

SN

中华人民共和国出入境检验检疫行业标准

SN/T 0212.2—2017
代替 SN/T 0212.2—1993

出口禽肉中二氯二甲吡啶酚残留量测定

Determination of clopidol residues in poultry meat for export

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中 华 人 民 共 和 国
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前 言

本部分是 SN/T 0212 的第 2 部分。

本部分按照 GB/T 1.1—2009 给出的规则起草。

本部分代替 SN/T 0212.2—1993《出口禽肉中二氯二甲吡啶酚残留量检验方法(气相色谱法)》。

本部分与 SN/T 0212.2—1993 相比主要修改如下：

- 对标准的名称进行了修改；
- 略去了抽样步骤；
- 对原标准的检测范围进行了扩展,除鸡肉外增加了鸡肝；
- 对衍生化方法进行改进；
- 用气相色谱-质谱法和气相色谱串联质谱法替代气相色谱法。

本部分由国家认证认可监督管理委员会提出并归口。

本部分起草单位:中华人民共和国上海出入境检验检疫局。

本部分主要起草人:陈迪、朱坚、樊祥、吴启明、于瑞祥、郭德华、邓晓军、王敏、韩丽、伊雄海、彭觅、张浩。

本部分所代替标准历次版本发布情况为：

- SN/T 0212.2—1993。

出口禽肉中二氯二甲吡啶酚残留量测定

1 范围

SN/T 0212 的本部分规定了禽肉和肝脏中二氯二甲吡啶酚残留量的气相色谱-质谱和气相色谱串联质谱测定法。

本部分适用于禽肉和肝脏中二氯二甲吡啶酚残留量的测定。

2 方法提要

采用甲醇提取样品中二氯二甲吡啶酚,提取液经中性氧化铝柱净化,在碱性条件下的吡啶催化下,以丙酸酐衍生。用气相色谱-质谱仪和气相色谱串联质谱仪测定,外标法定量。

3 试剂和材料

除另有规定外,所有试剂均为分析纯,水为蒸馏水。

3.1 甲醇:液相色谱纯。

3.2 正己烷:残留级。

3.3 吡啶。

3.4 丙酸酐:纯度 $\geq 98.0\%$ 。

3.5 四硼酸钠。

3.6 0.1 mol/L 四硼酸钠溶液:称取 38.0 g 四硼酸钠(3.5),加水溶解并定容至 1 000 mL。

3.7 氧化铝:中性,层析用,粒度 100 目~200 目,在 300 °C 灼烧 3 h,并在 130 °C 烘箱中烘烤 1 h 后,放入干燥器中备用。

3.8 二氯二甲吡啶酚标准物质:(Clopidol, $C_7H_7Cl_2NO$, CAS 号:2971-90-6),纯度 $>98\%$ 。

3.9 二氯二甲吡啶酚标准储备液:准确称取适量的二氯二甲吡啶酚标准物质,以甲醇配成 0.10 mg/mL 标准储备溶液,于 4 °C 冰箱内避光保存。保存期为 12 个月。

3.10 二氯二甲吡啶酚标准工作液:根据需要用甲醇将二氯二甲吡啶酚标准储备液(3.9)稀释成适当浓度的标准工作溶液,于 4 °C 冰箱内避光保存。保存期为 6 个月。

4 仪器和设备

4.1 气相色谱-质谱仪,配 EI 源。

4.2 气相色谱串联质谱仪,配 EI 源。

4.3 组织捣碎机。

4.4 均质器。

4.5 离心机,4 000 r/min。

4.6 涡旋混合器。

4.7 旋转蒸发器。

4.8 氮吹仪。

- 4.9 带螺旋盖塑料离心管,15 mL。
- 4.10 带螺旋盖塑料离心管,50 mL。
- 4.11 玻璃层析柱:20 mm(id)×400 mm。装入16 g 氧化铝(3.7)轻轻敲打装实。
- 4.12 电子天平:感量0.1 mg和0.01 g。
- 4.13 梨形瓶:250 mL。

