SN

中华人民共和国出入境检验检疫行业标准

SN/T 2453—2010

进出口动物源性食品中二硝托胺残留量的测定　液相色谱-质谱/质谱法

Determination of zoalene residues in foodstuffs of animal origin for import and export—LC-MS/MS method

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前　言

本标准的附录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。

本标准由中华人民共和国黑龙江出入境检验检疫局负责起草。

本标准主要起草人：刘永、吴岩、杨长志、康庆贺、王雪飞、鞠文东。

本标准系首次发布的出入境检验检疫行业标准。

进出口动物源性食品中二硝托胺残留量的测定　液相色谱-质谱/质谱法

1　范围

本标准规定了动物源性食品中二硝托胺的制样和液相色谱 质谱/质谱检测及确证方法。

本标准适用于动物肉、肝脏、肾脏、牛奶中二硝托胺残留量的测定。

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。凡是注明日期的引用文件，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面研究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　方法提要

用乙腈提取试样中的二硝托胺，提取液经中性氧化铝固相萃取柱净化，洗脱液浓缩后定容，液相色谱 质谱/质谱仪测定，外标法定量。

4　试剂和材料

所有试剂除特殊注明外，所用试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1　乙腈：色谱纯。

4.2　二氯甲烷。

4.3　甲醇。

4.4　丙酮。

4.5　氯化钠。

4.6　无水硫酸钠：经 650 ℃灼烧 4 h，冷却后在干燥器中储存备用。

4.7　无水硫酸钠柱：用内径约为 1.5 cm 的下部收口玻璃管，收口处填装少量玻璃棉，然后填装无水硫酸钠，高度约为 1.5 cm 左右。

4.8　二氯甲烷 丙酮(7＋3)溶液：量取 100 mL 丙酮，用 400 mL 二氯甲烷稀释。

4.9　甲醇 丙酮(8＋2)溶液：量取 100 mL 甲醇，用 400 mL 丙酮稀释。

4.10　乙腈 水(4＋6)溶液：量取 400 mL 乙腈，用水稀释至 1 000 mL，过 0.22 μm 微孔滤膜。

4.11　乙腈饱和的正己烷溶液：20 mL 乙腈中加入 100 mL 正己烷，充分振荡后，静置分层，取上层正己烷层备用。

4.12　中性氧化铝固相萃取柱：1 000 mg，6 mL，或相当者。使用前分别用 10 mL 丙酮、10 mL 二氯甲烷 丙酮(7＋3)溶液预处理，并保持柱体湿润。

注：中性氧化铝固相萃取柱开封后应保持干燥。

4.13　标准物质：二硝托胺纯度大于 98.0%，$C_8H_7N_3O_5$，CAS：148 01 6。

4.14　标准储备溶液：准确称取按其纯度折算为 100%质量的二硝托胺标准品 0.010 g，用乙腈溶解并定容至 100 mL，浓度相当于 100 μg/mL。储备液在 0 ℃～4 ℃冰箱避光保存，可使用 6 个月。

4.15　标准中间溶液：准确量取标准储备溶液 1.0 mL，用乙腈 水(4＋6)溶液溶解并定容至 100 mL，浓

度相当于 1.0 μg/mL，在 0 ℃～4 ℃冰箱避光保存，可使用 1 周。

4.16 标准工作溶液：根据需要用 1.0 mL 适当浓度的标准工作溶液溶解空白样品浓缩残渣，配制成适当浓度的标准工作溶液，标准工作溶液应当天配制。

5 仪器和设备

5.1 高效液相色谱 质谱/质谱仪：配电喷雾离子源(ESI)或相当者。

5.2 天平：感量 0.01 g。

5.3 分析天平：感量 0.1 mg。

5.4 固相萃取装置。

5.5 氮吹仪。

5.6 旋涡混匀器。

5.7 均质器。

5.8 离心机。

5.9 0.22 μm 微孔滤膜。

6 试样的制备与保存

固态样品取有代表性的约 200 g，均分成两份作为试样，分别装入洁净容器，密封并做好标识，于－18 ℃冰箱内保存；液态样品取有代表性的约 200 mL，均分成两份作为试样，分别装入洁净容器，密封并做好标识，于 0 ℃～4 ℃冰箱内保存。

