SN

中华人民共和国出入境检验检疫行业标准

SN/T 2231—2008

进出口食品中呋虫胺残留量检测方法 液相色谱-质谱/质谱法

Determination of dinotefuran residues in food for import and export—LC-MS/MS

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中华人民共和国
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行　业　标　准
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液相色谱-质谱/质谱法
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前　言

本标准的附录A和附录B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：中国检验检疫科学研究院、龙大食品集团有限公司。

本标准主要起草人：李立、付建、初玉圣、娄喜山、王杰。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中呋虫胺残留量检测方法 液相色谱-质谱/质谱法

1 范围

本标准规定了进出口食品中呋虫胺残留量的制样和液相色谱-质谱/质谱检测方法。

本标准适用于小麦、花生、玉米、菠菜、苹果、胡萝卜、紫苏叶、猪肉及马哈鱼中呋虫胺残留量的检测和确认。

2 方法提要

样品用乙腈提取，提取液加入无水硫酸钠脱水后，用石墨化非多孔碳柱(Envi-Carb)/酰胺丙基甲硅烷基化硅胶柱(LC-NH_2)净化，液相色谱-质谱/质谱法测定，外标法定量。

3 试剂和材料

除另有规定外，试剂均为色谱纯，水为超纯水。

3.1 乙腈。

3.2 正己烷。

3.3 丙酮。

3.4 乙醚。

3.5 氯化钠：优级纯。

3.6 无水硫酸钠：分析纯，650 ℃灼烧 4 h，自然冷却后贮于密封瓶中备用。

3.7 磷酸氢二钾(K_2HPO_4)：分析纯。

3.8 磷酸二氢钾(KH_2PO_4)：分析纯。

3.9 氢氧化钠：分析纯。

3.10 盐酸：优级纯。

3.11 1 mol/L 氢氧化钠：称取 40 g 氢氧化钠(3.9)，溶于 1 L 水中。

3.12 1 mol/L 盐酸：称取 36.5 g 纯盐酸(3.10)，溶于 1 L 水中。

3.13 正己烷饱和的乙腈：在 250 mL 的分液漏斗中分别加入 100 mL 乙腈(3.1)和 100 mL 正己烷(3.2)，充分振摇，静置分层，下层为正己烷饱和乙腈。

3.14 丙酮-正己烷(1+1，体积比)：将等体积丙酮(3.3)和正己烷(3.2)混合，充分振摇。

3.15 磷酸缓冲液：0.5 mol/L(pH=7.0)，称取 52.7 g 磷酸氢二钾和 30.2 g 磷酸二氢钾，加入约 500 mL 水溶解，用 1 mol/L 氢氧化钠或 1 mol/L 盐酸调整 pH7.0 后，加水定容至 1 L。

3.16 呋虫胺标准物质：dinotefuran，$C_7H_{14}N_4O_3$，CAS 号：165252-70-0，纯度大于等于 98%。

3.17 呋虫胺标准储备液：称取适量呋虫胺标准品，用丙酮-正己烷(1+1，体积比)配制成 1.0 mg/mL 的标准储备液；0 ℃～4 ℃保存。保存期 1 年。

3.18 呋虫胺标准工作液：根据需要用丙酮-正己烷(1+1，体积比)将储备液稀释配制成适用浓度的标准工作液。0 ℃～4 ℃保存。保存期 1 个月。

3.19 Envi-Carb/LC-NH_2 固相萃取柱：500 mg/500 mg。Supelclean ENVI-Carb SPE9(石墨化非多孔碳)与 Supelclean LC-NH_2 SPE(酰胺丙基甲硅烷基化硅胶)串联。

3.20 滤膜：0.2 μm。

4 仪器和设备

4.1 液相色谱-质谱/质谱联用仪:配备电喷雾离子源(ESI)。
4.2 振荡器。
4.3 旋涡混合器。
4.4 旋转蒸发仪。
4.5 高速均质器。10 000 r/min。
4.6 离心机。
4.7 离心管:100 mL。
4.8 分液漏斗:250 mL、150 mL。
4.9 粮谷粉碎机。
4.10 食品捣碎机。
4.11 花生粉碎机。