5 试样的制备与保存

5.1 试样制备

取样品中有代表性的约500 g,用组织捣碎机捣碎,混匀,平均分为两份,分别装入洁净容器中,密封并标明标记。

5.2 试样保存

将试样于-18℃以下冷冻保存。

6 测定步骤

6.1 提取

称取5 g 试样(精确至0.01 g)于50 mL 塑料离心管中,加入25 mL 甲醇(3.1),均质1 min,以4 000 r/min离心5 min。

6.2 净化

用40 mL 甲醇活化氧化铝柱(4.11),用移液管移取20 mL 样品提取液(6.1)于氧化铝柱中,当液面与氧化铝上表面相切时加入10 mL 甲醇淋洗,弃去流出液。然后用55 mL 甲醇洗脱,收集洗脱液于250 mL 梨形瓶(4.13)中。在50℃水浴下旋转蒸发至干。用2 mL 甲醇溶解残渣,转入15 mL 带螺旋盖离心管(4.9),并用1 mL 甲醇洗涤梨形瓶,合并至15 mL 带螺旋盖离心管中。用氮气流吹至大约0.5 mL。

6.3 衍生化

在上述试管中依次准确加入4 mL 0.1 mol/L 四硼酸钠溶液(3.6),0.25 mL 正己烷(3.2)(使用气相色谱串联质谱仪时加入1.00 mL),25 μL 吡啶(3.3)和50 μL 丙酸酐(3.4)。加塞,涡旋混合1 min。在4 000 r/min下离心5 min,将上层正己烷移入进样小瓶中,供气相色谱-质谱或气相色谱串联质谱分析。

6.4 测定

6.4.1 气相色谱质谱条件

6.4.1.1 气相色谱条件

气相色谱条件如下:

- a) 色谱柱:毛细管柱 DB-5MS,长30 m,内径0.25 mm,膜厚0.25 μm,或相当者;
- b) 升温程序:初始温度80℃,保持1 min,以10℃/min升高到250℃,保持5 min;
- c) 进样口温度:260℃;
- d) 载气:氮气,纯度≥99.999%;

- e) 载气流速:1.0 mL/min;
- f) 进样模式:不分流进样,1 min 后开阀;
- g) 进样量:1 μ L。

6.4.1.2 质谱条件

质谱条件如下:

- a) 接口温度:280 $^{\circ}$ C;
- b) 离子源:电子轰击源(EI);
- c) 电子能量:70 eV;
- d) 离子源温度:230 $^{\circ}$ C;
- e) 检测方式:选择离子监测(SIM);
- f) 选择离子(m/z):191(100),193(65),195(11),其中 191 为定量离子。

6.4.2 气相色谱串联质谱条件

6.4.2.1 气相色谱条件

气相色谱条件如下:

- a) 色谱柱:毛细管柱 DB-5MS;长 30 m,内径 0.25 mm,膜厚 0.25 μ m,或相当者;
- b) 升温程序:初始温度 50 $^{\circ}$ C,保持 1 min,以 20 $^{\circ}$ C/min 升高到 250 $^{\circ}$ C,保持 5 min;
- c) 进样口温度:260 $^{\circ}$ C;
- d) 流速:1.0 mL/min;
- e) 载气:氮气,纯度 \geq 99.999%;
- f) 进样模式:不分流进样,1 min 后开阀;
- g) 进样量:1 μ L。

6.4.2.2 串联质谱条件

串联质谱条件如下:

- a) 接口温度:280 $^{\circ}$ C;
- b) 离子源:电子轰击源(EI);
- c) 碰撞能量:15 eV;
- d) 离子源温度:230 $^{\circ}$ C;
- e) 检测方式:多反应监测(MRM);
- f) 离子对(m/z):191/128, 191/156。

6.4.3 色谱测定

根据样液中二氯二甲吡啶酚含量的情况,选定与样液中二氯二甲吡啶酚浓度相近的标准工作溶液。标准工作溶液和样液中二氯二甲吡啶酚的响应值应在仪器检测的线性范围内。标准溶液和样液等体积穿插进样测定。在上述气相色谱-质谱条件下(6.4.1.1),二氯二甲吡啶酚的保留时间约为 11.60 min,标准物质的色谱图参见附录 A 中图 A.1,全扫描质谱图参见图 A.2,在上述气相色谱串联质谱条件下(6.4.2.1),二氯二甲吡啶酚的保留时间约为 9.1 min,标准物质的色谱图参见图 A.3。

