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中华人民共和国出入境检验检疫行业标准

SN/T 2314—2009

进出口动物源性食品中二苯脲类残留量检测方法

Determination of diphenylurea residues in foodstuffs of animal origin for import and export

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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。

本标准起草单位：中华人民共和国广东出入境检验检疫局、中华人民共和国黑龙江出入境检验检疫局。

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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苯脲类残留量检测方法

1 范围

本标准规定了进出口动物源性食品中尼卡巴嗪(标志残留物为双硝基均二苯脲)和双咪苯脲残留量的液相色谱和液相色谱-质谱/质谱法测定方法。

本标准适用于牛肉、鸡肉、牛肝、鸡肝和鸡蛋中尼卡巴嗪和双咪苯脲残留量的测定和确证。

2 方法提要

试样在碱性条件下用乙腈提取,正己烷液液分配,双咪苯脲需经弱阳离子交换固相萃取柱净化,用液相色谱测定,外标法定量;用液相色谱-质谱/质谱测定和确证,内标法定量双硝基均二苯脲,外标法定量双咪苯脲。

3 试剂和材料

除另有说明外,所有试剂均为分析纯,水为去离子水。

3.1 乙腈:色谱纯。

3.2 正己烷。

3.3 甲醇:色谱纯。

3.4 冰乙酸:色谱纯。

3.5 氢氧化钾。

3.6 *N*,*N*-二甲基甲酰胺。

3.7 无水硫酸钠:经 650 ℃灼烧 4 h,置于干燥器内备用。

3.8 乙腈饱和正己烷:正己烷与足够的乙腈混合至饱和。

3.9 0.1%乙酸水溶液:取冰乙酸 1 mL,用水定容至 1 L。过 0.45 μm 滤膜备用。

3.10 1%乙酸水溶液:取冰乙酸 10 mL,用水定容至 1 L。过 0.45 μm 滤膜备用。

3.11 2%乙酸水溶液:取冰乙酸 2 mL,用水定容至 100 mL。过 0.45 μm 滤膜备用。

3.12 5%乙酸甲醇溶液:取冰乙酸 5 mL,用甲醇定容至 100 mL。

3.13 50%氢氧化钾溶液:取 50 g 氢氧化钾,溶于 100 mL 水。

3.14 甲醇-0.1%乙酸水溶液(1+1,体积比):量取 50 mL 甲醇,加 50 mL 0.1%乙酸水溶液(3.9),混匀备用。

3.15 空白样品提取溶液-水溶液(1+9,体积比):量取 1 mL 空白样品提取溶液,加 9 mL 水,混匀备用。

3.16 双硝基均二苯脲标准物质(Dinitrocarbanilide,CAS 号:587-90-6):纯度大于等于 97%。

3.17 双咪苯脲标准物质(Imidocarb,CAS 号:27885-92-3):纯度大于等于 97%。

3.18 氘代双硝基均二苯脲(双硝基均二苯脲-d_8,Dinitrocarbanilide-d_8):纯度大于等于 99%。

3.19 双硝基均二苯脲标准贮备溶液(100 mg/L):准确称取适量双硝基均二苯脲标准物质,加 *N*,*N*-二甲基甲酰胺 5 mL 溶解,用乙腈配成浓度为 100 mg/L 的标准贮备溶液。该溶液在 4 ℃保存。

3.20 双咪苯脲标准贮备溶液(100 mg/L):准确称取适量双咪苯脲标准物质,用乙腈配成浓度为 100 mg/L 的标准贮备溶液。该溶液在 4 ℃保存。

3.21 氘代双硝基均二苯脲标准贮备溶液(100 mg/L):准确称取适量氘代双硝基均二苯脲标准物质,

加 *N*,*N*-二甲基甲酰胺 5 mL 溶解,用乙腈配成浓度为 100 mg/L 的标准贮备溶液。该溶液在 4 ℃保存。

3.22 双硝基均二苯脲标准中间溶液(1.00 mg/L):准确移取 1.00 mL 双硝基均二苯脲标准储备溶液(3.19)于 100 mL 容量瓶,用乙腈稀释至刻度配成浓度为 1.00 mg/L 的标准中间溶液。该溶液在 4 ℃保存。

3.23 双咪苯脲标准中间溶液(1.00 mg/L):准确移取 1.00 mL 双咪苯脲标准贮备溶液(3.20)于 100 mL 容量瓶,用 1%乙酸水溶液(3.10)稀释至刻度配成浓度为 1.00 mg/L 的标准中间溶液。该溶液在4 ℃保存。

3.24 氘代双硝基均二苯脲标准中间溶液(0.20 mg/L):准确移取 0.20 mL 氘代双硝基均二苯脲标准贮备溶液(3.21)于 100 mL 容量瓶,用乙腈稀释至刻度配成浓度为 0.20 mg/L 的标准中间溶液。该溶液在 4 ℃保存。

3.25 双硝基均二苯脲液相色谱测定用标准工作溶液:根据需要移取适量标准贮备液(3.19),用乙腈释成合适的标准工作溶液,现用现配。

3.26 双咪苯脲液相色谱测定用标准工作溶液:根据需要移取适量标准贮备液(3.20),用 2%乙酸水溶液(3.11)稀释成适当浓度的标准工作溶液,现用现配。

3.27 双硝基均二苯脲液相色谱-质谱/质谱测定用标准工作溶液:根据需要移取一定量标准中间溶液(3.22)和氘代双硝基均二苯脲标准中间溶液(3.24),用甲醇-0.1%乙酸水溶液(3.14)配成适当浓度的混合标准工作溶液,每毫升该混合标准工作溶液含有 10 ng 氘代双硝基均二苯脲,现用现配。

3.28 双咪苯脲液相色谱-质谱/质谱测定用标准工作溶液:根据需要吸取一定量的标准中间溶液(3.23),以空白样品提取溶液-水溶液(3.15)稀释成适当浓度的标准工作液,现用现配。

3.29 弱阳离子交换固相萃取柱:Waters Oasis® WCX SPE(羧基键合二乙烯基苯、*N*-二乙烯基吡咯烷酮聚合物),60 mg/3 mL,或相当者。临用时用 3 mL 甲醇活化,3 mL 水平衡。

3.30 微孔滤膜:0.20 μm,有机相,水相;0.45 μm,水相。

4 仪器和设备

4.1 液相色谱仪:配紫外检测器。

4.2 液相色谱-质谱/质谱仪:配电喷雾离子源。

4.3 分析天平:感量为 0.1 mg 和 0.01 g。

4.4 组织捣碎机。

4.5 涡旋振荡器。

4.6 均质机。

4.7 离心机:3 500 r/min。

4.8 旋转蒸发仪。

4.9 固相萃取装置。

4.10 具塞离心管:50 mL,塑料。

5 试样的制备和保存

5.1 动物肌肉、肝脏

从原始样品中取出代表性的样品 500 g,用组织捣碎机捣碎混匀,均分成两份,分别装入洁净容器作为试样,密封,并标明标记。将试样放于−18 ℃以下保存。

5.2 蛋

从原始样品中取出代表性的样品 500 g,去除蛋壳后,用组织捣碎机捣碎混匀,均分成两份,分别装入洁净容器作为试样,密封,并标明标记。将试样放于 0 ℃～4 ℃保存。