5 试样的制备与保存

5.1 试样制备

5.1.1 小麦、玉米

取有代表性样品约500 g,用粉碎机全部粉碎并通过2.0 mm圆孔筛。混匀,装入洁净的容器内,密闭,标明标记。

5.1.2 水果、蔬菜、鱼、肉

取有代表性样品约500 g,将其可食用部分切碎后,用食品捣碎机将样品加工成浆状。混匀,装入洁净的容器内,密闭,标明标记。

5.1.3 花生

取有代表性样品500 g,用磨碎机全部磨碎。混匀,装入洁净的容器内,密闭,标明标记。

5.2 试样保存

小麦、玉米、花生试样于0 ℃～4 ℃保存;鱼、肉及水果和蔬菜类试样于－18 ℃以下冷冻保存。

在抽样及制样的操作过程中,应防止样品受到污染或发生残留物含量的变化。

6 测定步骤

6.1 提取

6.1.1 小麦、玉米、花生

称取5 g试样(精确至0.01 g)于100 mL离心管中,加20 mL水,放置5 min。加30 mL乙腈(3.1),于10 000 r/min均质提取1 min,离心3 min,提取液过滤至250 mL分液漏斗中,残留物再用20 mL乙腈重复提取一次。合并提取液于分液漏斗中,依次加入20 g氯化钠和60 mL磷酸缓冲液,振摇3 min。静置后,弃去水层。乙腈层加入5 g无水硫酸钠脱水后于40 ℃旋转浓缩至近干。

6.1.2 水果、蔬菜

称取10 g试样(精确至0.01 g)于100 mL离心管中,加30 mL乙腈,于10 000 r/min均质提取1 min,离心3 min,提取液过滤至250 mL分液漏斗中,残留物再用20 mL乙腈重复提取一次。合并提取液于分液漏斗中,依次加入20 g氯化钠和60 mL磷酸缓冲液,振摇3 min。静置后,弃去水层。乙腈层加入5 g无水硫酸钠脱水后于40 ℃旋转浓缩至近干。

6.1.3 鱼、肉

称取10 g试样(精确至0.01 g)于100 mL离心管中,加30 mL乙腈,旋涡混合均匀后于超声波中提取15 min,离心3 min,提取液过滤至250 mL分液漏斗中,残留物再用20 mL乙腈重复提取一次。合

并提取液于分液漏斗中，依次加入 20 g 氯化钠和 60 mL 磷酸缓冲液，振摇 3 min。静置后，弃去水层。乙腈层加入 5 g 无水硫酸钠脱水后于 40 ℃旋转浓缩至近干。

6.2 净化

6.2.1 小麦、玉米、花生、鱼、肉

提取物用 20 mL 正己烷溶解残留物，转入 150 mL 分液漏斗中，分别用 20 mL 正己烷饱和乙腈对溶解液萃取 2 次，取下层溶液合并萃取液，40 ℃减压浓缩干，加入 2 mL 乙腈溶解。

用 10 mL 乙腈预淋洗 Envi-carb/LC-NH_2(500 mg/500 mg)小柱，弃去流出液。注入上述所得溶液，再用 20 mL 乙腈淋洗，收集洗脱液，于 40 ℃旋转浓缩至近干，残留物用乙腈(3.1)溶解，定容至 2.0 mL，过 0.2 μm 滤膜，供液相色谱-质谱/质谱测定。

6.2.2 蔬菜、水果

用 10 mL 乙腈预淋洗 Envi-carb/LC-NH_2(500 mg/500 mg)小柱，弃去流出液。注入上述所得溶液，再用 20 mL 乙腈淋洗，收集洗脱液，于 40 ℃旋转浓缩至近干，残留物用乙腈(3.1)溶解，定容至 2.0 mL，过 0.2 μm 滤膜，供液相色谱-质谱/质谱测定。