6.4.4 定性测定

按照气相色谱质谱条件测定样品和标准工作溶液,如果检测的色谱峰保留时间与标准品一致,定性

离子对的相对丰度,是用相对于最强离子丰度的强度百分比表示,应当与浓度相当标准工作溶液的相对丰度一致,相对丰度允许偏差不得超过表 1 规定的范围,则可判断样品中存在对应的被测物。

表 1 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/% | ±10 | ±15 | ±20 | ±50 |

6.4.5 空白实验

除不加试样外,按上述测定步骤进行。

6.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中二氯二甲吡啶酚的残留量,计算结果应扣除空白值:

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

式中:

- X ——样品中二氯二甲吡啶酚含量,单位为毫克每千克(mg/kg);
- A ——样液中二氯二甲吡啶酚丙酯峰面积;
- c ——标准工作溶液中二氯二甲吡啶酚浓度,单位为微克每毫升(μg/mL);
- V ——样液最终定容体积,单位为毫升(mL);
- A_s ——标准工作溶液中二氯二甲吡啶酚丙酯峰面积;
- m ——试样量,单位为克(g);
- R ——浓缩倍数。

7 方法的测定低限(LOQ)和回收率

7.1 测定低限

本方法二氯二甲吡啶酚的测定低限(LOQ):鸡肉中的测定低限为 5 μg/kg,鸡肝中的测定低限为 10 μg/kg。

7.2 回收率

回收率的实验数据(在不同添加浓度范围内)见表 2。

表 2 不同基质中二氯二甲吡啶酚添加浓度和回收率范围

| 样品名称 | 添加浓度/(μg/kg) | 回收率/% | |
|------|--------------|-----------|-----------|
| | | GC-MS | GC-MS/MS |
| 鸡肉 | 5 | 70.1~96.5 | 71.5~93.6 |
| | 10 | 71.1~94.6 | 72.8~92.7 |
| | 20 | 70.3~88.4 | 70.1~86.9 |
| | 5 000 | 75.7~93.8 | 76.8~96.8 |

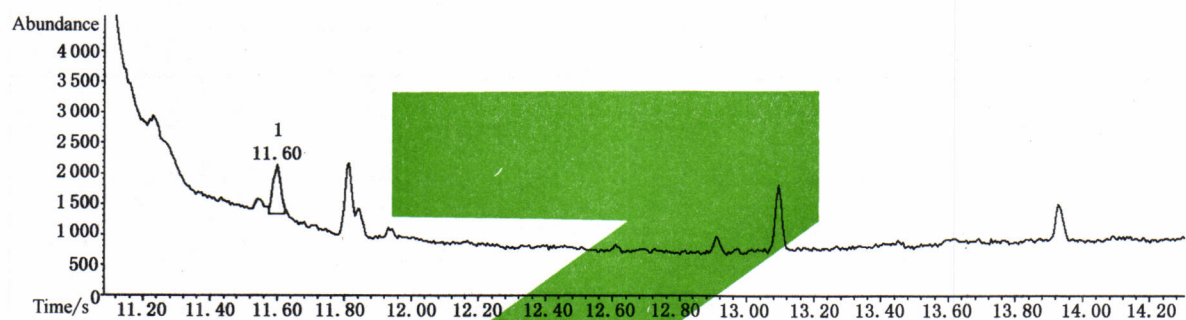
表 2 (续)

| 样品名称 | 添加浓度/($\mu\text{g}/\text{kg}$) | 回收率/% | |
|------|----------------------------------|-----------|-----------|
| | | GC-MS | GC-MS/MS |
| 鸡肝 | 10 | 70.5~91.2 | 70.2~90.8 |
| | 15 | 73.4~93.5 | 72.7~90.1 |
| | 30 | 76.5~96.7 | 73.9~91.4 |
| | 15 000 | 75.3~93.1 | 78.6~96.4 |

附录 A
(资料性附录)

二氯二甲吡啶酚标准品衍生物的选择性离子流色谱图、质谱图和多反应监测色谱图

二氯二甲吡啶酚标准品衍生物的选择性离子流色谱图、质谱图和多反应监测色谱图见图 A.1~图 A.3。



说明:

