——标准工作液中链格孢霉毒素的色谱峰面积；

m ——最终样液所代表的试样量,单位为克(g)。

8 测定低限、回收率

8.1 测定低限

本方法中 4 种链格孢霉毒素的测定低限均为 0.01 mg/kg 。

8.2 回收率

4 种链格孢霉毒素的添加水平及回收率数据参见附录 C 中表 C.1。

附 录 A
(资料性附录)
质谱检测参考条件

A.1 参考条件

- a) 电离方式:ESI⁻;
- b) 毛细管电压:3 kV;
- c) 萃取电压:3 V;
- d) 脱溶剂气:氮气,纯度≥99.9%,600 L/h;
- e) 锥孔气:氮气,纯度≥99.9%,50 L/h;
- f) 碰撞气:氩气,纯度≥99.999%,0.2 mL/min;
- g) 脱溶剂气温度:400 ℃;
- h) 离子源温度:115 ℃;
- i) 采集模式:多重反应监测模式(MRM);
- j) 多重反应监测条件见表 A.1。

表 A.1 4 种链格孢霉毒素测定的 MRM 条件

化合物	MRM 离子对	驻留时间 s	锥孔电压 V	碰撞能量 eV
链格孢霉素	291.1/229.0*	0.05	25	15
	291.1/247.1	0.05		15
链格孢酚	257.1/213.1*	0.05	45	22
	257.1/146.9	0.05		30
腾毒素	413.4/141.0*	0.05	35	22
	413.4/271.3	0.05		18
链格孢酚甲醚	271.1/256.1*	0.05	38	22
	271.1/228.1	0.05		30
* 为定量离子对。				

注: 以上条件为采用 Waters Quattro Premier XE 串联四级杆质谱仪时的质谱检测参数,若选用其他仪器,可根据具体情况进行调整。

附录 B

(资料性附录)

4 种链格孢霉毒素的液相色谱-串联质谱选择性离子流图

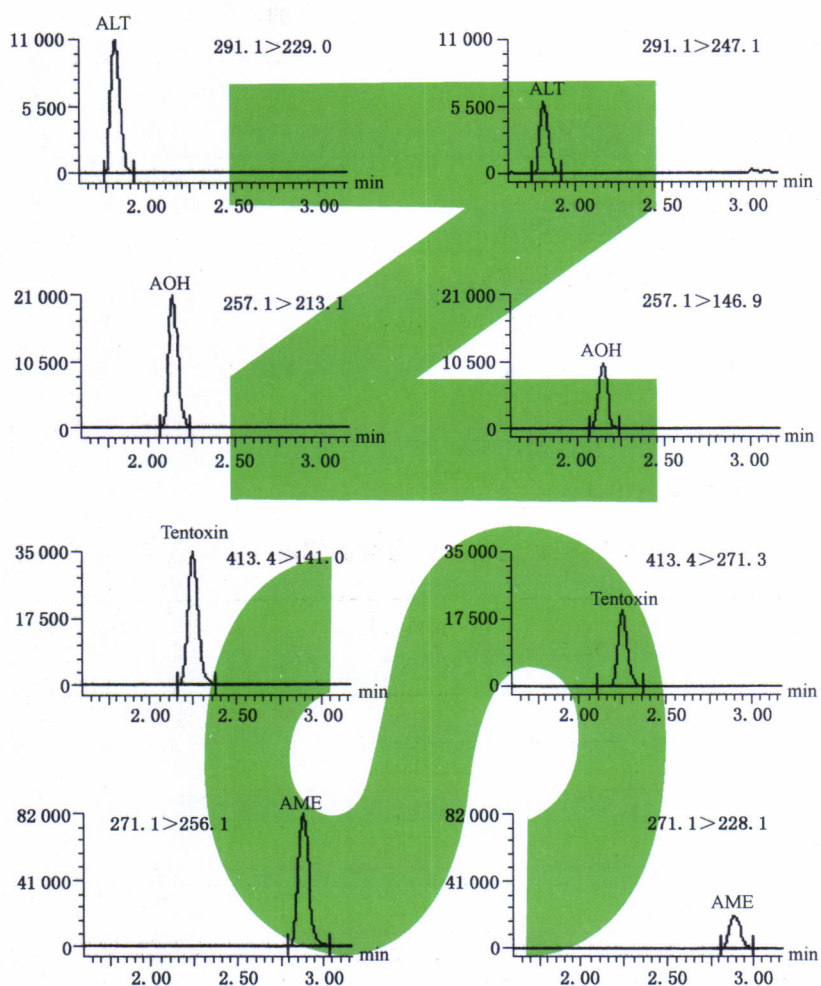


图 B.1 4 种链格孢霉毒素标准品液相色谱-串联质谱选择性离子流图

附 录 C

(资料性附录)