制样操作过程中,应防止样品污染或发生残留物含量的变化。

6 测定步骤

6.1 提取

称取5 g试样(精确到0.01 g)于50 mL离心管中,加入10 mL乙腈、0.2 mL 50%氢氧化钾溶液(3.13)和3 g无水硫酸钠,均质30 s,3 500 r/min离心5 min,上清液转移到25 mL容量瓶中,残渣加入10 mL乙腈和0.2 mL 50%氢氧化钾溶液,涡旋振荡提取2 min,3 500 r/min离心5 min,合并上清液,用乙腈定容至刻度,待净化。

6.2 净化

6.2.1 液液分配

移取10.0 mL提取液(6.1)于25 mL浓缩瓶中,加入5 mL乙腈饱和正己烷(3.8),涡旋振荡,静置分层,弃去上层。再加入5 mL乙腈饱和正己烷,重复操作。

6.2.2 双硝基均二苯脲测定

移取2.0 mL分配液(6.2.1)于40 ℃下旋转蒸发至近干,用乙腈溶解残渣,定容至2.0 mL,过0.20 μm滤膜,供液相色谱测定,或移取1.0 mL滤液,加入0.5 mL氘代双硝基均二苯脲标准中间溶液(3.24),用甲醇-0.1%乙酸水溶液(3.14)定容至10.0 mL,供液相色谱-质谱/质谱测定。

6.2.3 双咪苯脲测定

移取5.0 mL分配液于已活化、平衡处理的固相萃取柱中(3.29),控制流速小于0.5 mL/min,依次用3 mL水、3 mL甲醇淋洗,15 mL 5%乙酸甲醇溶液(3.12)洗脱,控制洗脱液流速小于1.0 mL/min。洗脱液转移到50 mL浓缩瓶,40 ℃下旋转浓缩至近干,用水溶解残渣,定容至2.0 mL,过0.20 μm滤膜,供液相色谱测定,或移取1.0 mL滤液,用水定容至10.0 mL,供液相色谱-质谱/质谱测定。

6.3 空白样品提取溶液制备

称取5 g空白样品,按6.1、6.2.1和6.2.3操作进行到水定容至2.0 mL,过0.20 μm滤膜,得到滤液作为空白样品提取溶液。

6.4 液相色谱测定

6.4.1 色谱条件

a) 色谱柱:Discovery C_{18}柱,250 mm×4.6(内径)mm,5 μm,或相当者;

b) 流动相:测定双硝基均二苯脲为乙腈-1%乙酸水溶液(53+47,体积比);测定双咪苯脲为乙腈-1%乙酸水溶液(10+90,体积比);

c) 流速:1.0 mL/min;

d) 检测波长:测定双硝基均二苯脲为350 nm;测定双咪苯脲为260 nm;

e) 柱温:35 ℃;

f) 进样量:20 μL。

6.4.2 色谱测定

根据样液中待测物的含量情况,选取响应值相近的标准工作溶液进行色谱分析,标准工作溶液和待测样液中双硝基均二苯脲及双咪苯脲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插进样测定。在上述色谱条件下,双硝基均二苯脲和双咪苯脲的保留时间分别约为10.3 min和7.88 min,标准物质色谱图分别参见附录A中图A.1和图A.2。

6.5 液相色谱-质谱/质谱测定

6.5.1 色谱条件

a) 色谱柱:SunFire C_{18},100 mm×2.1(内径) mm,3.5 μm,或相当者;

b) 流动相:测定双硝基均二苯脲为甲醇-0.1%乙酸水溶液(75+25,体积比);测定双咪苯脲为甲醇-0.1%乙酸水溶液(10+90,体积比);

c) 流速:0.25 mL/min;

d) 柱温:35 ℃;

e) 进样量:测定双硝基均二苯脲为 5 μL;测定双咪苯脲为 10 μL。

6.5.2 质谱条件

a) 离子源:电喷雾离子源(ESI);

b) 扫描方式:测定双硝基均二苯脲为负离子扫描;测定双咪苯脲为正离子扫描;

c) 检测方式:多反应选择离子检测(MRM);

d) 电喷雾电压(IS):测定双硝基均二苯脲为−4 500 V;测定双咪苯脲为 5 500 V;

e) 雾化气、气帘气、辅助加热气、碰撞气均为高纯氮气及其他合适气体;使用前应调节各气体流量以使质谱灵敏度达到检测要求;各参数参见附录 B 中表 B.1;

f) 辅助气温度(TEM):500 ℃;

g) 定性离子对、定量离子对、采集时间、去簇电压及碰撞能量等主要质谱参数参见附录 B 中表 B.1。

6.5.3 测定

6.5.3.1 定性测定

在相同实验条件下,如果样品中待测物质与标准溶液中对应的保留时间一致,定性离子对的相对丰度与浓度相当的标准溶液的相对丰度一致,偏差不超过表 1 规定的范围,则可判断样品中存在相应的待测物。

表 1 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

6.5.3.2 定量测定

根据样液中待测物的含量情况,选取响应值相近的标准工作溶液进行分析,标准工作溶液和待测样液中的响应值均应在仪器线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述仪器条件下,双硝基均二苯脲和双咪苯脲的保留时间分别约为 2.86 min 和 1.56 min,标准物质的二级质谱图分别参见附录 C 中图 C.1 和图 C.2,多反应监测(MRM)色谱图分别参见附录 C 中图 C.3 和图 C.4。

6.6 空白试验

除不加试样外,均按上述测定步骤进行。

7 结果计算和表述

用数据处理软件或液相色谱、液相色谱-质谱/质谱分别按式(1)和式(2)计算试样中双硝基均二苯脲残留量,用数据处理软件或按式(1)计算试样中双咪苯脲残留量,计算结果需扣除空白值。

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

式中:

X——试样中待测组分残留量,单位为毫克每千克(mg/kg);

A——样液中待测组分峰面积;

c——标准工作液中待测组分的浓度,单位为毫克每升(mg/L);

V——样液最终定容体积,单位为毫升(mL);

A_s——标准工作液中待测组分峰面积;

m——最终样液代表的试样质量,单位为克(g)。

$$X = \frac{c \times c_i \times A \times A_{si} \times V}{c_{si} \times A_i \times A_s \times m} \qquad \cdots\cdots(2)$$