6.3 测定

6.3.1 液相色谱-质谱/质谱条件

a) 色谱柱：Waters Acquity Uplc BEH C_8 1.7 μm；

b) 流动相：水(A)和乙腈(B)梯度见表 1；

表 1 流动相梯度

时间/min	流速/(mL/min)	A/%	B/%
0.00	0.25	60	40
0.30	0.25	60	40
2.00	0.25	30	70
2.50	0.25	60	40

c) 柱温：30 ℃；

d) 流速：0.2 mL/min；

e) 进样量：10 μL；

f) 质谱条件：参见附录 A。

6.3.2 液相色谱-质谱/质谱检测

根据样液中被测物含量，选定浓度相近的标准工作溶液，对标准工作溶液与样液等体积参插进样测定，标准工作溶液和待测样液中呋虫胺的响应值均应在仪器检测的线性范围内。

6.3.3 确证

如果样液与标准工作溶液的质量色谱图中，在相同保留时间有色谱峰出现，允许偏差小于±2.5%，所选择离子的丰度比与标准品对应离子的丰度比，其值在允许范围内(允许范围见表 2)。则可判断样品中存在相应的被测物。在 6.3.1 条件下，呋虫胺标准物的液相色谱-质谱谱图参见附录 B。

表 2 使用定性液相色谱-质谱时相对离子丰度最大允许偏差

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

6.4 空白试验

除不加样品外，均按上述步骤进行。

7 结果计算与表述

用色谱数据处理机或按式(1)计算样品中呋虫胺残留量。

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

式中：

X——试样中呋虫胺残留量，单位为微克每千克(μg/kg)；

A——样品溶液中呋虫胺的峰面积；

c——呋虫胺标准工作液的浓度，单位为微克每升(μg/L)；

V——样品溶液最终定容体积，单位为毫升(mL)；

A_s——呋虫胺标准工作溶液的峰面积；

m——最终样液代表的试样质量，单位为克(g)。

8 测定低限和回收率

8.1 测定低限

本方法呋虫胺的测定低限：小麦、花生、玉米、菠菜、苹果、胡萝卜、紫苏叶、肉及鱼中呋虫胺残留量的检测低限为 10 μg/kg。

8.2 回收率

样品的添加浓度及回收率的实验数据见表 3。

表 3 添加浓度及回收率的实验数据

样品名称	添加浓度/(μg/kg)	回收率/%
小麦	10	65.00～72.00
	20	70.45～80.00
	40	75.75～85.00
胡萝卜	10	69.70～80.20
	20	77.80～87.60
	40	80.40～92.00
玉米	10	69.00～78.00
	20	76.50～85.20
	40	78.40～87.50
花生	10	63.20～71.60
	20	68.40～81.00
	40	78.50～85.00
苹果	10	73.60～82.20
	20	78.40～85.45
	40	82.00～90.20
紫苏	10	69.50～85.70
	20	78.45～90.00
	40	83.00～93.50
菠菜	10	75.40～82.60
	20	78.00～87.00
	40	79.40～95.50
猪肉	10	65.50～78.00
	20	74.50～84.30
	40	78.50～90.00
马哈鱼	10	64.00～78.50
	20	74.50～85.45
	40	79.20～94.20

附 录 A
（资料性附录）
质谱/质谱和多反应监测条件[1)]

表 A.1 质谱/质谱条件

电离方式	ESI+
毛细管电压	3.0 kV
源温度	120 ℃
去溶剂温度	350 ℃
锥孔气流	氮气，50 L/h
去溶剂气流	氮气，600 L/h
碰撞气压	氩气，3.10×10^{-6} Pa
监测模式	多反应监测

表 A.2 多反应监测条件

化合物	母离子	子离子	驻留时间/s	锥孔电压/V	碰撞能量/eV
呋虫胺	202.9	128.8[a] 156.8	0.10 0.10	20 20	12 10
[a] 离子用于定量。					

1) 非商业性声明：附录 A 所列参数是在 Waters Quattro Premier 质谱仪上完成的，此处列出试验用仪器型号仅是为了提供参考，并不涉及商业目的，鼓励标准使用者尝试采用不同厂家或型号的仪器。