4 种链格孢霉毒素的添加回收率数据

表 C.1 水果蔬菜中 4 种链格孢霉毒素的添加回收率数据

样品	加标水平 mg/kg	回收率范围/%			
		链格孢霉素	链格孢酚	腾毒素	链格孢酚甲醚
苹果	0.01	72.9~99.5	75.6~101.1	82.9~112.4	73.5~98.4
	0.02	81.0~101.8	79.8~104.3	83.6~105.2	78.9~103.3
	0.04	84.0~105.2	82.9~103.5	84.3~113.9	79.8~102.6
梨	0.01	74.8~108.2	77.5~101.2	83.7~106.5	86.4~113.5
	0.02	81.0~109.5	81.0~105.6	82.8~110.2	85.7~115.8
	0.04	82.5~110.8	82.5~102.0	83.6~108.6	86.3~108.2
葡萄	0.01	85.3~108.7	77.6~106.2	81.2~106.5	79.3~105.3
	0.02	84.5~110.5	83.6~110.8	85.0~109.8	84.6~108.9
	0.04	86.3~108.4	84.5~108.9	86.5~109.7	85.2~109.0
猕猴桃	0.01	76.2~107.4	83.5~108.6	79.8~105.6	79.2~105.3
	0.02	79.8~108.9	81.9~107.8	86.5~110.9	83.1~106.8
	0.04	81.0~106.5	84.9~108.8	84.9~109.1	79.6~106.9
柑桔	0.01	78.9~103.8	81.3~106.4	81.5~105.8	82.3~107.0
	0.02	81.2~104.7	83.5~107.8	84.5~107.3	84.1~108.5
	0.04	82.5~106.2	85.4~109.0	84.4~110.8	87.6~111.5
西红柿	0.01	78.5~102.6	75.1~99.3	79.5~109.5	79.4~113.1
	0.02	79.5~106.9	77.8~103.3	81.3~110.8	82.2~111.1
	0.04	80.8~108.6	79~102.8	86.2~108.0	84.0~109.0
黄瓜	0.01	77.6~102.2	82.3~101.6	83.9~107.5	77.6~104.1
	0.02	78.6~101.2	83.2~103.6	84.2~106.7	81.5~105.0
	0.04	81.5~102.4	84.6~104.5	85.1~109.2	82.8~107.2
白菜	0.01	78.4~110.2	75.8~105.2	85.6~115.4	79.2~100.2
	0.02	80.8~109.5	79.6~105.8	86.0~113.7	82.1~103.4
	0.04	82.7~109.6	83.8~104.9	85.5~116.7	83.9~105.6
辣椒	0.01	74.1~102.3	78.8~100.0	84.5~106.2	78.2~107.4
	0.02	79.3~101.4	80.2~103.6	85.4~110.5	82.0~106.9
	0.04	84.3~102.5	81.6~103.8	84.9~106.9	84.5~107.8

Foreword

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

Please note that this document may involve some of the contents of the patent, the standard file publishing institutions does not assume the responsibility of identifying these patents.

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

This standard is drafted by Shaanxi entry-exit inspection and quarantine bureau of the People's Republic of China.

The standard is mainly drafted by Li Jianhua, He Qiang, Kong Xianghong, Zou Yang, Zhang Lu, Li Ying, Fu Chengyu.

Determination of alternaria toxins residues in fruit and vegetables for export—LC-MS/MS method

1 Scope

This standard specifies the methods of qualified and quantified determination of alternuene(ALT), alternariol(AOH), alternariol monomethyl ether(AME) and tentoxin in fruits and vegetables by LC-MS/MS.

This standard is applicable to determination and corroboration of ALT, AOH, AME and tentoxin contents in apple, pear, grape, kiwi fruit, citrus, tomato, cucumber, cabbage and hot pepper.

2 Normative references

The following documents are essential for the application of this document. For dated references, only dated version apply to this document. For undated references, the latest edition (including all the amendments) apply to this document.