式中：

X——试样中双硝基均二苯脲残留量，单位为毫克每千克(mg/kg)；

c——标准工作溶液中双硝基均二苯脲的浓度，单位为毫克每升(mg/L)；

c_i——样液中氘代双硝基均二苯脲的浓度，单位为毫克每升(mg/L)；

A——样液中双硝基均二苯脲的峰面积；

A_{si}——标准工作溶液中氘代双硝基均二苯脲的峰面积；

V——样液最终定容体积，单位为毫升(mL)；

c_{si}——标准工作溶液中氘代双硝基均二苯脲的浓度，单位为毫克每升(mg/L)；

A_i——样液中内标物的峰面积；

A_s——标准工作溶液中双硝基均二苯脲的峰面积；

m——最终样液代表的试样质量，单位为克(g)。

8 测定低限、回收率

8.1 测定低限

液相色谱法和液相色谱-质谱/质谱法测定双硝基均二苯脲和双咪苯脲残留量的测定低限均为0.050 mg/kg。

8.2 回收率

液相色谱法和液相色谱-质谱/质谱法测定鸡肉、鸡肝、鸡蛋、牛肉和牛肝中双硝基均二苯脲和双咪苯脲残留量的添加浓度及回收率数据见表2。

表2 双硝基均二苯脲和双咪苯脲的回收率数据($n=10$)

基质	添加浓度/(mg/kg)	药物名称	回收率/%	
			液相色谱法	液相-质谱/质谱法
鸡肉	0.050	双硝基均二苯脲	92.1～110	83.6～100
	0.200		97.2～106	84.5～99.0
	0.500		86.0～110	85.0～98.6
	0.050	双咪苯脲	96.4～110	87.6～101
	0.200		92.3～104	87.7～108
	0.500		83.5～91.2	79.7～94.8
鸡肝	0.050	双硝基均二苯脲	93.0～108	82.8～99.6
	0.200		90.4～110	84.0～98.0
	0.500		80.0～102	83.6～95.6
	0.050	双咪苯脲	86.4～110	81.0～103
	0.200		84.3～101	80.0～96.8
	0.500		84.2～97.3	81.0～92.5
鸡蛋	0.050	双硝基均二苯脲	89.6～108	84.0～99.4
	0.200		92.5～110	83.5～97.5
	0.500		93.0～104	83.2～99.2
	0.050	双咪苯脲	88.6～107	82.8～108
	0.200		91.3～99.0	88.3～104
	0.500		84.8～92.6	82.2～103

表 2（续）

基质	添加浓度/(mg/kg)	药物名称	回收率/%	
			液相色谱法	液相-质谱/质谱法
牛肉	0.050 0.200 0.500	双硝基均二苯脲	91.1～107 87.7～109 94.6～102	84.0～99.8 80.0～96.0 85.8～96.4
		双咪苯脲	92.8～110 90.7～103 88.3～104	89.6～101 92.3～106 80.0～97.8
牛肝	0.050 0.200 0.500	双硝基均二苯脲	87.9～108 83.9～95.7 88.8～102	82.0～98.2 87.0～97.0 82.8～98.6
		双咪苯脲	82.0～98.2 87.0～97.0 82.8～98.6	92.6～101 82.2～100 85.5～96.0

附 录 A
（资料性附录）
液相色谱图

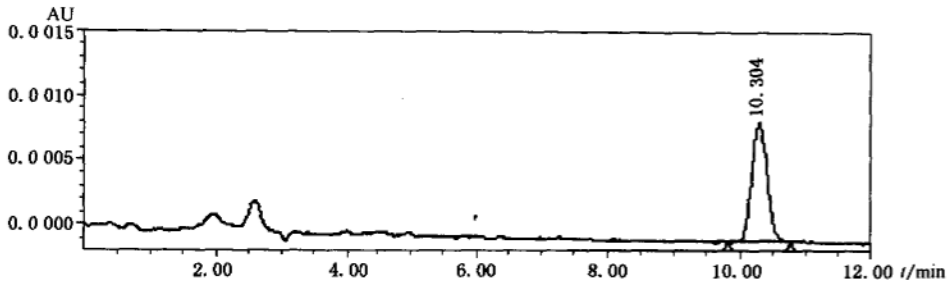


图 A.1 双硝基均二苯脲标准物质的液相色谱图

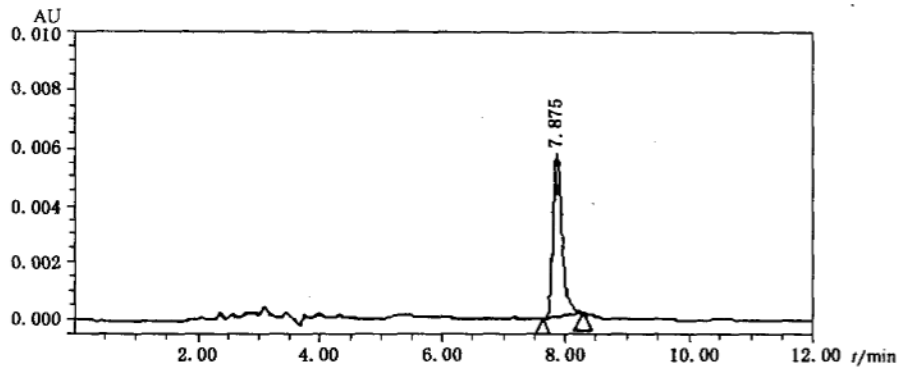


图 A.2 双咪苯脲标准物质的液相色谱图

附 录 B
（资料性附录）
参考质谱条件[1)]

表 B.1 主要质谱参数

被测物名称	定性离子对（m/z）	定量离子对（m/z）	采集时间（DP）/ ms	去簇电压/ V	碰撞能量（CE）/ V	雾化气（NEB）	气帘气（CUR）	碰撞气（CAD）	辅助加热气/（L/min）
双硝基均二苯脲	301.1/137.1 301.1/106.8	301.1/137.1	200	−45	−25 −55	7	12	12	7
氘代双硝基均二苯脲	309.3/141.0	309.3/141.0	200	−45	−17	7	12	12	7
双咪苯脲	349.4/188.2 349.4/162.2	349.4/188.2	200	60	41 35	15	10	12	7

1) 非商业性声明：附录 B 所列参考质谱条件是在 API3000 型液相色谱-质谱/质谱仪上完成的。此处所出试验用仪器仅供参考，并不涉及商业目的。鼓励标准使用者尝试不同厂家或型号的仪器。