在抽样和制样操作过程中，应防止样品受到污染或发生二硝托胺含量的变化。

7 测定步骤

7.1 提取

7.1.1 固态高含量样品

准确称取 0.5 g 试样(精确到 0.01 g)于 50 mL 离心管中，加入 2 g 无水硫酸钠、20 mL 乙腈，用均质器以 10 000 r/min 均质 1 min，以 4 000 r/min 离心 5 min，上清液转移至 50 mL 具塞离心管中，样品用 10 mL 乙腈重复上述提取、离心操作，合并两次提取的上清液，加入经乙腈饱和的正己烷溶液10 mL，用旋涡振荡器混匀 2 min 后，以 4 000 r/min 离心 3 min，弃去上清液，下层过无水硫酸钠柱，转移至鸡心瓶，浓缩近干后待净化。

7.1.2 固态低含量样品

准确称取 5.0 g 试样(精确到 0.01 g)于 50 mL 离心管中，以下同 7.1.1。

7.1.3 液态样品

准确量取 5.0 mL 试样于 50 mL 离心管中，加入 2 g 氯化钠、10 mL 乙腈，旋涡振荡 3 min，以 4 000 r/min离心 5 min，取上清液 5 mL 于 15 mL 离心管中，加入经乙腈饱和的正己烷溶液 10 mL，用旋涡振荡器混匀 2 min 后，以 4 000 r/min 离心 3 min，弃去上清液，下层用氮吹仪浓缩至近干，待净化。

7.2 净化

残渣用 2.0 mL 二氯甲烷 丙酮(8＋2)溶液溶解，以 1 mL/min～2 mL/min 的速度过中性氧化铝固相萃取柱，再用 2.0 mL 二氯甲烷 丙酮(7＋3)溶液分两次洗涤浓缩瓶，过中性氧化铝固相萃取柱，弃去流出液，待上样液流出后，用 10 mL 甲醇 丙酮(8＋2)溶液洗脱，洗脱液于氮吹仪浓缩至近干，用 1.0 mL 乙腈 水(4＋6)溶液溶解残渣，经 0.22 μm 滤膜过滤，供液相色谱 质谱/质谱测定。

7.3 测定

7.3.1 液相色谱条件

a) 色谱柱：C_{18} 1.7 μm，2.1(内径)×50 mm，或相当者；

b) 流动相:乙腈∶水=4∶6;

c) 流速:0.25 mL/min 或根据仪器条件优化;

d) 柱温:30 ℃;

e) 进样量:5 μL。

7.3.2 质谱条件

a) 离子源:电喷雾离子源;

b) 扫描方式:负离子;

c) 检测方式:多反应监测(MRM);

d) 其他质谱/质谱参考条件参见附录 A 中表 A.1。

7.3.3 液相色谱-质谱/质谱测定

7.3.3.1 定量测定

根据样液中二硝托胺的浓度大小,选定峰高相近的标准工作溶液,标准工作溶液和样液中二硝托胺的响应值均应在仪器的检测线性范围内。对标准工作溶液和样液等体积参差进样测定,在上述色谱条件下,二硝托胺的参考保留时间约为 0.86 min,标准溶液的多反应监测色谱图参见附录 B 中图 B.1。

7.3.3.2 定性测定

在相同的试验条件下,样品与标准工作液中待测物质的质量色谱峰相对保留时间在 2.5%以内,并且在扣除背景后的样品质量色谱图中,所选择的离子对均出现,同时与标准品的相对丰度允许偏差不超过表 1 规定的范围,则可判断样品中存在对应的被测物。

表 1 使用定性液相色谱-质谱/质谱时相对离子丰度最大允许偏差

相对离子丰度(K)	$K>50$	$20<K<50$	$10<K<20$	$K\leqslant10$
允许的最大偏差/%	±20	±25	±30	±50

8 空白试验

除不加试样外,均按上述操作步骤进行。

9 结果计算和表述

用数据处理软件中的外标法,或绘制标准曲线,按照式(1)计算样品中二硝托胺的含量。

$$X=\frac{c\cdot V}{m\cdot 1\,000} \qquad \cdots\cdots(1)$$

式中:

X 试样中待测组分含量,单位为毫克每千克(μg/kg);

c 由标准曲线而得的样液中待测组分的浓度,单位为纳克每毫升(ng/ mL);

V 样液最终定容体积,单位为毫升(mL);

m 最终样液所代表的试样质量,单位为克(g)。

10 测定低限、回收率

10.1 测定低限

本方法二硝托胺测定低限为 5 μg/kg。

10.2 回收率

回收率试验数据参见附录 C 中表 C.1。

附 录 A
（资料性附录）
串联质谱条件[1)]