1——二氯二甲吡啶酚标准品衍生物。

图 A.1 二氯二甲吡啶酚标准品衍生物选择性离子流色谱图(80 ng/mL)

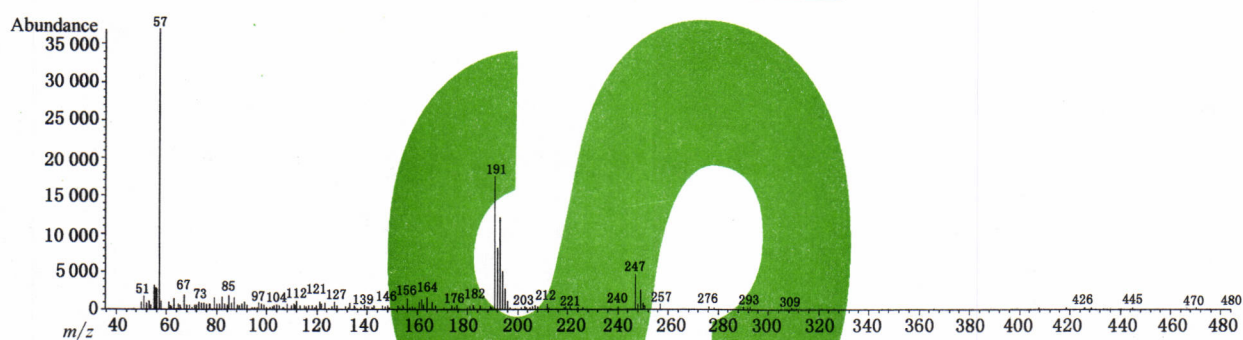
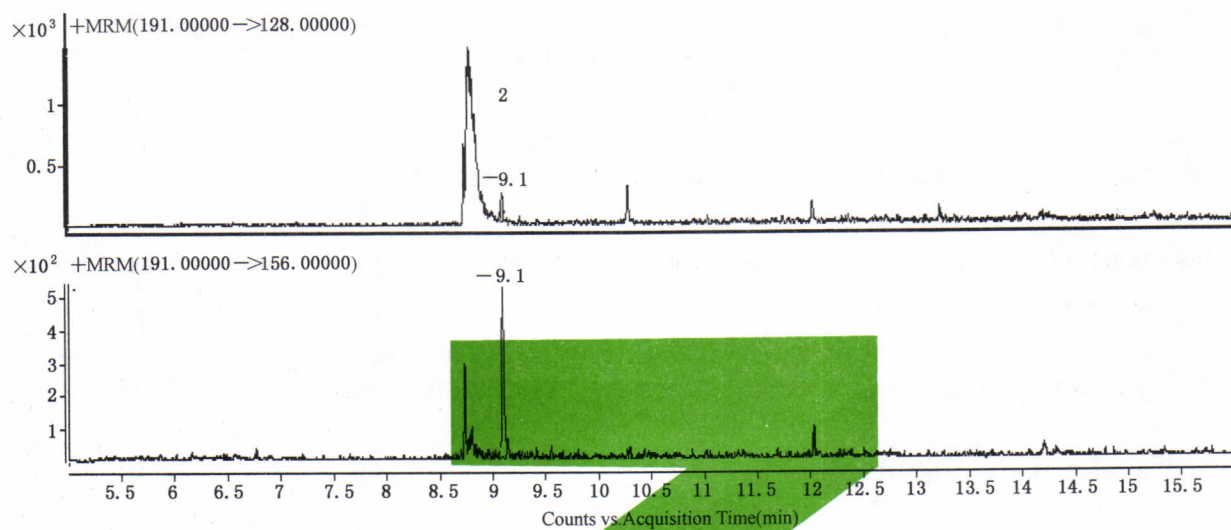


图 A.2 二氯二甲吡啶酚标准品衍生物全扫描质谱图



说明:

2——二氯二甲吡啶酚标准品衍生物。

图 A.3 二氯二甲吡啶酚标准品衍生物多反应监测色谱图(20 ng/mL)

Foreword

This standard is drafted according to rules of GB/T 1.1—2009.

This standard replace SN/T 0212.2—1993 GC method for determination of clopidol residues in poultry meat for export.

