SN

中华人民共和国出入境检验检疫行业标准

SN/T 3139—2012

出口农产品中噻虫嗪及其代谢物噻虫胺残留量的测定 液相色谱-质谱/质谱法

Determination of thiamethoxam and its metabolite clothianidin residues in agricultural products for export—LC-MS/MS method

2012-05-07 发布　　2012-11-16 实施

中华人民共和国
国家质量监督检验检疫总局　发布

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：中华人民共和国北京出入境检验检疫局、中华人民共和国山东出入境检验检疫局。

本标准主要起草人：王金花、张蓉、冯骞、李小林、刘岩、汤志旭。

出口农产品中噻虫嗪及其代谢物噻虫胺残留量的测定 液相色谱-质谱/质谱法

1 范围

本标准规定了农产品中噻虫嗪和噻虫胺残留量的液相色谱-质谱/质谱检测方法。

本标准适用于大米、大豆、栗子、菠菜、油麦菜、洋葱、茄子、马铃薯、柑橘、蘑菇、茶等植物源性产品和鸡肝、猪肉、牛奶等动物源性产品中噻虫嗪、噻虫胺残留量的检测和确证。

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 方法原理

用0.1%乙酸-乙腈超声提取试样中的噻虫嗪和噻虫胺残留物，采用基质分散固相萃取剂净化，超高效液相色谱-质谱/质谱仪测定，外标法定量。

4 试剂和材料

除另有说明外，所用试剂均为色谱级，水为符合GB/T 6682规定的一级水。

4.1 乙腈。

4.2 乙酸铵。

4.3 乙酸：分析纯。

4.4 甲酸。

4.5 甲醇。

4.6 正己烷。

4.7 无水硫酸镁($MgSO_4$)：分析纯。

4.8 无水硫酸钠(Na_2SO_4)：分析纯。

4.9 *N*-丙基乙二胺(PSA)吸附剂：粒径40 μm～60 μm。

4.10 石墨化炭黑吸附剂(GCB)：粒径120 μm～400 μm。

4.11 十八烷基硅烷(ODS)键合相吸附剂(C_{18})：粒径40 μm～60 μm。

4.12 基质分散固相萃取剂：

a) 针对大米、茄子、洋葱、马铃薯、柑橘样品：50 mg PSA (4.9)、150 mg $MgSO_4$(4.7)；

b) 针对菠菜、油麦菜样品：50 mg PSA、150 mg $MgSO_4$、10 mg GCB (4.10)；

c) 针对栗子、蘑菇、牛奶样品：50 mg PSA、150 mg $MgSO_4$、50 mg C_{18}(4.11)；

d) 针对茶叶、大豆样品：50 mg PSA、150 mg $MgSO_4$、50 mg C_{18}、10 mg GCB；

e) 针对鸡肝、猪肉样品：50 mg PSA、150 mg $MgSO_4$、50 mg C_{18}。

4.13 0.1%乙酸-乙腈溶液：取 1 mL 乙酸(4.3)，加入乙腈(4.1)定容到 1 000 mL，混匀。

4.14 10 mmol/L 乙酸铵溶液(含 0.1%甲酸)：准确称取 0.16 g 乙酸铵(4.2)于 200 mL 容量瓶中，先用少量水溶解后加入 200 μL 甲酸(4.4)，再用水定容至刻度，混匀，现用现配。

4.15 噻虫嗪标准品(thiamethoxam)：CAS 号 153719-23-4，分子式 $C_8H_{10}ClN_5O_3S$，相对分子质量 291.7，纯度大于或等于 99%。

4.16 噻虫胺标准品(clothianidin)：CAS 号 205510-53-8，分子式 $C_6H_8ClN_5O_2S$，分子量 250.2，纯度大于或等于 99%。

4.17 噻虫嗪标准储备溶液：准确称取适量的噻虫嗪标准品(精确至 0.01 mg)，用乙腈溶解，配制成 1 000 μg/mL的标准储备溶液，−18 ℃下保存。

4.18 噻虫胺标准储备溶液：准确称取适量的噻虫胺标准品(精确至 0.01 mg)，用乙腈溶解，配制成 1 000 μg/mL的标准储备溶液，−18 ℃下保存。

4.19 噻虫嗪和噻虫胺混合标准工作溶液：根据需要分别取适量噻虫嗪标准储备溶液和噻虫胺标准储备溶液，用乙腈稀释成 1 μg/mL 标准工作溶液，−18 ℃下保存。