串联质谱条件：

a） 电离方式：ESI ；

b） 毛细管电压：3.0 kV；

c） 源温度：110 ℃；

d） 去溶剂温度：350 ℃；

e） 锥孔气流：50 L/h；

f） 去溶剂气流：550 L/h；

g） 碰撞气：氩气，碰撞气压 0.33 Pa(3.30×10^{-3} mbar)；

h） 监测模式：多反应监测，多反应监测条件见表 A.1。

表 A.1 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
二硝托胺	224.3	181.2[a]	0.1	20	10
		151.2	0.1	20	18
[a] 离子用于定量。					

1） 非商业性声明：表 A.1 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）
标准样品色谱图

二硝托胺高效液相色谱 质谱/质谱色谱图，见图 B.1。

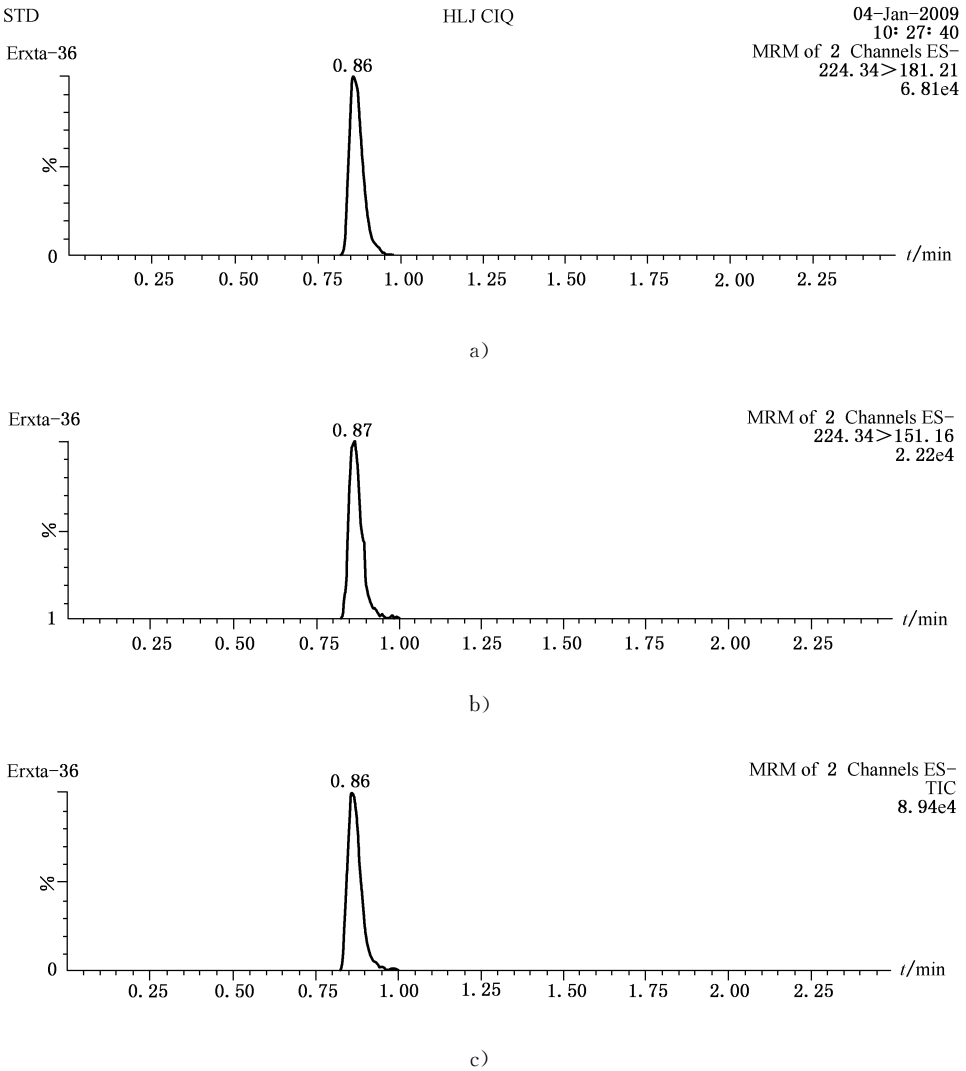


图 B.1 二硝托胺高效液相色谱-质谱/质谱色谱图

附 录 C
（资料性附录）
添加回收率

添加回收率见表 C.1。

表 C.1 添加回收率（*n* 10）

食品名称	添加浓度/（mg/kg 或 mg/L）	平均测定值/（mg/kg）	平均回收率/%	相对标准偏差/%
鸡肉	0.1 1.0 6.0	0.087 0.894 5.663	87.2 83.8 94.4	10.54 8.62 4.50
鸡肝	0.1 1.0 6.0	0.088 0.820 5.32	85.6 81.99 88.62	9.08 7.48 4.83
鸡肾	0.1 1.0 6.0	0.088 0.859 5.31	88.5 85.88 88.60	7.45 10.79 5.43
牛奶	0.1 1.0 6.0	0.087 0.926 5.394	87.0 92.6 89.9	7.43 5.56 6.10

Foreword

Annex A，annex B and annex C of this standard is informative annexes.