The main defferences between this standard and SN/T 0212.2—1993 are:

- the topic of standard is modified;
- cancel the procedure of sampling;
- the applicable scope is extended and add the matrix of liver;
- improve the method of deriviation;
- GC/MS and GC/MS/MS replace GC method.

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

This standard was drafted by Shanghai Entry-Exit Inspection and Quarantine Bureau of the Pepople's Republic of China.

The main drafters of this standard are ChenDi, ZhuJian, FanXiang, YuRuixiang, DengXiaojun, GuoDehua, HanLi, YiXionghai, PengMi, Zhanghao.

This standard is promulgated for the first time in 1993, and modified for the first time.

Determination of clopidol residues in poultry meat for export

1 Scope

This standard specifies the methods of determination by GC-MS and GC-MS/MS of clopidol residues in poultry meat.

This standard is applicable to the determination of clopidol residues in poultry meat.

2 principle

Clopidol residues in poultry meat is extracted with methanol and cleaned up by neutral alumina column. It is derived by propionic acid anhydride with the catalyst of pyridine in alkali condition. The aliquot is determined by GC/MS and GC/MS /MS, using external standard method.

3 Reagents and materials

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

3.1 Methanol: HPLC grade.

3.2 Hexane: Residue grade.

3.3 Pyridine.

3.4 Propionic acid anhydride, purity $\geq 98\%$.

3.5 Sodium tetraborate.

3.6 Sodium tetraborate solution (0.1 mol / L): Weigh 38.0 g sodium tetraborate (3.5) and desolve in 1 000 mL water.

3.7 Neutral alumina: for chromatography, 100~200 mesh.

3.8 Clopidol standard: $C_7H_7Cl_2NO$, CAS NO.: 2971-90-6 purity $> 98.0\%$.

3.9 Standard stock solution of clopidol: Weigh clopidol standard, dissolved it with methanol to

form a standard stock solution of 0.1 mg/mL in concentration. The standard stock solution should be stored at 4 °C in refrigerator and is stable for 12 months.

3.10 Standard working solution of clopidol: According to the requirement, dilute a standard working solution of clopidol(3.9) to appropriate concentration with methanol. The standard working solution should be stored at 4 °C in refrigerator and is stable for 6 months.

4 Apparatus and equipment

- 4.1 Gas chromatography-mass spectrometry equipped with electron impact ionization.
- 4.2 Gas chromatography tandem mass spectrometry equipped with electron impact ionization.
- 4.3 Tissue triturator.
- 4.4 Homogenizer.
- 4.5 Centrifuger: 4 000 rpm.
- 4.6 Vortex mixer.
- 4.7 Rotary evaporator.
- 4.8 Nitrogen blowing instrument.
- 4.9 Plastic centrifuge tubes, 15 mL.
- 4.10 Plastic centrifuge tubes, 50 mL.
- 4.11 Glass chromatography column: 20 mm(id) × 400 mm, filled with 16g neutral alumina(3.7).
- 4.12 Balance: sensitive 0.1 mg and 0.01 g.
- 4.13 Pear-shaped bottle, 250 mL.

5 Sample preparation and storage

5.1 Preparation of test sample

Representative samples should be taken from all samples, the edible parts are selected, about 500 g is selected by Criss and cross method, put into a tissue triturator and homogenized. Then divide the

pulp into two equal portions, each portion is put in a clean container which is sealed and labeled.

5.2 Storage of test sample

Samples should be stored below $-18\text{ }^{\circ}\text{C}$.

6 Procedure

6.1 Extraction

Weigh about 5.0 g (accurate to 0.01 g) of the test sample into 50 mL plastic centrifuge tubes, add 25 mL of methanol(3.1) homogenized for 1 min, centrifuge for 5 min in 4 000 r/min.

6.2 Cleanup

Activate alumina column(4.11) with 40 mL of methanol, then transfer 20 mL the extracted solution (6.1) into it. When extracted solution tangent to alumina, add 10 mL methanol, do not collect eluted solution. Add 55 mL methanol and collect eluted solution into pear-shaped bottle(4.13). The solution is rotary evaporated to dryness in a water bath at $50\text{ }^{\circ}\text{C}$. Dissolve the residues with 2 mL methanol, transfer it to plastic centrifuge tube (4.9), then add 1 mL methanol to wash the pear-shaped bottle, also transfer it to plastic centrifuge tube, then concentrate it to 0.5 mL with nitrogen blowing instrument.