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

3 Method summary

ALT, AOH, AME and tentoxin in the test sample are extracted with acetonitrile. After concentrated, the solution was cleaned with polystyrene-divinylbenzene copolymer SPE column and NH_2 SPE columns, or cleaned with GPC and NH_2 SPE columns. Then detected by LC-MS/MS and quantitated using external standard method.

4 Reagents and materials

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

4.1 Acetonitrile: HPLC Grade.

4.2 Methanol: HPLC Grade.

4.3 Methylene dichloride.

4.4 Ethyl acetate.

4.5 Cyclohexane.

4.6 Formic acid.

4.7 Sodium chloride.

4.8 Anhydrous sodium sulfate; ignite at 650 °C for 4 h and store in a desiccator.

4.9 Ethylacetate-cyclohexane solution (1+1, V/V): add 1 000 mL of ethylacetate and 1 000 mL cyclohexane, mix homogeneously.

4.10 Methanol-water solution (1+4, V/V): add 100 mL of methanol and 400 mL water, mix homogeneously.

4.11 Formic acid-methanol-ethyl acetate solution (0.5+50+50, V/V): add 1 mL of formic acid add 100 mL of methanol and 100 mL ethyl acetate, mix homogeneously.

4.12 Acetonitrile-water solution (1+1, V/V): Acetonitrile dissolved in the same volume of water to homogenization.

4.13 Alternuene standard (Alternuene, $C_{15}H_{16}O_6$, CAS:29752-43-0): purity $\geq 99.0\%$.

4.14 Alternariol standard (Alternariol, $C_{14}H_{10}O_5$, CAS:641-38-3): purity $\geq 98.5\%$.

4.15 Tentoxin standard (Tentoxin, $C_{22}H_{30}N_4O_4$, CAS:28540-82-1): purity $\geq 99.5\%$.

4.16 Alternariol monomethyl-ether standard (Alternariol monomethyl-ether, $C_{15}H_{12}O_5$, CAS:26894-49-5): purity $\geq 99.0\%$.

4.17 Standard stock solution: accurately weigh alternuene, alternariol, alternariol monomethyl ether and tentoxin respectively, dissolve with acetonitrile to 100 $\mu\text{g/mL}$ standard stock solution. Store at the temperature of 0 °C ~4 °C.

4.18 Mixed standard working solution: according to the requirement, accurately measure an adequate volume of standard stock solution dilute stepwise with acetonitrile-water solution (4.12) to obtain mixed standard working solution. Store at the temperature of 0 °C ~4 °C.

4.19 Polystyrene-divinylbenzene copolymer SPE column: Oasis HLB column, 60 mg, 3 mL or equivalent. Condition with 3 mL of methanol and 5 mL water respectively before use.

4.20 Amino(-NH₂) SPE column: 500 mg, 3 mL. add 2 cm of anhydrous sodium sulfate (4.8) and condition with 5 mL of methylene dichloride before use.

4.21 Millipore filter: 0.2 μm, organic.

5 Apparatus and equipment

5.1 Liquid chromatography-mass spectrometry, equipped with electrospray ion source.

5.2 Gel permeation chromatography clean system.

5.3 Analytical balance, sensitivity:0.01 mg and 0.01 g.

5.4 Tissue blender.

5.5 Mechanical Shaker.

5.6 Centrifuge: 5 000 r/min.

5.7 Solid phase extraction with mechanical vacuum pump.

5.8 Rotary evaporator.

5.9 Nitrogen evaporator.

5.10 Polypropylene centrifuge tube: 50 mL.

5.11 evaporating bottle: 50 mL and 100 mL.

6 Sample preparation and storage

Take approximately 500 g of representative sample (without wash by water). The edible parts are blended and homogenized in a high speed blender. Divide into two equal portions. Each portion is placed into a clean container as test sample, sealed and labeled.

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

7 Analytical procedure

7.1 Extraction

7.1.1 For apple, pear, grape, kiwi fruit, citrus, tomato, cucumber, and cabbage

Weigh 5 g of the test sample (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube, add 10 mL water, 4 g sodium chloride and 15 mL acetonitrile, vortex for 10 min., and then centrifuge at 3 000 r/min for 5 min. Transfer the supernatant layer into a 100 mL evaporate flask. The residues are extracted again with 15 mL of acetonitrile. Combine the supernatant layer into the same 100 mL evaporate flask. Evaporate to approach dryness in a rotary evaporator with a bath temperature about 40 °C. Dissolve the residues with 1 mL of methanol and dilute with 9 mL of water for next clean up.