附 录 C
（资料性附录）
二级质谱图与多反应监测（MRM）离子色谱图

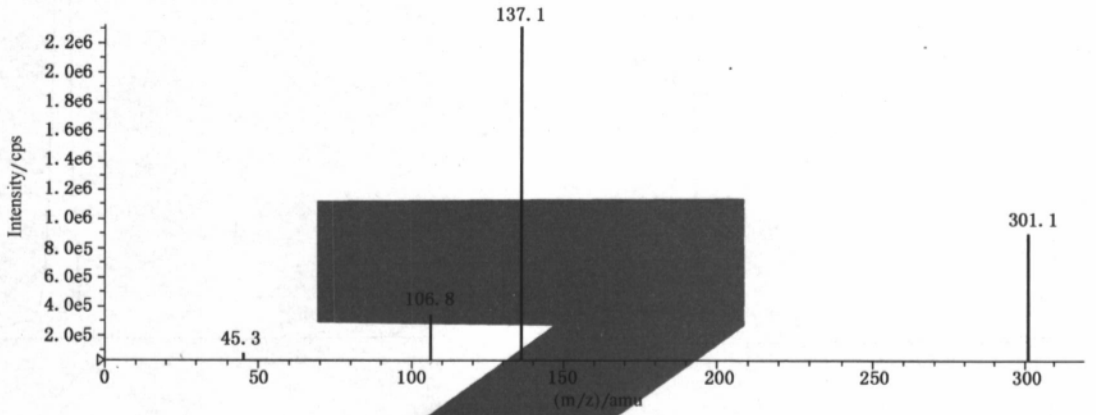


图 C.1 双硝基均二苯脲标准物质的二级质谱图

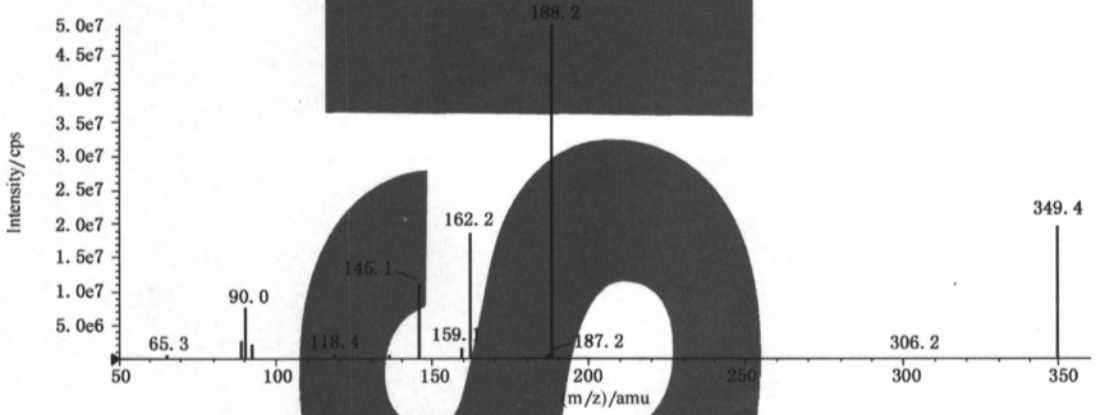


图 C.2 双咪苯脲标准物质的二级质谱图

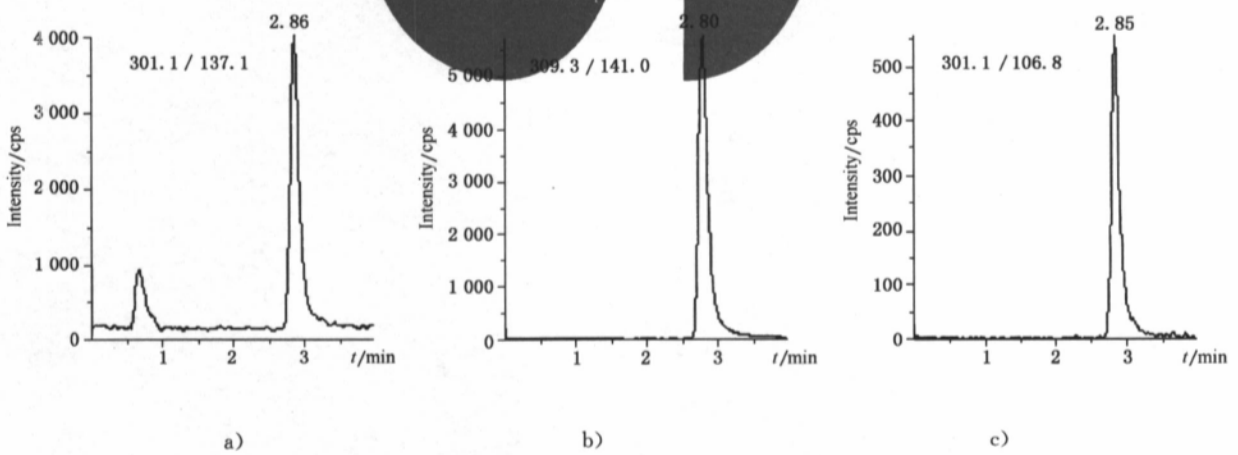
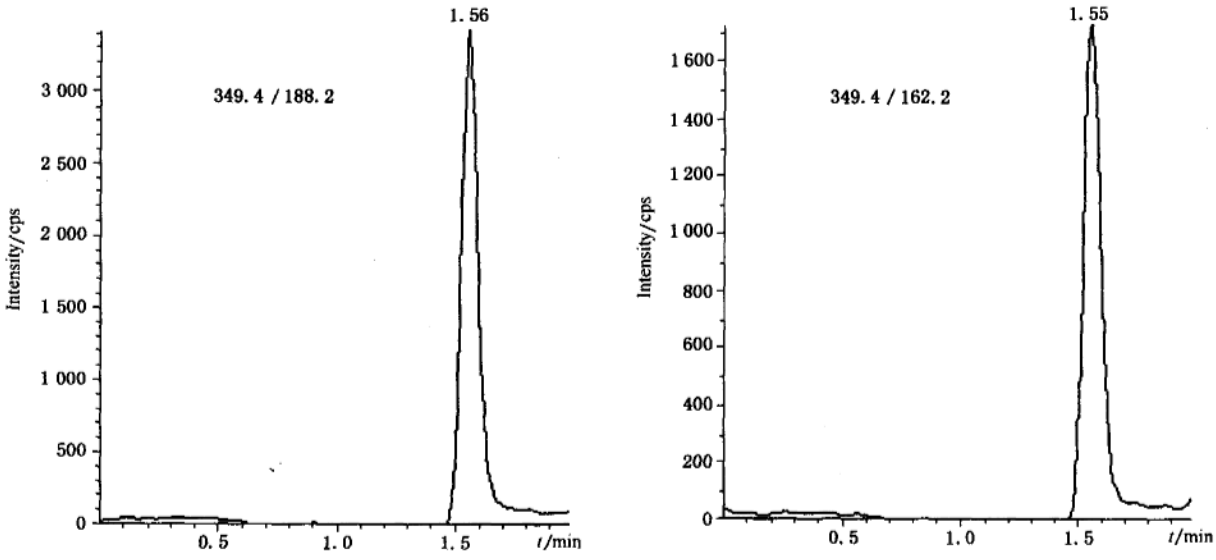


图 C.3 双硝基均二苯脲和氘代双硝基均二苯脲标准物质液相-质谱/质谱多反应监测色谱图



a) b)

图 C.4 双咪苯脲标准物质液相-质谱/质谱多反应监测色谱图

Foreword

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

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

This standard is drafted by Guangdong Entry-Exit Inspection and Quarantine Bureau and Heilongjiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

Main drafters of this standard are Lin Haidan, Yao Yangxun, Shao Linzhi, Lin Feng, Xie Shouxin, Qin Yan, Kang Qinghe and Wu Yingxuan.