4.20 基质空白溶液：将不同基质的阴性样品分别按照 7.1 处理后得到的溶液。

4.21 基质标准工作液：根据实验需要吸取适量混合标准工作溶液(4.19)、用基质空白溶液稀释成适当浓度的标准工作液，现用现配。

4.22 微孔滤膜：尼龙，13 mm(内径)，0.2 μm。

5 仪器和设备

5.1 超高效液相色谱-串联质谱仪：配有电喷雾离子源(ESI)。

5.2 粉碎机。

5.3 组织捣碎机。

5.4 天平：感量分别为 0.01 mg 和 0.01 g。

5.5 超声波振荡器。

5.6 旋涡混匀器。

5.7 离心机：转速不低于 4 000 r/min。

5.8 离心管：具塞，聚四氟乙烯，50 mL。

5.9 分液漏斗：100 mL，具塞。

6 试样制备与保存

6.1 要求

在制样的操作过程中，应防止样品受到污染或发生农药残留含量的变化。

6.2 大米、大豆、栗子和茶叶

取代表性样品 500 g，用粉碎机粉碎。混匀，均分成两份作为试样，分装，密闭，于 0 ℃～4 ℃保存。

6.3 菠菜、油麦菜、洋葱、茄子、马铃薯、柑橘和蘑菇

取代表性样品 1 000 g，将其可食用部分(不可用水洗涤)切碎后，依次用捣碎机将样品加工成浆状。混匀，均分成两份作为试样，分装，密闭，于 0 ℃～4 ℃保存。

6.4 鸡肝、猪肉

取代表性样品500 g，将其切碎后，依次用捣碎机将样品加工成浆状，混匀，均分成两份作为试样，分装，密闭，于－18 ℃保存。

6.5 牛奶

取代表性样品约500 g，混匀，均分成两份作为试样，分装，密闭，于0 ℃～4 ℃保存。

7 分析步骤

7.1 提取与净化

7.1.1 大米、大豆、栗子、洋葱、菠菜、油麦菜、茄子、马铃薯、桔子、蘑菇、茶叶和牛奶

称取5 g(精确至0.01 g)试样于50 mL离心管(5.8)中，如果为干燥样品则加5 mL～8 mL水，视具体样品而定，浸泡0.5 h。加入乙酸-乙腈溶液(4.13)使乙腈和水总体积为20 mL，均质30 s，摇匀，在40 ℃以下超声提取30 min，4 000 r/min离心10 min。取上清液1.0 mL，加适量基质分散固相萃取剂(4.12)净化，剧烈振摇1 min，4 000 r/min离心10 min，取上清液用0.2 μm(4.22)滤膜过滤，用液相色谱-质谱/质谱仪测定。

7.1.2 鸡肝、猪肉

称取5 g(精确至0.01 g)试样于50 mL离心管中，加入约3 g无水硫酸钠(4.8)，混匀，加入10 mL乙酸-乙腈溶液，10 mL正己烷，均质3 min，4 000 r/min离心10 min，转移提取液到分液漏斗中，充分摇匀后静置分层，收集乙腈层。按同样操作重复提取一次。用10 mL乙腈洗涤正己烷层，摇匀后静置分层，收集乙腈层。合并三次收集的乙腈层，在40 ℃下旋蒸至近干，用0.1%乙酸-乙腈溶液定容到20 mL，取1.0 mL，加适量基质分散固相萃取剂(4.12)净化，剧烈振摇1 min，4 000 r/min离心10 min，取上清液用0.2 μm滤膜过滤，用液相色谱-质谱/质谱仪测定。

7.2 液相色谱-质谱/质谱仪测定

7.2.1 液相色谱参考条件

a) 液相色谱柱：ACQUITY UPLC BEH C_{18}，50 mm×2.1 mm(内径)，粒度1.7 μm，或性能相当者；
b) 柱温：35 ℃；
c) 流动相梯度：见表1；
d) 进样量：2.0 μL；
e) 样品室温度：10 ℃。

表1 流动相洗脱梯度表

	时间/min	流速/(mL/min)	10 mmol/L乙酸铵溶液(4.10)/%	甲醇/%	曲线类型
1	0.00	0.25	90.0	10.0	
2	0.50	0.25	90.0	10.0	1
3	2.50	0.25	50.0	50.0	6
4	3.00	0.25	90.0	10.0	1

7.2.2 质谱参数

质谱参考条件：参见附录 A。

7.2.3 标准曲线的配制

以 5.0 g 空白样品通过前处理步骤(7.1)制备基质空白溶液，将标准中间溶液稀释至 2.0 ng/mL、5.0 ng/mL、10.0 ng/mL、20.0 ng/mL、40.0 ng/mL、100.0 ng/mL，不过原点做基质标准工作曲线，宜现用现配。

7.3 定量测定

本方法中采用外标校准曲线法定量测定。为减少基质对定量测定的影响，定量用标准曲线应采用基质混合标准工作溶液绘制的标准工作曲线，并且保证所测样品中农药的响应值均在线性范围以内。在上述仪器条件下，噻虫嗪和噻虫胺的保留时间分别约为 2.51 min 和 2.92 min，多反应离子监测色谱图参见图 B.1。

7.4 定性确证

在上述条件下进行测定，试液中待测物的保留时间应在标准溶液保留时间的时间窗内，各离子对的相对丰度应与标准品的相对丰度一致，误差不超过表 2 中规定的范围。噻虫嗪和噻虫胺标准品的多反应离子监测色谱图参见图 B.1。