7.1.2 For hot pepper

Weigh 5 g of the test sample (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube, add 10 mL water, 4 g sodium chloride, 15 mL acetonitrile and 10 mL n-hexane, vortex for 10 min., and then centrifuge at 3 000 r/min for 5 min. Transfer the middle supernatant acetonitrile layer into a 50 mL evaporate flask. The residues are extracted again with 15 mL of acetonitrile. Combine the supernatant acetonitrile layer into the same 50 mL evaporate flask. Evaporate to approach dryness in a rotary evaporator with a bath temperature about 40 °C. Dissolve the residues with 10 mL of ethylacetate-cyclohexane solution for next clean up.

7.2 Clean up

7.2.1 For apple, pear, grape, kiwi fruit, citrus, tomato, cucumber, and cabbage

Load 5.0 mL extract(7.1.1) into HLB SPE column (4.19). Wash the column with 5 mL methanol-water solution (4.10). Pump the cartridge to dryness. Connect the amino SPE column (4.20) under the HLB SPE column, wash the columns with 5 mL methylene dichloride. Elute with 7 mL Formic acid-methanol-ethyl acetate solution (4.11), Keeps the flow speed at 2 mL/min~3 mL/min during the whole cleaning up. Collect all the eluted solution in a 50 mL evaporating bottle and evaporate to approach dryness in a rotary evaporator with a bath temperature about 40 °C and blow dryness with nitrogen. Dissolve the residue and dilute exactly to 1.0 mL with acetonitrile-water solution (4.12) and filtered through 0.2 μm millipore filter for LC-MS/MS determination.

7.2.2 For hot pepper

Load 5.0 mL extract(7.1.2) into gel permeation chromatography (GPC) clean system, collect the eluted solution and evaporate to approach dryness in a rotary evaporator with a bath temperature about 40 °C. Dissolve the residues with 2 mL of methylene dichloride. Load the methylene dichloride solution into amino SPE column (4.20), wash the flask with 2 mL methylene dichloride and load into the

same column. Wash the column with 5 mL methylene dichloride. Then elute with 7 mL Formic acid-methanol-ethyl acetate solution (4.11), Keeps the flow speed at 2 mL/min~3 mL/min during the whole cleaning up. Collect all the eluted solution in a 50 mL evaporating bottle and evaporate to approach dryness in a rotary evaporator with a bath temperature about 40 °C and blow dryness with nitrogen. Dissolve the residue and dilute exactly to 1.0 mL with acetonitrile-water solution (4.12) and filtered through 0.2 μm millipore filter for LC-MS/MS determination.

The reference conditions of GPC clean up:

- a) GPC gel column: inner diameter is 25 mm, length is 400 mm, the padding model is Bio-Beads-S-X3 Beads (200 mesh~400 mesh); or the equivalent.
- b) Mobile phase: ethylacetate-cyclohexane(4.9).
- c) Flow speed: 5.0 mL/min.
- d) Injection volume: 5.0 mL.
- e) Collection mode: time mode, collect 19.0 min~30.0 min eluent.

7.3 Determination conditions

7.3.1 LC operation conditions

LC operation conditions are as following:

- a) Column: C₁₈, 50 mm×2.1 mm (i.d.), 1.7 μm, or the equivalent.
- b) Mobile phase: A: acetonitrile, B: water, the elution gradient is listed in table 1.
- c) Column temperature: 35 °C.
- d) Injection volume: 2.0 μL.

Table 1—Elution gradient of LC

Time min	Flow rate mL/min	A %	B %
0.00	0.30	15	85
3.00	0.30	70	30
3.50	0.30	70	30
3.60	0.30	15	85
5.00	0.30	15	85

7.3.2 MS operation conditions

The MS operation conditions see Annex A.

7.3.3 LC/MS/MS determination

7.3.3.1 Quantitative determination

According to the approximate concentration of alternaria toxins in the test sample solution, the standard working solution is selected with similar peak height to that of sample solution. The response of alternaria toxins in the standard working solution and sample solution should be in the linear range of the instrumental detection. If not, the sample solution should be diluted before injected. The standard working solution should be injected randomly in between the injections of sample solution of equal volume. Under the above chromatograph conditions, reconstituted ion chromatogram of standard working solution of alternaria toxins can be found in annex B.