This standard is a professional standard for Entry-Exit inspection and quarantine of the People's Republic of China promulgated for the first time.

Determination of diphenylurea residues in foodstuffs of animal origin for import and export

1 Scope

This standard specifies the method for the determination of nicarbazin (marker residue is dinitrocarbanilide) and imidocarb residues in foodstuffs of animal origin by high performance liquid chromatography and high performance liquid chromatography tandem mass spectrometry.

This standard is applicable to determine nicarbazin and imidocarb residues in bovine meat, chicken meat, bovine liver, chicken liver and egg.

2 Principle

Test sample was extracted with acetonitrile under alkali condition, after liquid-liquid extraction with hexane, for dinitrocarbanilide determination, followed by a clean step on a weak cation exchange SPE column. The extract was analyzed by HPLC and quantified by external standard method. For LC-MS/MS determination and confirmation, dinitrocarbanilide was quantified by internal standard method, imidocarb was quantified by external standard method.

3 Reagents and materials

Unless otherwise specified, all reagents used are A. R., and pure "water" is redistilled water.

3.1 Acetonitrile: HPLC grade.

3.2 *N*-hexane.

3.3 Methanol: HPLC grade.

3.4 Acetic acid: HPLC grade.

3.5 Potassium hydroxide.

3.6 *N*, *N*-dimethylformamide.

3.7 Anhydrous sodium sulfate: Burn at 650 ℃ for 4 h and then store in a desiccator.

3.8 *N*-hexane saturated with acetonitrile: Mix *n*-hexane with sufficient acetonitrile till saturation.

3.9 0.1% acetic acid solution: Pipette 1 mL of acetic acid to a 1 L volumetric flask, dilute to volume with water. Filter the solution for use by 0.45 μm aqueous filter.

3.10 1% acetic acid solution: Pipette 10 mL of acetic acid to a 1 L volumetric flask, dilute to volume with water. Filter the solution for use by 0.45 μm aqueous filter.

3.11 2% acetic acid solution: Pipette 2 mL of acetic acid to a 100 mL volumetric flask, dilute to volume with water. Filter the solution for use by 0.45 μm aqueous filter.

3.12 5% acetic acid in methanol: Pipette 5 mL of acetic acid to a 100 mL volumetric flask, dilute to volume with methanol.

3.13 50% potassium hydroxide solution: Weigh 50 g of potassium hydroxide and dissolve in 100 mL of water.

3.14 Methanol-0.1% acetic acid solution (1+1, *V*/*V*): Add 50 mL of methanol and 50 mL of 0.1% acetic acid solution (3.9), mix for use.

3.15 Blank sample extraction solution-water (1+9, *V*/*V*): Pipette 1 mL of blank sample extraction solution and 9 mL of water, mix for use.

3.16 Standards of dinitrocarbanilide (CAS Number: 587-90-6): Purity ≥97%.

3.17 Standards of imidocarb (CAS Number: 27885-92-3): Purity ≥97%.

3.18 Standards of dinitrocarbanilide deuterated (dinitrocarbanilide $-d_8$): Purity≥99%.

3.19 Dinitrocarbanilide standard stock solution (100 mg/L): Accurately weigh an adequate amount of dinitrocarbanilide standard, add 5 mL *N*, *N*-dimethylformamide and dissolve in acetonitrile to prepare a solution of 100 mg/L as the standard stock solution, stored at 4 ℃.

3.20 Imidocarb standard stock solution (100 mg/L): Accurately weigh an adequate amount of imidocarb standard, dissolve in acetonitrile to prepare a solution of 100 mg/L as the standard stock solution, stored at 4 ℃.

3.21 Dinitrocarbanilide deuterated standard stock solution (100 mg/L): Accurately weigh an adequate amount of dinitrocarbanilide deuterated standard, add 5 mL *N*, *N*-dimethylformamide and dissolve in acetonitrile to prepare a solution of 100 mg/L as the standard stock solution, stored at 4 ℃.

3.22 Dinitrocarbanilide intermediate standard solution (1.00 mg/L): Accurately pipette 1.00 mL of dinitrocarbanilide standard stock solution (3.19) to a 100 mL volumetric flask, dilute to volume with acetonitrile to prepare a solution of 1.00 mg/L as the intermediate standard stock solution, stored at 4 ℃.

3.23 Imidocarb intermediate standard solution (1.00 mg/L): Accurately pipette 1.00 mL of imidocarb standard stock solution (3.20) to a 100 mL volumetric flask, dilute to volume with 1% acetic acid solution (3.10) to prepare a solution of 1.00 mg/L as the intermediate standard stock solution, stored at 4 ℃.

3.24 Dinitrocarbanilide deuterated intermediate standard solution (0.20 mg/L): Accurately pipette 0.20 mL of dinitrocarbanilide deuterated standard stock solution (3.21) to a 100 mL volumetric flask, dilute to volume with acetonitrile to prepare a solution of 0.20 mg/L as the intermediate standard solution, stored at 4 ℃.

3.25 Dinitrocarbanilide working LC standard solution: According to the requirement, pipette an adequate volume of dinitrocarbanilide standard solution (3.19), dilute with acetonitrile to prepare propriety concentration standard working solution, prepare before use.

3.26 Imidocarb working LC standard solution: According to the requirement, pipette an adequate volume of imidocarb standard solution (3.20), dilute with 2% acetic acid solution (3.11) to prepare propriety concentration standard working solution, prepare before use.

3.27 Dinitrocarbanilide LC-MS/MS working standard solution: According to the requirement, separately pipette an adequate volume of dinitrocarbanilide intermediate standard solution (3.22) and dinitrocarbanilide deuterated intermediate standard solution (3.24), dilute with methanol-0.1% acetic acid solution (3.14) to prepare propriety concentration standard working solution, which containing 10 ng/mL dinitrocarbanilide deuterated, prepare before use.

3.28 Imidocarb LC-MS/MS working standard solution: According to the requirement, pipette an adequate volume of imidocarb intermediate standard solution (3.23) dilute with blank sample extraction solution-water (3.15) to prepare propriety concentration standard working solution. Prepare before use.

3.29 Weak cation exchange SPE column: Waters Oasis® WCX SPE (carboxylic acid poly divinylbenzene-co-*N*-vinylpyrrolidone) or equivalent, 60 mg/3 mL. Condition the SPE column with 3 mL of methanol followed by 3 mL of water just before use.