表 2 定性测定时相对离子丰度最大容许偏差

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

7.5 空白试验

除不加试样外，均按上述操作步骤进行。

8 结果计算和表述

液相色谱-质谱/质谱法测定试样中噻虫嗪或噻虫胺农药的残留量采用标准曲线法定量，标准曲线法定量结果按式(1)计算，计算结果应扣除空白值：

$$X_i = c_i \times \frac{V}{m} \qquad \cdots\cdots(1)$$

式中：

X_i ——试样中噻虫嗪或噻虫胺农药的残留量，单位为毫克每千克(mg/kg)；

c_i ——从标准曲线上得到的被测组分溶液浓度，单位为微克每毫升(μg/mL)；

V ——样品提取液总体积，单位为毫升(mL)(本方法中为 20.0 mL)；

m ——最终样品溶液所代表试样的质量，单位为克(g)。

9 测定低限、回收率

9.1 测定低限

本方法对噻虫嗪和噻虫胺的测定低限为 0.010 mg/kg。

9.2 添加回收率

本方法的添加浓度和回收率范围参见表 C.1。

附 录 A
（资料性附录）
噻虫嗪和噻虫胺质谱分析参考条件[1)]

噻虫嗪和噻虫胺质谱分析参考条件：

a） 电离方式：ESI+；

b） 检测方式：MRM；

c） 毛细管电压：3.00 kV；

d） 氮吹气流量：800 L/h；

e） 锥孔气流量：50 L/h；

f） 去溶剂气温度：350 ℃；

g） 放大器电压：650 V；

h） 离子源温度：110 ℃；

i） 监测离子、碰撞能量和锥孔电压（见表 A.1）。

表 A.1 噻虫嗪和噻虫胺的多反应离子监测分析参数

组 分	监测离子对（m/z）	驻留时间/s	锥孔电压/V	碰撞能量/V
噻虫嗪	*292/211	0.050	20.0	19.0
	292/181	0.050	20.0	10.0
噻虫胺	*250/169	0.050	16.0	15.0
	250/131	0.050	16.0	10.0
*定量离子对。				

1） 非商业声明：以上所列参数是在 Waters Acquity UPLC TM-Quattro Premier 质谱仪上完成的，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）
噻虫嗪和噻虫胺标准溶液谱图