7.3.3.2 Qualitative determination

Under the above operating conditions(6.3), if the variation range of the retention time for the peak of analyte in test sample solution and in the standard working solution within the range of $\pm 2.5\%$, and the relative ion abundance of the selected ions according with that of the calibration standard, at comparable concentrations, within the range of table 2, the analytes are present in the test sample.

Table 2—Maximum permitted tolerances for relative ion abundance while confirmation

Relative abundance (base peak) / %	>50	>20~50	>10~20	≤ 10
Permitted tolerances/%	± 20	± 25	± 30	± 50

7.4 Blank test

Blank sample is treated according to the above procedure.

7.5 Calculation and expression of result

The calculation of alternaria toxins concentration in the sample is according to chromatograph data processor or formula (1), the blank value should be subtracted from the result of calculation:

$$X = \frac{A \times c_s \times V}{A_s \times m} \dots\dots\dots (1)$$

Where:

X ——the residue content of analyte in the test sample, $\mu\text{g}/\text{kg}$;

c_s —— the concentration of analyte which is quantified by standard calibration curve, $\mu\text{g/L}$;

A —— peak area of phenothiazine in the sample solution;

V —— the final volume of sample solution, mL;

A_s —— peak area of phenothiazine in the standard working solution;

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

8 Limit of quantification and recovery

8.1 Limit of quantification

The limit of determination of this method for the four alternaria toxins are 0.010 mg/kg.

8.2 Recovery

The recovery data in different samples see Table C. 1 in Annex C.

Annex A
(Informative Annex)
MS operation conditions

A.1 Conditions

- a) Ion source: ESI⁻;
- b) Capillary voltage: 3 kV;
- c) Extractor voltage: 3 V;
- d) Desolvation gas: nitrogen, purity $\geq 99.9\%$, 600 L/h;
- e) Cone gas: nitrogen, purity $\geq 99.9\%$, 50 L/h;
- f) Collision gas: argon, purity $\geq 99.999\%$, 0.2 mL/min;
- g) Desolvation temperature: 400 °C;
- h) Source temperature: 115 °C;
- i) Measure mode: multiple reaction monitoring (MRM);
- j) Multiple reaction monitoring conditions: the MS detection conditions see Table A.1.

Table A.1—MRM conditions of the 4 alternaria toxins

Compound	Ion pair	Dwell time msec	Cone voltage V	CE eV
ALT	291.1/229.0*	0.05	25	15
	291.1/247.1	0.05		15
AOH	257.1/213.1*	0.05	45	22
	257.1/146.9	0.05		30
AME	413.4/141.0*	0.05	35	22
	413.4/271.3	0.05		18
Tentoxin	271.1/256.1*	0.05	38	22
	271.1/228.1*	0.05		30

* stand for quantitative ion pair

Note: The reference mass parameters are accomplished by Waters Quattro Premier XE LC/MS/MS, for the different MS equipment, the parameters may be different, and the MS parameters should be optimized to the best before analysis.

Annex B
(Informative Annex)
Multiple reaction monitoring(MRM)chromatogram of standard