This standard was proposed by and is under the charge of China National Regulatory Commission for Certification and Accreditation.

The standard was drafted by Hei longjiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Liu Yong et al.

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

Determination of zoalene residues in foodstuffs of animal origin for import and export—LC-MS/MS method

1 Scope

This standard specifies the methods of sample preparation and determination by liquid chromatography mass spectrometry of zoalene residues in foodstuffs of animal origin.

This standard is applicable to the determination and qualification of zoalene residue in animal-derived foods including pork, poultry and their liver, kidney.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this national standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based in this national standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies.

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

3 Principles

The residues of zoalene residues in the test sample are extracted with acetonitrile, *n*-hexane is used to remove lipid soluble matters. The test sample are cleaned-up with Al_2O_3 solid-phase extractioncartridges. After being concentrated, the residues are dissolved volume with Acetonitrile-water, and determined by liquid chromatography mass spectrometry with electrospray ionization and triquadruple mass spectrometer, quantified by external standard method.

4 Reagents and materials

Unless otherwise 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 Dichloromethane.

4.3 Methanol.

4.4 Acetone.

4.5 Sodium chloride.

4.6 Sodium sulfate: Be heat in 650 ℃ 4 h, then cooling to room temperature in desiccator.

4.7 Sodium sulfate column: Be used glass tube of bottom contraction and filled 1.5 cm sodium chloride.

4.8 Dichloromethane-acetone (7 + 3) solution: 100 mL acetone diluted to 400 mL with dichloromethane.

4.9 Methanol-acetone (8+2) solution: 100 mL methanol diluted to 400 mL with acetone.

4.10 Acetonitrile-water (4+6) solution: 400 mL acetonitrile diluted to 1 000 mL with water.

4.11 *n*-hexane saturated with acetonitrile: Add 20 mL *n*-hexane into 100 mL acetonitrile, mix adequately, then wait for delamination separation, use the substrate layer.

4.12 Al_2O_3 SPE cartridges (6 mL, 1 000 mg) or equivalent: Pretreat the cartridge with 10 mL acetone, 10 mL dichloromethane-acetone (7+3) solution before using, and keep the cartridge wet.

4.13 Standards: Zoalene, purity should be no less than 98.0%.

4.14 Stock standard solution: Dissolve 0.010 g zoalene with acetonitrile in 100 volumetric flask and the concentration of standard is 100 μg/mL. The solution should be stored at the temperature 0 ℃ ~ 4 ℃ for more than six months.

4.15 Intermediate standard solution: Add 1.0 mL stock standard solution to a 100 mL volumetric flask and dilute to volume with acetonitrile-water (4+6) solution. The concentration of intermediate standard solution is 1 μg/mL. The solution should be stored at the temperature 0 ℃ ~4 ℃ for more than one week.

4.16 Working standard solution: According to the requirement, dilute 1.0 mL intermediate standards solution with blank matrix extraction solution to proper concentration. The solution should be prepared just before using.

5 Apparatus and equipment

5.1 Liquid chromatography-mass spectrometry, equipped with electrospray ion source and triquadruple mass spectrometer or equivalent.

5.2 Blance: Sensitivity at 0.01 g.

5.3 Analytical balance: Sensitivity at 0.1 mg.

5.4 SPE vacuum container.

5.5 Nitrogen flow appearance.

5.6 Vortex mixer.

5.7 Tissue blender.

5.8 Centrifuge.

5.9 Membrane filter: 0.22 μm.

6 The preparation and storing of the sample

About 200 g representative samples should be taken from all samples, then grinded and blended by a tissue blender to produce homogenous samples, put in suitable clean container. After being sealed and labeled, the samples should be stored at below 18 ℃ in refrigerator.