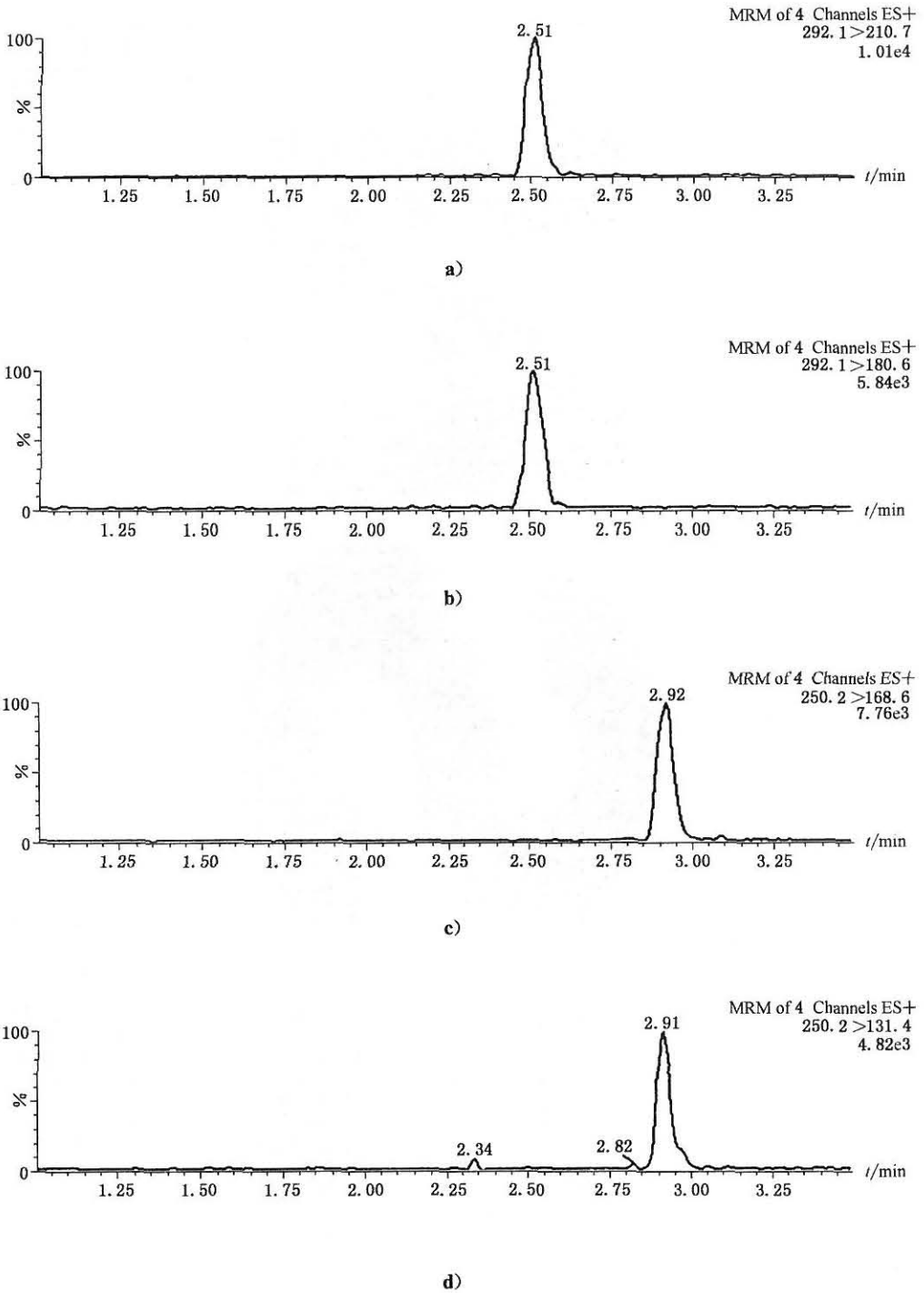


图 B.1 噻虫嗪/噻虫胺标准溶液（浓度为 10 ng/mL）LC-MS/MS 的多反应监测（MRM）色谱图

附 录 C
（资料性附录）
噻虫嗪、噻虫胺回收率范围

表 C.1 噻虫嗪和噻虫胺在不同基质样品中的添加浓度及回收率范围($n=10$)

样 品	添加浓度/(mg/kg)	噻虫嗪回收率范围/%	噻虫胺回收率范围/%
大米	0.010	72.4～79.6	84.4～106.4
	0.020	88.2～103.6	92.2～106.6
	0.040	92.3～102.1	92.4～108.7
大豆	0.010	73.2～93.6	76.8～94
	0.020	71.6～76.2	79.2～93.2
	0.050	70～78.1	90.16～102.8
	0.10	87.2～105.8	87.8～100.6
栗子	0.010	82.0～99.2	74.0～94.4
	0.020	83.2～98.2	83.2～95.8
	0.040	88.5～95.5	85.9～95.1
菠菜	0.010	104.4～109.2	84.4～97.2
	0.020	94.4～109.6	90.4～107.2
	0.040	84.7～108.7	72.6～89.3
	2.0	91.4～95.5	78.2～89.7
油麦菜	0.010	74.8～101.2	73.6～99.6
	0.020	78.2～99.0	75.2～100.2
	0.040	86.1～99.1	80.6～104.2
茄子	0.010	90～102.4	72.8～97.2
	0.50	99.3～103.4	94.7～104.9
	1.0	100.3～105	98.5～107.8
洋葱	0.010	96.8～109.6	82.4～100.8
	0.020	98.0～110.0	86.2～103.2
	0.040	101.1～109.3	84.0～91.0
马铃薯	0.010	75.2～98.8	92.0～100.0
	0.25	72.5～84.0	91.1～100.2
	0.50	70.6～89.0	71.8～87.7
蘑菇	0.010	73.6～99.2	87.6～106.0
	0.020	102.8～107.4	100.6～109.4
	0.040	103.0～112.8	101.1～109.8

表 C.1（续）

样　品	添加浓度/(mg/kg)	噻虫嗪回收率范围/%	噻虫胺回收率范围/%
柑橘	0.010	75.6～85.2	84.0～95.6
	0.040	93.0～105.5	94.9～107.1
	0.50	89.6～108.0	96.4～112.4
	1.0	97.6～104.4	98.2～101.8
茶	0.010	71.6～95.2	83.6～101.6
	0.050	82.6～91.1	90.7～101.4
	0.10	80.9～88.1	95.0～100.7
	20(噻虫嗪)/50(噻虫胺)	79.2～97.2	80.3～95.5
鸡肝	0.010	78.0～93.2	88.8～107.6
	0.020	80.4～96.6	83.4～96.8
	0.040	79.6～97.0	74.1～93.3
猪肉	0.010	70.8～86.4	80.8～98.4
	0.020	70.2～84.6	73.4～91.8
	0.040	82.1～92.7	80.5～90.4
牛奶	0.010	83.2～92.8	94.4～109.2
	0.020	76.2～86.8	92.6～103.8
	0.040	95.0～105.3	97.6～108.8

注：因有些样品的 MRL 值较高，为确保定量结果准确以及避免污染仪器，将较高水平的添加浓度在上机前进行如下稀释：

a) 添加水平为 2 000 μg/kg 时，稀释 10 倍后上机检测；

b) 添加水平为 1 000 μg/kg 时，稀释 10 倍后上机检测；

c) 添加水平为 500 μg/kg 时，稀释 5 倍后上机检测；

d) 添加水平为 20 mg/kg(噻虫嗪)、50 mg/kg(噻虫胺)时，稀释 200 倍后上机检测。

Foreword

This standard was drafted in accordance with the rule of GB/T 1.1—2009.