3.30 Membrane filter: 0.20 μm organic and aqueous filter; 0.45 μm aqueous filter.

4 Apparatus and equipment

4.1 High performance liquid chromatography with UV detector.

4.2 Liquid chromatography-electrospray ionization tandem mass spectrometry.

4.3 Balance: sensitivity: 0.1 mg and 0.01 g.

4.4 Tissue blender.

4.5 Vortex mixer.

4.6 Homogenizer.

4.7 Centrifuge:3 500 r/min.

4.8 Rotary vacuum evaporator.

4.9 Solid phase extraction vacuum manifold.

4.10 Centrifuge tube with cap:50 mL,plastic.

5 Sample preparation and storage

5.1 Animal muscle and animal liver

All primary sample is reduced to 500 g as the representative sample,which is blended and homogenized,and then divided into two equal portions. Each portion is placed in clean containers as the test sample,which is sealed and labeled. The test sample should be stored at below −18 ℃.

5.2 Egg

All primary sample is reduced to 500 g as the representative sample,discarded eggshells and homogenized,and then divided into two equal portions. Each portion is placed in clean containers as the test sample,which is sealed and labeled. The test sample should be stored at below 0 ℃ ~4 ℃.

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 Procedure

6.1 Extraction

Weigh 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube,add 10 mL of acetonitrile,0.2 mL 50% potassium hydroxide solution (3.13) and about 3 g of anhydrous sodium sulfate. Homogenize the sample for 30 s. After centrifugation at 3 500 r/min for 5 min,the supernatant layer was transferred to a 25 mL volumetric flask. Add 10 mL of acetonitrile and 0.2 mL of 50% potassium hydroxide solution to the residues,vortex to extract for 2 min. Centrifuge at 3 500 r/min for 5 min and combined the supernatants to the same volumetric flask. Dilute to the mark with acetonitrile for clean up.

6.2 Clean up

6.2.1 Liquid and liquid partition

Measure 10.0 mL of extraction solution (6.1) in a 25 mL concentration flask, add 5 mL of acetonitrile saturated *n*-hexane (3.8), vortex to mix and stay for separation, discard supernatant layer. Repeat step with another 5 mL of acetonitrile saturated *n*-hexane.

6.2.2 Clean up for dinitrocarbanilide

Measure 2.0 mL of partition solution, evaporate to nearly dryness with rotary evaporator at 40 ℃. Dissolve the residue with acetonitrile and make up to 2.0 mL. Filter through the 0.20 μm membrane filter and analyzed by LC system, or pipette 1.0 mL of filtered solution, then add 0.50 mL dinitrocarbanilide deuterated intermediated standard solution (3.24), dilute to 10.0 mL with methanol-0.1% acetic acid solution (3.14) for LC-MS/MS determination.

6.2.3 Clean up for imidocarb

Load 5.0 mL of partition solution to the conditioned and equibrated SPE column (3.29) at a rate less than 0.5 mL/min. Wash the SPE with 3 mL of water, followed by 3 mL of methanol. Elute the SPE with 15 mL 5% acetic acid in methanol (3.12) at a rate less than 1.0 mL/min. Transfer the eluate to a 50 mL concentration flask.

The eluate is evaporated nearly dryness with rotary evaporator at 40 ℃, made up to 2.0 mL with water, filter through the 0.20 μm membrane filter and analyzed by LC system, or pipette 1.0 mL of filtered solution, dilute to 10.0 mL with water for LC-MS/MS determination.

6.3 Prepare for blank sample extraction solution

Weigh 5 g blank sample, follow step 6.1, 6.2.1 and 6.2.3 till making up to 2.0 mL with water, filter through the 0.20 μm membrane filter to make a blank sample extraction solution.

6.4 LC determination

6.4.1 LC operating conditions

a) Column: Discovery C_{18}, 250 mm × 4.6 (i.d.) mm, 5 μm particle size, or equivalent;

b) Mobile phase: For dinitrocarbanilide is acetonitrile-1% acetic acid (53 + 47, *V*/*V*), for imidocarb is acetonitrile-1% acetic acid (10 + 90, *V*/*V*);

c) Flow rate: 1.0 mL/min;

d) Detection wavelength:350 nm for dinitrocarbanilide;260 nm for imidocarb;

e) Column temperature:35 ℃;

f) Injection volume:20 μL.

6.4.2 LC determination

According to the approximate concentration of analyte in sample solution, select the standard working solution with similar responses to that of sample solution. The responses of the analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. The standard working solution and the sample solution should be injected with equal volume alternatively. Under the above HPLC operating condition, the reference retention times of dinitrocarbanilide and imidocarb are 10.3 and 7.88 separately. The chromatograms of the standards are shown in Figure A.1 and Figure A.2.

6.5 LC-MS/MS determination

6.5.1 LC operating conditions

a) Column:Sunfire C_{18}, 3.5 μm particle size, 100 mm × 2.1 mm(i.d.)or equivalent;

b) Mobile phase:For dinitrocarbanilide is acetonitrile-0.1% acetic acid (75 + 25, *V*/*V*), for imidocarb is acetonitrile-0.1% acetic acid (10 + 90, *V*/*V*);

c) Flow rate:0.25 mL/min;

d) Column temperature:35 ℃;

e) Injection volume:5 μL for dinitrocarbanilide, 10 μL for imidocarb.

6.5.2 MS operation conditions

a) Ion source:ESI;

b) Scan mode:Negative mode for dinitrocarbanilide, positive mode for imidocarb;

c) Monitor mode:Multiple reaction monitoring;

d) Ionspray voltage: −4 500 V for dinitrocarbanilide, 5 500 V for imidocarb;

e) Nebulizer gas, Curtain gas, Heater gas, Nitrogen and Collision gas and are high purity nitrogen gases or equivalent, optimize the flow rate of each gas to reach the requirement of the sensitivity

of mass spectrometry. The reference parameters are listed in table B. 1 of annex B.

f) Source temperature:500 ℃ ;

g) Main mass parameters including quality ions,quantity ions,declustering potential,dwell time and collision energy are listed in table B. 1 of annex B.

6. 5. 3 LC/MS-MS determination

6. 5. 3. 1 LC/MS-MS confirmation

Under the same determination conditions. The chromatographic retention time of the analyte shall correspond to that of the calibration solution. The relative intensities of the quality ions of analyst, shall correspond to those of the calibration standard at comparable concentrations,within the tolerances shown in table 1,then the corresponding analyte must be present in the sample.

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

6. 5. 3. 2 LC/MS-MS quantification

According to the approximate concentration of analyte in sample solution,select the standard working solution with similar responses to that of sample solution. The responses of the analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. The standard working solution and the sample solution should be injected with equal volume alternatively. Under the above chromatography operating condition, the reference retention times of dinitrocarbanilide and imidocarb are 2. 86 and 1. 56 separately. The chromatograms of the standards are shown in Figure C. 1 and Figure C. 2 in annex C,Multiple reaction monitoring chromatogram of the standards are shown in Figure C. 3 and Figure C. 4 in annex C.

6. 6 Blank test

The operation of the blank test is the same as the described in the method of determination,but with the omission of sample addition.

7 Calculation and expression of result

Calculation the content of dinitrocarbanilide residue in the test sample by chromatography data processor or according to the formula (1) for LC and (2) for LC-MS/MS separately. Calculation the content of imidocarb residue in the test sample by chromatography data processor or according to the formula (1). The blank value should be subtracted from the above result of calculation.