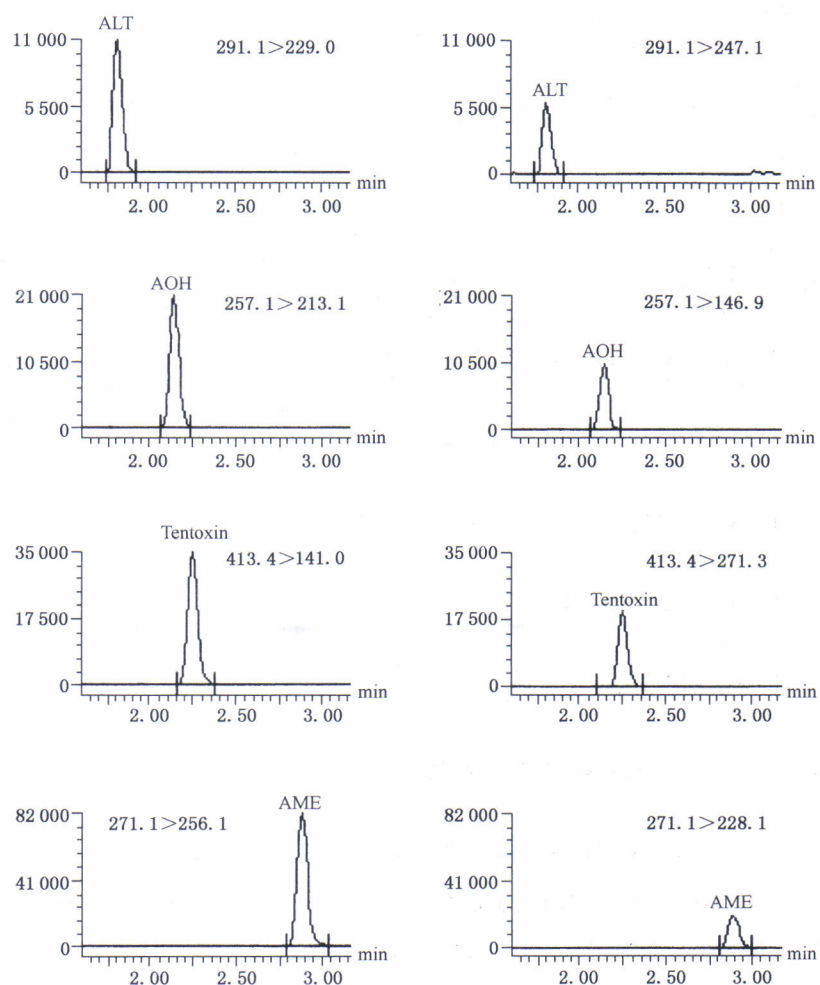


Figure B.1—Multiple reaction monitoring(MRM)chromatogram of alternaria toxins

Annex C
(Informative Annex)
The recovery data in different samples

Table C.1—Recoveries of the residues in fruit and vegetable samples

Sample	Fortifying concentration mg/kg	Range of recovery/%			
		ALT	AOH	AME	Tentoxin
Apple	0.01	72.9~99.5	75.6~101.1	82.9~112.4	73.5~98.4
	0.02	81.0~101.8	79.8~104.3	83.6~105.2	78.9~103.3
	0.04	84.0~105.2	82.9~103.5	84.3~113.9	79.8~102.6
Pear	0.01	74.8~108.2	77.5~101.2	83.7~106.5	86.4~113.5
	0.02	81.0~109.5	81.0~105.6	82.8~110.2	85.7~115.8
	0.04	82.5~110.8	82.5~102.0	83.6~108.6	86.3~108.2
Grape	0.01	85.3~108.7	77.6~106.2	81.2~106.5	79.3~105.3
	0.02	84.5~110.5	83.6~110.8	85.0~109.8	84.6~108.9
	0.04	86.3~108.4	84.5~108.9	86.5~109.7	85.2~109.0
Kiwi fruit	0.01	76.2~107.4	83.5~108.6	79.8~105.6	79.2~105.3
	0.02	79.8~108.9	81.9~107.8	86.5~110.9	83.1~106.8
	0.04	81.0~106.5	84.9~108.8	84.9~109.1	79.6~106.9
Citrus	0.01	78.9~103.8	81.3~106.4	81.5~105.8	82.3~107.0
	0.02	81.2~104.7	83.5~107.8	84.5~107.3	84.1~108.5
	0.04	82.5~106.2	85.4~109.0	84.4~110.8	87.6~111.5
Tomato	0.01	78.5~102.6	75.1~99.3	79.5~109.5	79.4~113.1
	0.02	79.5~106.9	77.8~103.3	81.3~110.8	82.2~111.1
	0.04	80.8~108.6	79~102.8	86.2~108.0	84.0~109.0
cucumber	0.01	77.6~102.2	82.3~101.6	83.9~107.5	77.6~104.1
	0.02	78.6~101.2	83.2~103.6	84.2~106.7	81.5~105.0
	0.04	81.5~102.4	84.6~104.5	85.1~109.2	82.8~107.2
cabbage	0.01	78.4~110.2	75.8~105.2	85.6~115.4	79.2~100.2
	0.02	80.8~109.5	79.6~105.8	86.0~113.7	82.1~103.4
	0.04	82.7~109.6	83.8~104.9	85.5~116.7	83.9~105.6
hot pepper	0.01	74.1~102.3	78.8~100.0	84.5~106.2	78.2~107.4
	0.02	79.3~101.4	80.2~103.6	85.4~110.5	82.0~106.9
	0.04	84.3~102.5	81.6~103.8	84.9~106.9	84.5~107.8