Please note that some of the elements of this standard may involve patents, but the Standards Organization does not assume responsibility for identifying these patents.

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

This standard was drafted by Beijing and Shandong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Wang Jinhua, Zhang Rong, Feng Qian, Li Xiaolin, Liu Yan, Tang Zhixu.

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

Determination of thiamethoxam and its metabolite clothianidin residues in agricultural products for export—LC-MS/MS method

1 Scope

This standard specifies the determination of thiamethoxam and clothianidin residues in agricultural products for import and export by LC-MS/MS.

This standard is applicable to the determination and comfirmation of thiamethoxam and clothianidin residues in rice, soybean, chestnut, spinage, green lettuce, onion, eggplant, potato, orange, mushrooms, tea, pork, chicken liver and milk.

2 Cited normative documents

Following documents are necessary for the application of this present file. Only the version dated can be applied to the present documents, if not, the latest edition can do (including all the modified form).

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

3 Principle

The Thiamethoxam and Clothianidin residues from the above food samples are extracted with acetonitrile (0.1% Acetic acid). After the extract is cleaned up with PSA, C_{18} and graphitized carbon, The residues are then detected by LC-MS/MS and quantified by external standard method.

4 Reagents and materials

Unless otherwise specified, all reagents used should be HPLC grade, "Water" is first-level water regulated by GB/T 6682.

4.1 Acetonitrile.

4.2 Ammonium acetate.

4.3 Acetic acid: AR.

4.4 Formic acid.

4.5 Methanol.

4.6 *N*-Hexane.

4.7 Anhydrous magnesium sulfate ($MgSO_4$): AR.

4.8 Anhydrous sodium Acetate (Na_2SO_4): AR.

4.9 Primary secondary amine (PSA) sorbent: 40 μm～60 μm.

4.10 Graphitized carbon black sorbent (GCB): 120 μm～400 μm.

4.11 Octadecylsilane (ODS) bonded phase (C_{18}): 40 μm～60 μm.

4.12 Dispersive Solid Phase Extraction:

The usage of dispersive SPE sorbent:

a) rice, eggplant, onion, potato, orange: 50 mg PSA (4.9), 150 mg $MgSO_4$ (4.7);

b) spinach, green vegetable: 50 mg PSA, 150 mg $MgSO_4$, 10 mg GCB (4.10);

c) chestnut, mushroom, milk: 50 mg PSA, 150 mg $MgSO_4$, 50 mg C_{18} (4.11);

d) tea, soybean: 50 mg PSA, 150 mg $MgSO_4$, 50 mg C_{18}, 10 mg GCB;

e) chicken liver and pork: 50 mg PSA, 150 mg $MgSO_4$, 50 mg C_{18}.

4.13 0.1% acetic acid acetonitrile solution: Transfer 1 mL of acetic acid (4.3) into a 1 000 mL beaker, dilute to volume with acetonitrile (4.1) and mix well.

4.14 10 mmol/L ammonium acetate solution (0.1% formic acid): accurately weight 0.16 g ammonium acetate (4.2) in 200 mL volumetric flask, dilute to volume with water and 200 μL formic acid (4.4), mix well.

4.15 Thiamethoxam Standard:, CAS No.: 153719-23-4, molecular formula $C_8H_{10}ClN_5O_3S$, MW 291.7, purity ≥ 99%.

4.16 Clothianidin Standard:, GAS No.: 205510-53-8, molecular formula $C_6H_8ClN_5O_2S$, MW 250.2, purity ≥ 99%.

4.17 Thiamethoxam standard store solution: Accurately weight an adequate amount of thiamethoxam standard (accurating to 0.01 mg), dissolve in Acetonitrile and prepare a solution of 1 000 μg/mL as the standard store solution. This solution should be only stored in refrigerator below −18 ℃.

4.18 Clothianidin standard store solution: accurately weight an adequate amount of Clothianidin standard (accurating to 0.01 mg), dissolve in Acetonitrile and prepare a solution of 1 000 μg/mL as the standard store solution. This solution should be only stored in refrigerator below −18 ℃.

4.19 Mixed-Standard working solution: according to the concentration required, a mixed-standard working solution is prepared from the stock solution of Thiamethoxam and Clothianidin with Acetonitrile. A standard working solution of 1 μg/mL should be stored in refrigerator below −18 ℃.

4.20 Matrix blank solution: the solution of blank matrix handled by step 7.1.

4.21 Matrix standard solution: dilute standard working solution (4.19) by matrix blank solution to proper concentration.

4.22 Membrane filter: nylon, 13 mm (i.d.), 0.2 μm.

5 Apparatus and equipment

5.1 Ultra Performance liquid chromatography-mass spectrometry: equipped with electrospray ion source and triquadruple mass spectrometer.