附 录 B
（资料性附录）
标准物质色谱图

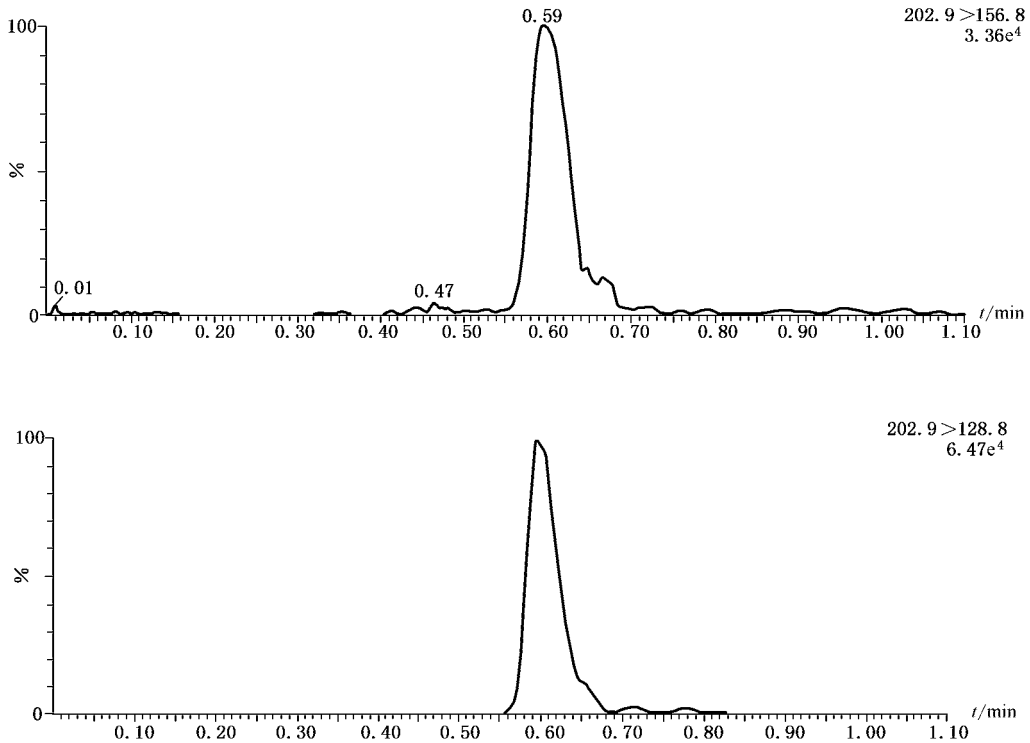

图 B.1 呋虫胺液相色谱-质谱/质谱多反应监测色谱图

Foreword

Annex A of this standard is an informative annex.

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 Chinese Academy Inspection and Quarantine Bureau of the People's Republic of China. longda foodstaff co. ,Ltd.

The main drafters of this standard are: Li Li,Fu Jian,ChuYusheng,Lou XiShan and Wang Jie.

This standard is an Entry-Exit Inspection and Quarantine professional standard promulgated for the first time.

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

Determination of dinotefuran residues in food for import and export—LC-MS/MS

1 Scope

This standard specifies the method of preparation and determination of dinotefuran residue in foodstuffs for import and export by LC-MS/MS.

This standard is applicable to the determination and confirmation of dinotefuran residue in wheat, maize, peanut, pineapple, apple, carrot, spinach, pork, and salmon.

2 Principle

The test sample is extracted by acetonitrile, and the extract is then dehydrated with anhydrous sodium sulfate. After cleaned up with Envi-Carb/LC-NH_2 cartridge or C_{18} solid phase extraction cartridge, the analyte is determined and confirmed by LC-MS/MS, using external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents used should be chromatography pure. "Water" is redistilled water.

3.1 Acetonitrile.

3.2 *n*-Hexane.

3.3 Acetone.

3.4 Aether.

3.5 Sodium chloride.

3.6 Anhydrous sodium sulfate: Analytically pure, ignite for 4 h at 650 ℃ and keep in a tightly container.

3.7 K_2HPO_4.

3.8 Potassium dihydrogen phosphate.

3.9 Sodium hydroxide.

3.10 Hydrochloric acid.

3.11 1 mol/L Sodium hydroxide: Weight 40 g sodium hydroxide(3.9), dissolve in 1 L water.

3.12 1 mol/L Hydrochloric acid: Weight 36.5 g hydrochloric acid (3.10), dissolve in 1 L water.

3.13 Acetonitrile saturated with *n*-hexane: Add 100 mL acetonitrile(3.1) into 100 mL *n*-hexane (3.2), mix adequately, then wait for delamination, use the substrate layer.