6.3 Esterification

Add reagents into the plastic centrifuge tube in turn: 4 mL Sodium tetraborate solution (3.6), 0.25 mL hexane(3.2) (add 1.00 mL hexane if use GC/MS/MS), 25 μL Pyridine(3.3) and 50 μL propionic acid anhydride(3.4). Vortex mix for 1 min and centrifuge at 4 000 r/min for 5 min. The hexane layer is transferred for GC-MS or GC-MS/MS determination.

6.4 Determination

6.4.1 GC-MS conditions

6.4.1.1 GC conditions

- a) Column: DB-5MS capillary column, $30\text{ m} \times 0.25\text{ mm (i.d.)} \times 0.25\text{ }\mu\text{m}$, or the equivalent;
- b) Temperature program: $80\text{ }^{\circ}\text{C}$ for 1 min, $10\text{ }^{\circ}\text{C/min}$ to $250\text{ }^{\circ}\text{C}$ for 5 min;

- c) Inlet temperature: 260 °C ;
- d) Carrier gas: Helium, purity $\geq 99.999\%$;
- e) Flow rate: 1.0 mL/min;
- f) Injection mode: Splitless;
- g) Injection volume: 1 μ L.

6.4.1.2 MS conditions

- a) Interface temperature: 280 °C ;
- b) Ion source: EI;
- c) Electron impact: 70 eV;
- d) Ionization source temperature: 230 °C ;
- e) Mode: Select ion monitoring (SIM);
- f) Selected monitoring ions (m/z): 191(100), 193(65), 195(11), among them 191 is the quantification ion.

6.4.2 GC-MS/MS conditions

6.4.2.1 GC conditions

- a) Column: DB-5MS capillary column, 30 m \times 0.25 mm (i.d.) \times 0.25 μ m, or the equivalent;
- b) Temperature program: 50 °C for 1 min, 20 °C/min to 250 °C for 5 min;
- c) Inlet temperature: 260 °C ;
- d) Flow rate: 1.0 mL/min;
- e) Carrier gas: Helium, purity $\geq 99.999\%$;
- f) Injection mode: Splitless;
- g) Injection volume: 1 μ L.

6.4.2.2 MS/ MS conditions

- a) Interface temperature: 280 °C ;
- b) Ion source: EI;
- c) Electron impact: 15 eV;
- d) Ionization source temperature: 230 °C ;
- e) Mode: Multiple reaction monitoring (MRM);
- f) Monitor ions (m/z): 191/128, 191/156.

6.4.3 GC-MS determination

According to the approximate concentration of the clopidol in the sample solution, select the standard working solution with similar concentration of the sample solution. The response of clopidol derivatization in the standard working solution and the sample solution should be within the linear range of the instrument detection. The standard working solution should be injected in-between the injections of the sample solution with one common volume. Under the above GC-MS operating conditions (6.4.1.1), the retention time of clopidol derivatization is about 11.60 min, and SIM chromatogram of the clopidol standard derivatization are shown by figure A.1 in annex A. Under the above GC-MS/MS operating conditions (6.4.2.1), the retention time of clopidol derivatization is about 9.1 min, and MRM chromatogram of the clopidol standard derivatization are shown by figure A.3 in annex A.

6.4.4 Confirmation

Under GC-MS conditions, the working solution and sample solution is injected. If the retention times of samples chromatogram peaks are consistent with that of standard solution, calibration curve method is used for quantitative measurement. The relative intensities of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of standard solution should be same with those of sample solution. The permitted tolerances listed in table 1, then the corresponding analyte must be present in sample.

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

| Relative ion intensities/% | >50 | >20~50 | >10~20 | ≤10 |
|--------------------------------|------|--------|--------|------|
| Maximum permitted tolerances/% | ± 10 | ± 15 | ± 20 | ± 50 |

6.4.5 Blank test

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

6.5 Calculation and expression of the result

Calculate the content of clopidol in the test sample by GC-MS or GC-MS/MS data processor or using the followed formula (1).