5.2 Grinder.

5.3 Homogenizer.

5.4 Balance: accurate to 0.01 mg and 0.01 g.

5.5 Ultrasonic water bath.

5.6 Vortex mixter.

5.7 Centrifuge: above 4 000 r/min.

5.8 Centrifuge tube: PTFE, 50 mL, with stopper.

5.9 Seperating funnel: 100 mL, with stopper.

6 Sample preparation and storage

6.1 Requirement

In the course of sample preparation, precautions should be taken to avoid contamination or any factors which may cause the change of residue content.

6.2 Rice, soybean, chestnut, tea

The original sample is mixed from which a 500 g is taken for analysis. The sample is divided to two potions equally after grinded thoroughly. Then the sample is placed in a clean container to be used as the test sample. The container having the test sample is well sealed and labeled. All the samples should be stored under 0 ℃～4 ℃.

6.3 Spinach, green vegetable, onion, eggplant, potato, orange, mushroom

One kg of the edible parts from the original sample without washing is blended thoroughly. The sample is then divided to two equal portions. The sample is placed in a clean container to be used as the test sample. The container having the test sample is well sealed and labeled. The test sample should be stored at 0 ℃～4 ℃.

6.4 Chicken liver, pork

Five hundred grams of the original sample is homogilised thoroughly. The sample is then divided to two equal portions. The sample is placed in a clean container to be used as the test sample. The container having the test sample is well sealed and labeled. The test sample shoul be stored at －18 ℃.

6.5 Milk

The original sample is mixed from which a 500 g is taken for analysis. Then the sample is placed in a clean container to be used as the test sample. The container having the test sample is well sealed and labeled. The sample is stored at 0 ℃～4 ℃.

7 Analysis procedure

7.1 Extraction and clean up

7.1.1 Rice, soybean, chestnut, onion, spinach, green vegetable, eggplant, potato, orange, mushroom, tea and milk

Weight 5 g (accurate to 0.01 g) in centrifuge tube (5.8), for dry samples add 5 mL~8 mL H_2O based on specific sample, then let stand for 0.5 h. Add 0.1% acetic acid acetonitrile solution (4.13) and make sure the toltal volumn of water and acetonitrile is 20 mL. vortex 30 s mix well, extract for 30 min in ultrasonic water bath under 40 ℃. Centrifuge at 4 000 r/min for 10 min. Transfer 1 mL upper layer to 1.0 mL tube, clean up by Dispersive solid phase extraction (MSPD) (4.12), shake for 1 min violently, Centrifuge at 4 000 r/min, 10 min. The supernatant layer is filtered through the 0.2 μm membrane filter (4.22) and ready for LC-MS/MS determination.

7.1.2 Chicken liver, pork

Weight 5 g (accurate to 0.01 g) in 50 mL centrifuge tube, add 3 g Na_2SO_4 (4.8), mix well, add 10 mL 0.1% acetic acid acetonitrile solution and 10 mL *n*-hexane, vortex for 3 min. Centrifuge at 4 000 r/min for 10 min, transfer extract solution to seperating funnel, shake violently and wait for it separate into two phase, then collect the ACN phase. Extract like above once again, then extract the *n*-hexane phase with 10 mL ACN collecting ACN phase. Combine all the ACN phase, viporate under 40 ℃ to dryness, Resolve with 20 mL 0.1% acetic acid acetonitrile solution, transfer 1 mL upper layer and clean up by dispersive solid phase extraction (MSPD) (4.12), shake violently for 1 min, centrifuge at 4 000 r/min for 10 min. The supernatant layer is filtered through the 0.2 μm membrane filter and ready for LC-MS/MS determination.

7.2 LC-MS/MS determination

7.2.1 LC analysis parameters

a) LC column: ACQUITY UPLC BEH C_{18}, 50 mm × 2.1 mm (i.d.), 1.7 μm, or equivalent column.

b) Column tempreture: 35 ℃.

c) Mobile phase: see table 1.

d) Injector volumn: 2.0 μL.

e) Sample tempreture: 10 ℃.

Table 1—Gradient program of mobile phase

	Time/min	Flow rate/(mL/min)	10 mmol/L ammonium acetate solution/%	methanol/%	curve
1	0.00	0.25	90.0	10.0	
2	0.50	0.25	90.0	10.0	1
3	2.50	0.25	50.0	50.0	6
4	3.00	0.25	90.0	10.0	1

7.2.2 MS/MS parameters

See Annex A.

7.2.3 Standard curve

Use matrix blank solution (7.1) to dilute standard working solution to 2.0 ng/mL, 5.0 ng/mL, 10.0 ng/mL, 20.0 ng/mL, 40.0 ng/mL, 100.0 ng/mL for matrix standard working curve.

7.3 LC-MS/MS determination

To reduce the influence of matrix, use matrix standard working curve to determinate target compounds. The responses of target compounds in the matrix standard working solution and the sample solution should be in the linear range of the instrumental detection. The matrix standard working solution should be injected randomly in-between the injections of the sample solution of equal volume.