3.14 Acetone-*n*-hexane(1+1, *V*/*V*): Mix adequately acetone(3.3)and *n*-hexane with the same volume.

3.15 Phosphate buffer solution: Dissolve 52.7 g dibasic potassium phosphate (K_2HPO_4)and 30.2 g of monobasic potassium phosphate in ca 500 mL water, adjust to pH 7.0 with 1 mol/L sodium hydroxide solution or 1 mol/L hydrochloric acid solution dilute to 1 L with water.

3.16 Dinotefuran standard: $C_7H_{14}N_4O_3$, CAS No: 165252-70-0, purity≥98%.

3.17 Dinotefuran standard stock solution: Weigh an asequate amount of dinotefuran standard, dissolve in acetone *n*-hexane(1+1, *V*/*V*) to prepare a solution of 1.0 mg/mL as the standard stock solution, stored at 0 ℃～4 ℃, avoiding sunlight for one year.

3.18 Dinotefuran standard working solution: According to the requirement, prepare a standard working solution by diluting the stock solution with acetone-*n*-hexane, stored at 0 ℃～4 ℃.

3.19 Envi-Card/LC-NH_2 solid phase extraction column: 500 mg/500 mg.

3.20 Filter: 0.2 μm.

4 Apparatus and equipment

4.1 Liquid chromatography-tandem mass spectrometry equipped with electrospray ion source.

4.2 Shaker.

4.3 Mixer.

4.4 Rotary vacuum evaporator.

4.5 High speed homogenizer:10 000 r/min.

4.6 Centrifuge.

4.7 Centrifuge tube 100 mL.

4.8 Separatory funnel: 150 mL,250 mL.

4.9 Grain disintegrator.

4.10 Tissue blender.

4.11 Peanut muller.

5 Sample preparation and storage

5.1 Preparation of test sample

5.1.1 Wheat,maize

Take approximately 500 g of representative sample. Grind with a grinder to pass through a 2.0 mm round-hole sieve. Mix thoroughly and placed into a clean container,sealed and labeled.

5.1.2 Fruits,vegetables,pork and salmon

Take approximately 500 g of representative sample. The edible part are blended in a high speed blender,mix thoroughly and placed into a clean container,sealed and labeled.

5.1.3 Peanut

Take approximately 500 g of representative sample. Grind and mix thoroughly,placed into a clean container,sealed and labeled.

5.2 Storage of the test sample

Test sample of wheat,maize,peanut,tea,the power of ginger,the power of bunge prickly ash should be stored at a range of 0 ℃～4 ℃. The test sample of fruits and vegetables should be stored below −18 ℃. 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.

6 Procedure

6.1 Extraction

6.1.1 Wheat, maize and peanut

Weigh 5 g of the test sample(accurate to 0.01 g) into a 100 mL centrifuge tube, add 20 mL water, let stand for 5 min, add 30 mL acetonitrile(3.1), Extract for 1 min in a speed of 10 000 r/min, certrifuge for 3 min. Transfer the extract into a 250 mL volumetric flask . The residue was extracted by 20 mL acetonitrile(3.1). Add 20 g sodium chloride and 60 mL phosphate buffer solution shake for 3 min. Place aside for separation. Discard the water phase. Add 5 g anhydrous sodium sulfate into acetonitrile phase. Condense acetonitrile to nearly dryness by a rotary evaporator at 40 ℃.

6.1.2 fruit and vegetable

Weigh 10 g of the test sample(accurate to 0.01 g)into a 100 mL centrifuge tube, add 30 mL acetonitrile(3.1), Extract for 1 min in a high speed of 10 000 r/min, certrifuge for 3 min. Transfer the extract into a 250 mL separatory funnel. Extract the residue with 20 mL acetonitrile, combine the extract, Add 20 g sodium chloride and 60 mL phosphate buffer solution. Shake for 3 min, place aside for separation . Discard the water phase. Add 5 g anhydrous sodium sulfate into acetonitrile phase. Condense acetonitrile to nearly dryness by a rotary evaporator at 40 ℃.

6.1.3 pork and salmon

Weigh 10 g of the test sample (accurate to 0.01 g) into a 100 mL centrifuge tube, add 30 mL acetonitrile(3.1), then vorter, Extract with ultrasonic for 15 min, certrifuge for 3 min. Transfer the extract into a 250 mL separatory funnel. Extract the residue with 20 mL acetonitrile, combine the extract, Add 20 g sodium chloride and 60 mL phosphate buffer solution. Shake for 3 min, place aside for separation . Discard the water phase. Add 5 g anhydrous sodium sulfate into acetonitrile phase. Condense acetonitrile to nearly dryness by a rotary evaporator at 40 ℃.