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

Where:

X —the residue content of analyte in the test sample, mg/kg;

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

c —the concentration of analyte in the standard working solution, mg/L;

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

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

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

$$X = \frac{c \times c_i \times A \times A_{si} \times V}{c_{si} \times A_i \times A_s \times m} \quad \cdots\cdots(2)$$

Where:

X —the residue content of dinitrocarbanilide in the test sample, mg/kg;

c—the concentration of a dinitrocarbanilide in the standard working solution, mg/L;

c_i—the concentration of dinitrocarbanilide deuterated in the sample solution, mg/L;

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

A_{si}—the peak area of dinitrocarbanilide deuterated in the standard working solution;

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

c_{si}—the concentration of dinitrocarbanilide deuterated in the standard working solution, mg/L;

A_i—the peak area of dinitrocarbanilide deuterated in the sample solution;

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

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

8 Limit of quantification (LOQ) and recovery

8.1 Limit of quantification

The limits of quantification of the method for dinitrocarbanilide and imidocarb residues are 0.05 mg/kg.

8.2 Recovery

According to the experimental data, the corresponding recoveries of fortified concentrations of chicken meat, chicken liver, bovine meat, bovine liver and egg are shown in table 2.

Table 2—Recoveries for dinitrocarbanilide and imidocarb ($n = 10$)

Matrix	Fortified/(mg/kg)	Analyte	Recovery/%	
			LC	LC-MS/MS
Chicken meat	0.050 0.200 0.500	Dinitrocarbanilide	92.1～110 97.2～106 86.0～110	83.6～100 84.5～99.0 85.0～98.6
		Imidocarb	96.4～110 92.3～104 83.5～91.2	87.6～101 87.7～108 79.7～94.8
Chicken liver	0.050 0.200 0.500	Dinitrocarbanilide	83.0～108 90.4～110 80.0～102	82.8～99.6 84.0～98.0 83.6～95.6
		Imidocarb	86.4～110 84.3～101 84.2～97.3	81.0～103 80.0～96.8 81.0～92.5
Egg	0.050 0.200 0.500	Dinitrocarbanilide	89.6～108 92.5～110 93.0～104	84.0～99.4 83.5～97.5 83.2～99.2
		Imidocarb	88.6～107 91.3～99.0 84.8～92.6	82.8～108 88.3～104 82.2～103
Bovine meat	0.050 0.200 0.500	Dinitrocarbanilide	91.1～107 87.7～109 94.6～102	84.0～99.8 80.0～96.0 85.8～96.4
		Imidocarb	92.8～110 90.7～103 88.3～104	89.6～101 92.3～106 80.0～97.8
Bovine liver	0.050 0.200 0.500	Dinitrocarbanilide	87.9～108 83.9～95.7 88.8～102	82.0～98.2 87.0～97.0 82.8～98.6
		Imidocarb	82.0～98.2 87.0～97.0 82.8～98.6	92.6～101 82.2～100 85.5～96.0

Annex A
(Informative)
Chromatogram

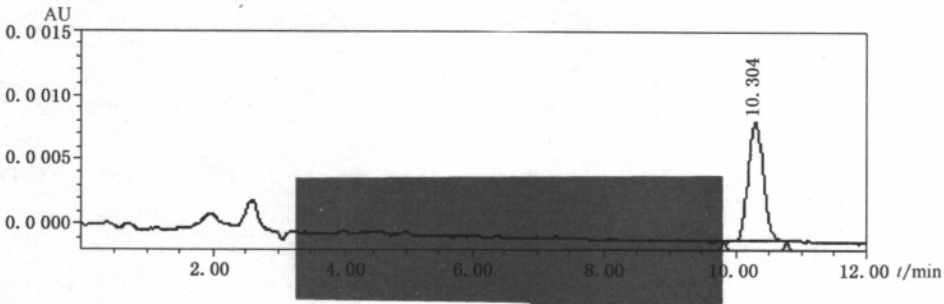


Figure A.1—Chromatogram of dinitrocarbanilide standard

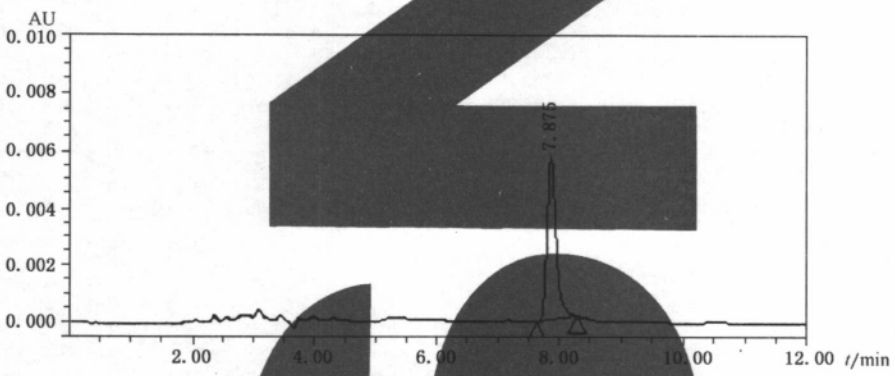


Figure A.2—Chromatogram of imidocarb standard

Annex B
(Informative)
Reference mass conditions[1)]

Table B. 1—Main MS parameters

Analyst	Quality ions (m/z)	Quantity ions (m/z)	Dwell time/ ms	Decluste -ring potential (DP)/ V	Collision energy (CE)/ V	Nebulizer gas (NEB)	Curtain gas (CUR)	Collision gas (CAD)	Heated gas (L/min)
Dinitrocarbanilide	301.1/137.1	301.1/137.1	200	−45	−25	7	12	12	7
	301.1/106.8				−55				
Dinitrocarbanilide deuterated	309.3/141.0	309.3/141.0	200	−45	−17	7	12	12	7
Imidocarb	349.4/188.2	349.4/188.2	200	60	41	15	10	12	7
	349.4/162.2				35				

1) Declaration for non-commercial: The reference mass conditions listed in annex B are performed on API3000 LC-MS/MS. The type of the equipment mentioned here is only for reference and not for commerial purpose. Encourage users to try different manufactures of models of equipment.