7 Procedure

7.1 Extraction

7.1.1 Solid fatty sample

An aliquot of 0.5 g of the test sample (accurate to 0.01 g) was weighed in a 50 mL centrifuge tube. Add 2 g sodium sulfate and 20 mL acetonitrile. For extraction, homogen at 10 000 r/min for 1 min, then centrifuge at 4 000 r/min for 5 min. Extract again with 10 mL acetonitrile. Combine the supernatants and mix the extraction with Acetonitrile-*n*-hexane saturated solution (5.11) 10 mL.

Vortexes for 2 min, then centrifuge at 4 000 r/min for 3 min. Abandon the supernatants, and the acetonitrile phase permeated the natrii sulfas exsiccatus. Concentrate with rotatory evaporator, then it is

ready for cleaned-up.

7.1.2 Solid a little fatty sample

An aliquot of 0.5 g of the test sample (accurate to 0.01 g) was weighed in a 50 mL centrifuge tube and bottom was same of 7.1.1.

7.1.3 Liquid sample

An aliquot of 5.0 mL of the test sample was measured in a 50 mL centrifuge tube. Add 2 g sodium chloride and 10 mL acetonitrile. Centrifuge at 4 000 r/min for 5 min. Extract again with 10 mL acetonitrile. Combine the supernatants and mix the extraction with Acetonitrile-*n*-hexane saturated solution 10 mL. Vortexes for 2 min, then centrifuge at 4 000 r/min for 3 min. Abandon the supernatants, and the acetonitrile phase permeated the natrii sulfas exsiccatus. Concentrate with rotatory evaporator, then it is ready for cleaned-up.

7.2 Clean-up

The residue is to dissolve with 2.0 mL Dichloromethane-acetone (8+2) solution. Load the solution to Al_2O_3 SPE cartridge (5.14) at the flow rate of 1 mL/min～2 mL/min, then wash the heart-bottle twice with 2.0 mL Dichloromethane-acetone (7+3) solution and permeate the Al_2O_3 SPE cartridge. Elute with 10 mL Methanol-acetone (8+2) solution, then dry the eluant with nitrogen flow appearance (at less than 45 ℃), then dissolved the residue to 1.0 mL with Acetonitrile-water (4+6) solution. After filtered with 0.22 μm filter, the final solution is ready for LC-MS/MS determination.

7.3 Determination

7.3.1 HPLC operatibottle

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

b) Mobile phase: acetonitrile : water 4 : 6;

c) Flow rate: 0.25 mL/min;

d) Column temperature: 30 ℃;

e) Injection volume: 5 μL.

7.3.2 MS operating condition

a) Ion source: ESI-;

b) Scanning model:Negaive ion;

c) Determination model:Multiple reaction monitor (MRM);

d) Other parameters are listed annex A.

7.3.3 LC-MS/MS determination

7.3.3.1 Quantitative determination

According to the method, detect the residues of zoalene in the test sample solution, the standard working solution, The response of Zoalene should be in the linear range of the instrumental detection. If the response out of the linear range, dilute with the extract of the blank sample to suitable concertration. Reconstituted ion chromatogram of standard working solution is listed in fig. B. 1 of annex B.

7.3.3.2 Quantifation determination

Under the same conditions of experiment, the retention time of the unknow sample is the same as the stand working solution, the qualification ions for every compound must be found. 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 the standard working solution at the similar concentration can not be out of range of table 1.

Table 1—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity/%	>50	>20～50	>10～20	≤10
Permitted tolerances/%	±20	±25	±30	±50

8 Blank tests

The operation of blank test is the same as the procedure that prescribed above, but omission of sample.

9 Calculation and expression of result

Calculate the content of zoalene residue concentration in the sample is carried out by LC/MS/MS data processor or according to the formula (1):

$$X \quad \frac{c \times V}{m \times 1\,000} \qquad \text{.....(1)}$$

where:

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

c—the concentration of Zoalene in the test sample calculated by calibration curve, ng/mL;

V—the final volume of sample solution, mL;

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

10 Limit of quantification and recovery

10.1 Limit of quantification

The limit of quantification for Zoalene is 5 μg/kg.

10.2 Recovery

The results of recoveries were showed on table C.1 of annex C.

Annex A
(informative)
LC-MS/MS condition[1)]

LC-MS/MS condition

a) Ion source:ESI-;

b) Capillary voltage:3.0 kV;

c) Source temperature:110 ℃;

d) Desolvation temperature:350 ℃;

e) Cone gas flow:Nitrogen,50 L/h;

f) Desolvation gas flow:Nitrogen,550 L/h;

g) Collision gas pressure:Argon,0.33 Pa (3.30×10^{-3} mbar);

h) Monitoring model:multiple reaction monitor (MRM),MRM condition see table A.1.