6.2 Clean-up

6.2.1 Wheat, maize, peanut, pork and salmon

Dissolve the residue with 20 mL *n*-hexane, transfer the solution to 150 mL separatory funnel, extract the residue for two times with 20 mL acetonitrile saturated with *n*-hexane. Combine the underlayer and evaporated to dryness at 40 ℃. Dissolve the residue with 2 mL acetonitrile.

Rines a Envi-Carb/LC-NH_2(500 mg/500 mg)with 10 mL acetonitrile. Load the above solution to column. Elute the column with 20 mL acetonitrile, collect the eluates and evaporated to nearly dryness at 40 ℃. Dissolve the regidue with acetonitrile (3.1), dilute to 2.0 mL, passing through a 0.2 μm filter, the solution is ready for LC-MS/MS determination and confirmation.

6.2.2 Fruit and vegetable

Rines a Envi-Carb/LC-NH_2(500 mg/500 mg)with 10 mL acetonitrile. Discard the effluent. Load the extract(6.1.2) to column . Elute the column with 20 mL acetonitrile,collect the eluates and evaporated to nearly dryness at 40 ℃. Dissolve the residue with acetonitrile (3.1),dilute to 2.0 mL,passing through a 0.2 μm filter,the solution is ready for LC-MS/MS determination and confirmation.

6.3 Determination

6.3.1 LC-MS/MS operating condition

a) LC column: Waters Acquity Uplc BEH C_8 1.7 μm;

b) Mobile phase:Water,acetonitrile;

Table 1—The grade of mobile phase

Time/min	Flow rate/(mL/min)	Water/%	Acetonitrile/%
0.00	0.25	60	40
0.30	0.25	60	40
2.00	0.25	30	70
2.50	0.25	60	40

c) Column temperature: 30 ℃;

d) Flow rate: 0.2 mL/min;

e) Injection volume: 10 μL;

f) MS/MS operating condition:See annex A.

6.3.2 Determination

Select appropriate standard working solution with similar concentration level to that in sample solution. The standard working solution should be injected before and between the injections of the sample solutions with same injection volume. The response value of dinotefuran in the standard working solution and sample solution should be within the linear range of the instrumental detection.

6.3.3 Confirmation

Under above determination condition,the variation range of the vetention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of ±0.25 min, for the same analysis batch and the same compound,the variation range of the ion ratio between the two daughter ions for the unknown sample and in the standard working solution at the similar concentration can not be out of table 1,and then the corresponding analyte must be present in the

sample. Under chromatographic condition above(6.3.1),mass spectrum of the dinotefuran standard are shown respectively as figure B.1 in annex B.

Table 2—Maximum permitted tolerance for relative ion intensities while confirmation

Relative intensity(base peak)/%	>50	>20～50	>10～20	≤10
LC-MS/MS(relative)/%	±20	±25	±30	±50

6.4 Blank test

The operation of the blank test is the same as the discribtion in the methed of determation,but with omition of sample addition.

7 Calculation and expression of result

Calculate the content of fipronil residue in the test sample by LC-MS/MS data processor or according to the formula (1).

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

Where:

X —the residue content of dinotefuran in the test sample, μg/kg;

A —the peak height of dinotefuran in the sample solution;

A_s —the peak height of dinotefuran in the standard working solution;

c —the concentration of dinotefuran in the standard working solution,μg/L;

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

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

8 Limit of determination and recovery

8.1 Limit of determination

The limit of determination of this method is 10 μg/kg.

8.2 Recovery

The experimental data of the concentrations of dinotefuran in the fortified sample and its corresponding recoveries are: seen in table 3.