Annex C
(Informative)
Mass spectrometry-mass spectra and multiple reaction monitoring chromatogram

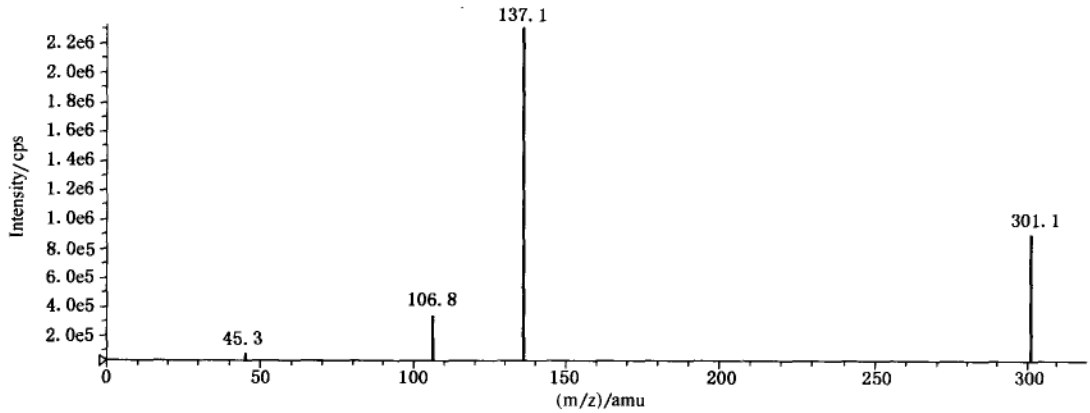


Figure C. 1—Mass spectrometry-mass spectra of dinitrocarbanilide standard

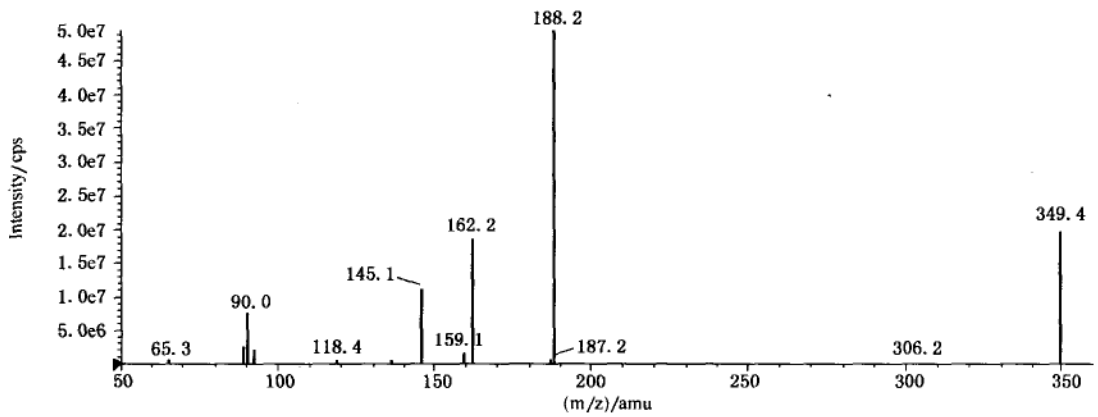
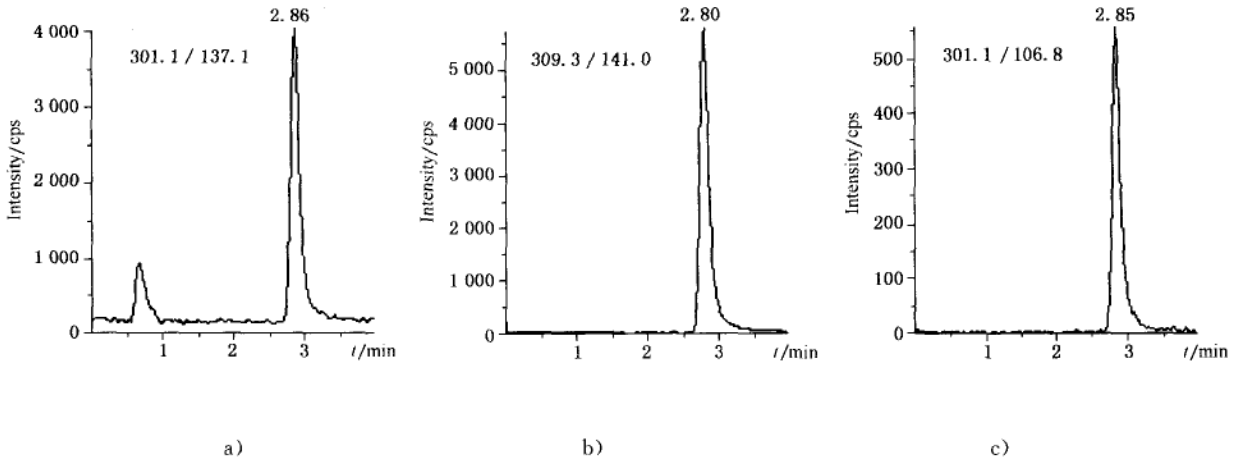


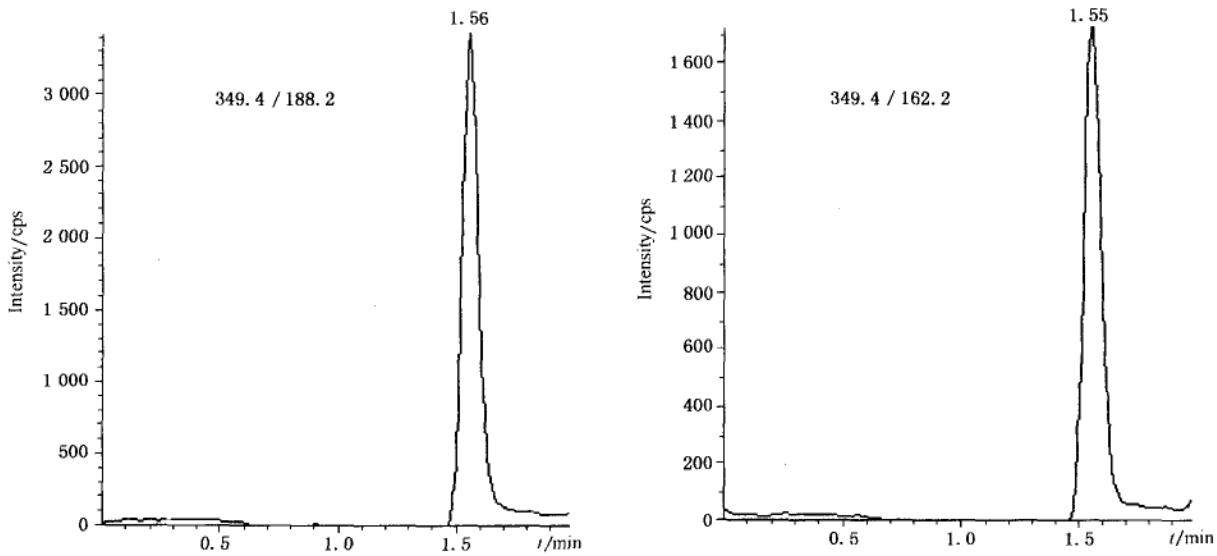
Figure C. 2—Mass spectrometry-mass spectra of imidocarb standard

SN/T 2314—2009



a) b) c)

Figure C.3—Multiple reaction monitoring chromatogram of dinitrocarbanilide and dinitrocarbanilide deuterated standard



a) b)

Figure C.4—Multiple reaction monitoring chromatogram of imidocarb standard