Under the above LC-MS/MS operating conditions, the retention time of Thiamethoxam and its metabolite Clothianidin are 2.51 min and 2.92 min. LC-MS/MS multiple reaction monitoring chromatogram of target compounds are shown respectively Figure B.1.

7.4 LC-MS/MS confirmation

The relative intensities of the detected ions of each analyte shall correspond to those of the calibration standard solution at comparable concentrations, the allowed relative deviation of relative intensity is less than within the table 2 tolerance, the same compound in sample must be confirmed.

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

Relative ion intensities/%	>50	>20~50	>10~20	≤10
permitted relative tolerances/%	±20	±25	±30	±50

7.5 Blank test

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

8 Calculation and expression of result

Calculate the content of Thiamethoxam and Clothianidin residue in the test sample by LC-MS/MS data processor or using the followed formula (1):

$$X_i = c_i \times \frac{V}{m} \quad \cdots\cdots(1)$$

X_i —the residue content of Thiamethoxam or Clothianidin in the test samples, mg/kg;

c_i —the concentration of Thiamethoxam or Clothianidin of sample solution determinated by matrix standard working solution, μg/mL;

V —the final volume of sample solution, mL (20.0 mL in this method);

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

9 Limit of Determination and recovery

9.1 Limit of determination

The limit of determination of target compound in this method is 0.010 mg/kg.

9.2 Recovery

The recovery and the spiked concentration of target compounds in different matrix are shown by table C.1.

Annex A
(informative annex)
Thiamethoxam and Clothianidin LC-MS/MS reference condition[1)]

Thiamethoxam and clothianidin LC-MS/MS reference condition:

a) Ionization mode:ESI+;

b) Scan mode:MRM;

c) Capillary Voltage:3.00 kV;

d) N_2 Desolvation Gas Flow:800 L/h;

e) Cone Gas Flow:50 L/h;

f) Desolvation Temperature:350 ℃;

g) Multiple voltage:650 V;

h) Source Temperature:110 ℃;

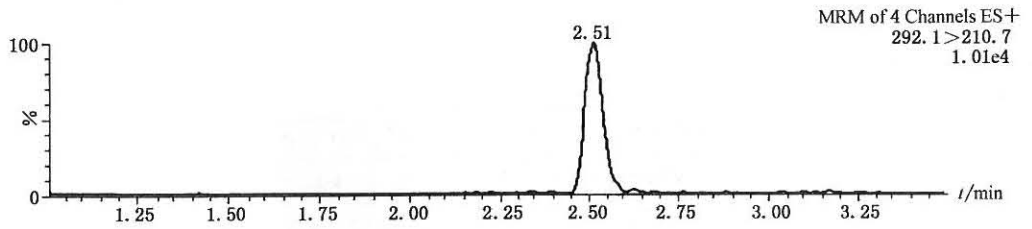
i) Multiple reaction monitoring (MRM) condition (see table A.1).

Table A.1—MRM analytical parameters for Thiamethoxam and Clothianidin

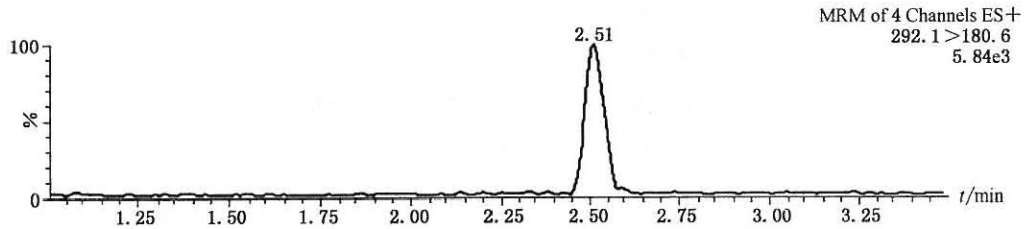
Target compound	Channel Reaction (m/z)	Dwell time/s	Cone voltage/V	Collision Energy/V
thiamethoxam	* 292/211	0.050	20.0	19.0
	292/181	0.050	20.0	10.0
clothianidin	* 250/169	0.050	16.0	15.0
	250/131	0.050	16.0	10.0
* quantitative ion pairs.				

1) Non-commercial statement:the equipments Waters Acquity™ UPLC-Quattro Premier involved in this standard method are not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different.