Table 3—The experimental data of the concentrations of dinotefuran in the fortified sample and its corresponding recoveries

Sample	Added concentrations/(μg/kg)	Recovery/%
Wheat	10	65.00～72.00
	20	70.45～80.00
	40	75.75～85.00
Carrot	10	69.70～80.20
	20	77.80～87.60
	40	80.40～92.00
Maize	10	69.00～78.00
	20	76.50～85.20
	40	78.40～87.50
Peanut	10	63.20～71.60
	20	68.40～81.00
	40	78.50～85.00
Apple	10	73.60～82.20
	20	78.40～85.45
	40	82.00～90.20
Perilla	10	69.50～85.70
	20	78.45～90.00
	40	83.00～93.50
Spinach	10	75.40～82.60
	20	78.00～87.00
	40	79.40～95.50
Pork	10	65.50～78.00
	20	74.50～84.30
	40	78.50～90.00
Salmon	10	64.00～78.50
	20	74.50～85.45
	40	79.20～94.20

Annex A
(informative)
MS/MS and MRM condition[1)]

Table A.1—MS/MS condition

Ion source	ESI+
Capillary voltage	3.0 kV
Source temperature	120 ℃
Desoluation temperature	350 ℃
Cone gas flow	nitrogen,50 L/h
Desoluation gas flow	nitrogen,600 L/h
Collision gas pressure	argon,3.10×10^{-6} Pa
Monitoring model	Multiple reaction monitor(MRM)

Table A.2—MRM condition

Compound	Precursor ion m/z	Product ion m/z	Dwell time/s	Cone voltage/V	Collision energy/eV
Dinotefuran	202.9	128.8[a] 156.8	0.10 0.10	20 20	12 10
[a] the produce ion is used for quantification.					

1) The equipment and their types Waters Quattro Premier involved in the standard method are not related to commercial aims,and the analysts are encouraged to use equipments of different corporation pr different type.

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Annex B

(informative)

MRM Chromatogram of the standard

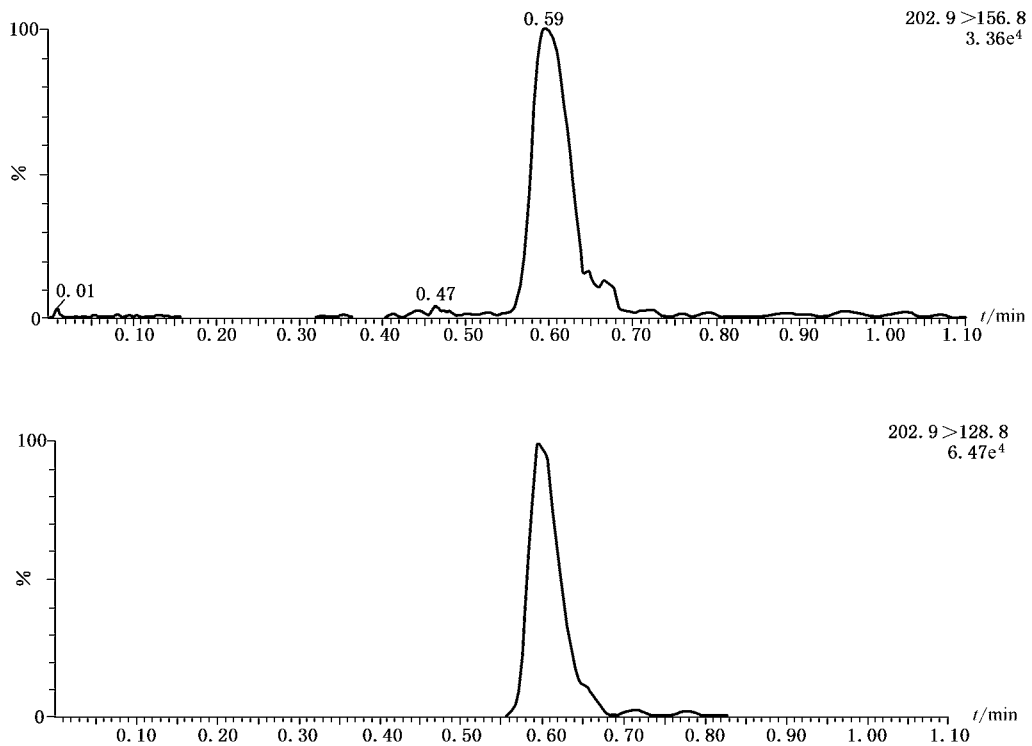

Figure B.1—The MRM Chromatogram of dinotefuran standard

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