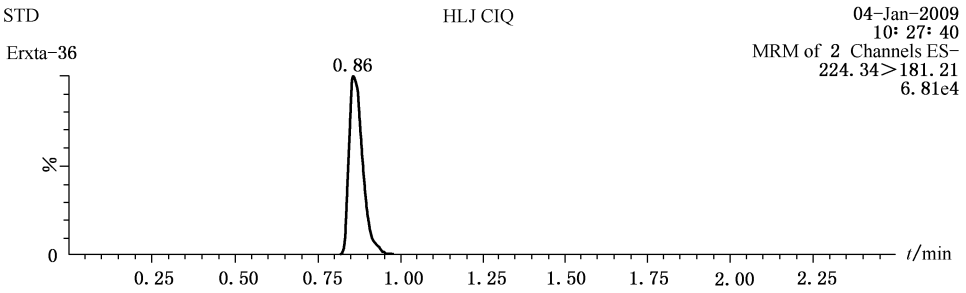
Table A.1—MRM condition

Compound	Precursor ion	Product ion	Dwell time/s	Cone voltage/V	Collision energy/eV
Zoalene	224.3	181.2[a]	0.1 s	20	10
		151.2	0.1 s	20	18
[a] quantification determination.					

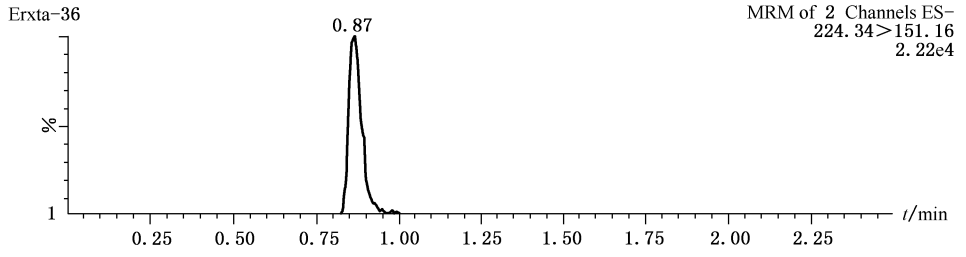
1) Non commercial statement:the equipments and their types Waters Quattro Premier involved in the standard method are not related to commercial aims,and the analysts are encouraged to equipments of different corporation or different type.

Annex B
(informative)
MRM chromatogram of the standard

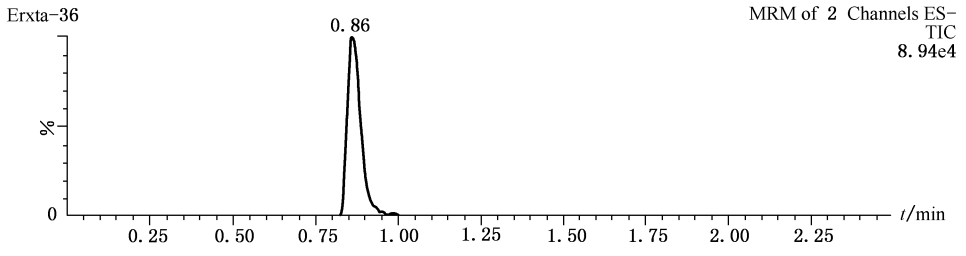
The MRM chromatogram of zoalene standard see figure B. 1.



a)



b)



c)

Figure B. 1—The MRM chromatogram of zoalene standard

Annex C
（informative）
The results of recovery in different matrix

The results of recovery in different matrix see table C. 1.

Table C. 1—The data of recovery（n = 10）

Samples	Added concentration/（mg/kg mg/L）	Mean of results/（mg/kg）	Range of recovery/%	RSD/%
Chicken	0. 1 1. 0 6. 0	0. 087 0. 894 5. 663	87. 2 83. 8 94. 4	10. 54 8. 62 4. 50
Chick Liver	0. 1 1. 0 6. 0	0. 088 0. 820 5. 32	85. 6 81. 99 88. 62	9. 08 7. 48 4. 83
Chick Kidney	0. 1 1. 0 6. 0	0. 088 0. 859 5. 31	88. 5 85. 88 88. 60	7. 45 10. 79 5. 43
Milk	0. 1 1. 0 6. 0	0. 087 0. 926 5. 394	87. 0 92. 6 89. 9	7. 43 5. 56 6. 10

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