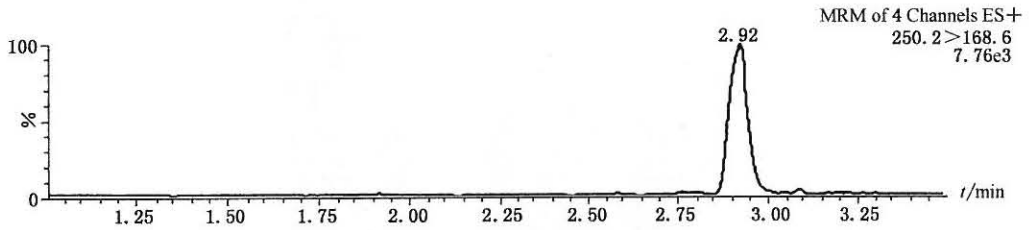
Annex B
(informative annex)
Multiple reaction monitoring chromatogram of the Thiamethoxam and Clothianidin standard



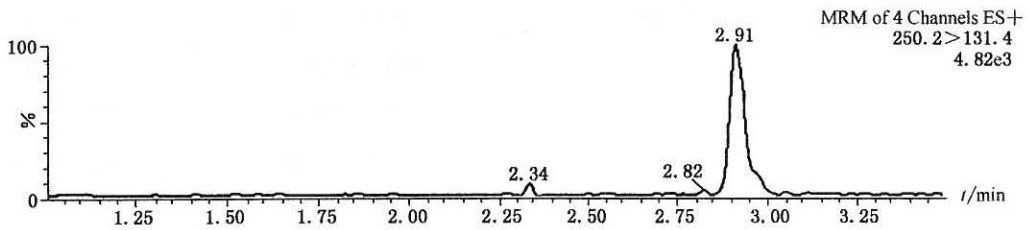
a)



b)



c)



d)

Figure B.1—The MRM chromatograph of Thiamethoxam and Clothianidin standard solution

Annex C
(informative annex)
The recovery range of Thiamethoxam and Clothianidin

Table C. 1—The spiked contration and recovery range of Thiamethoxam and Clothianidin ($n = 10$)

Sample	Spiked conc/(mg/kg)	Recovery range of Thiamethoxam/%	Recovery range of Clothianidin/%
rice	0.010	72.4～79.6	84.4～106.4
	0.020	88.2～103.6	92.2～106.6
	0.040	92.3～102.1	92.4～108.7
soybean	0.010	73.2～93.6	76.8～94
	0.020	71.6～76.2	79.2～93.2
	0.050	70～78.1	90.16～102.8
	0.10	87.2～105.8	87.8～100.6
chestnut	0.010	82.0～99.2	74.0～94.4
	0.020	83.2～98.2	83.2～95.8
	0.040	88.5～95.5	85.9～95.1
spinach	0.010	104.4～109.2	84.4～97.2
	0.020	94.4～109.6	90.4～107.2
	0.040	84.7～108.7	72.6～89.3
	2.0	91.4～95.5	78.2～89.7
green vegetable	0.010	74.8～101.2	73.6～99.6
	0.020	78.2～99.0	75.2～100.2
	0.040	86.1～99.1	80.6～104.2
eggplant	0.010	90～102.4	72.8～97.2
	0.50	99.3～108.4	94.7～104.9
	1.0	100.3～105	98.5～107.8
onion	0.010	96.8～109.6	82.4～100.8
	0.020	98.0～110.0	86.2～103.2
	0.040	101.1～109.3	84.0～91.0
potato	0.010	75.2～98.8	92.0～100.0
	0.25	72.5～84.0	91.1～100.2
	0.50	70.6～89.0	71.8～87.7
mushroom	0.010	73.6～99.2	87.6～106.0
	0.020	102.8～107.4	100.6～109.4
	0.040	103.0～112.8	101.1～109.8

Table C. 1 (continued)

Sample	Spiked conc/(mg/kg)	Recovery range of Thiamethoxam/%	Recovery range of Clothianidin/%
orange	0.010	75.6～85.2	84.0～95.6
	0.040	93.0～105.5	94.9～107.1
	0.50	89.6～108.0	96.4～112.4
	1.0	97.6～104.4	98.2～101.8
tea	0.010	71.6～95.2	83.6～101.6
	0.050	82.6～91.1	90.7～101.4
	0.10	80.9～88.1	95.0～100.7
	20 (Thiamethoxam)/ 50 (Clothianidin)	79.2～97.2	80.3～95.5
chicken liver	0.010	78.0～93.2	88.8～107.6
	0.020	80.4～96.6	83.4～96.8
	0.040	79.6～97.0	74.1～93.3
pork	0.010	70.8～86.4	80.8～98.4
	0.020	70.2～84.6	73.4～91.8
	0.040	82.1～92.7	80.5～90.4
milk	0.010	83.2～92.8	94.4～109.2
	0.020	76.2～86.8	92.6～103.8
	0.040	85.0～105.3	97.6～108.8

Note: For the high MRL of some samples, the extract is diluted as below before instrument detection.

a) spiked level 2 000 μg/kg, dilute 10 times before instrument detection;

b) spiked level 1 000 μg/kg, dilute 10 times before instrument detection;

c) spiked level 500 μg/kg, dilute 5 times before instrument detection;

d) spiked level 20 mg/kg (Thiamethoxam), 50 mg/kg (Clothianidin), dilute 200 times before instrument detection.