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

Where

X —the residue content of clopidol in the test sample, (mg/kg);

A —the area of clopidol derivatization in the test sample;

c —the concentration of clopidol in the standard working solution, (μg/mL);

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

A_s —the area of clopidol derivatization in the standard working solution;

m —the sample weight (g);

R —Dilution times.

7 Limit of determination and recovery

7.1 Limit of determination

The limit of determination of clopidol of this method is 5 μg/kg for chicken meat and 10 μg/kg for chicken liver.

7.2 Recovery

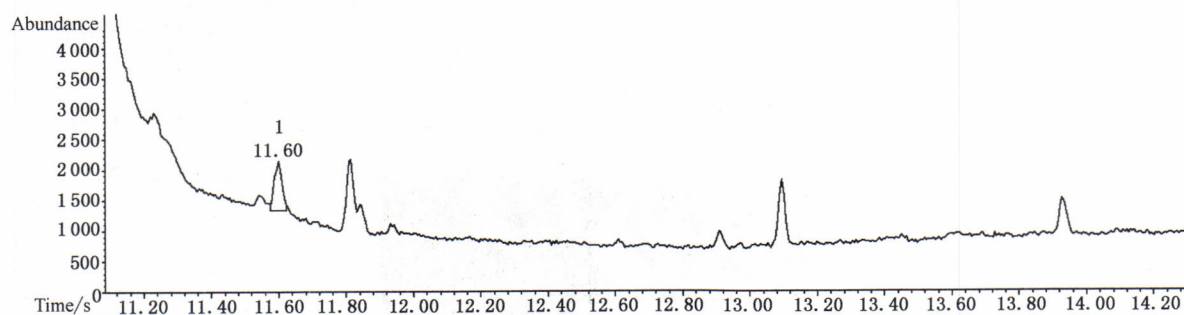
The recovery data was shown in table 2.

Table 2—The range of recoveries of clopidol in chicken meat and chicken liver

| Sample name | Added concentrations /($\mu\text{g/kg}$) | Recoveries/% | |
|---------------|---|--------------|-----------|
| | | GC-MS | GC-MS/MS |
| Chicken meat | 5 | 70.1~96.5 | 71.5~93.6 |
| | 10 | 71.1~94.6 | 72.8~92.7 |
| | 20 | 70.3~88.4 | 70.1~86.9 |
| | 5 000 | 75.7~93.8 | 76.8~96.8 |
| Chicken liver | 10 | 70.5~91.2 | 70.2~90.8 |
| | 15 | 73.4~93.5 | 72.7~90.1 |
| | 30 | 76.5~96.7 | 73.9~91.4 |
| | 15 000 | 75.3~93.1 | 78.6~96.4 |

Annex A
(Informative)

SIM/MS/MS chromatogram of the clopidol standard derivatization



1—clopidol standard derivatization

Figure A.1 SIM chromatogram of clopidol standard derivatization(80 ng/mL)

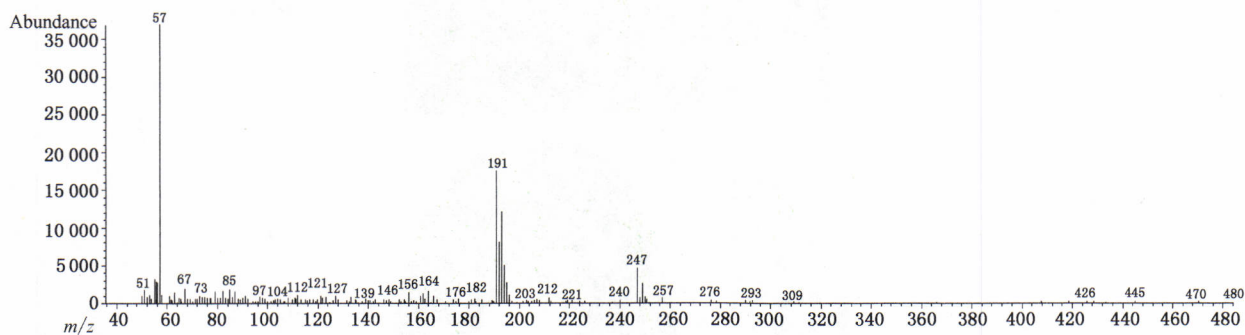
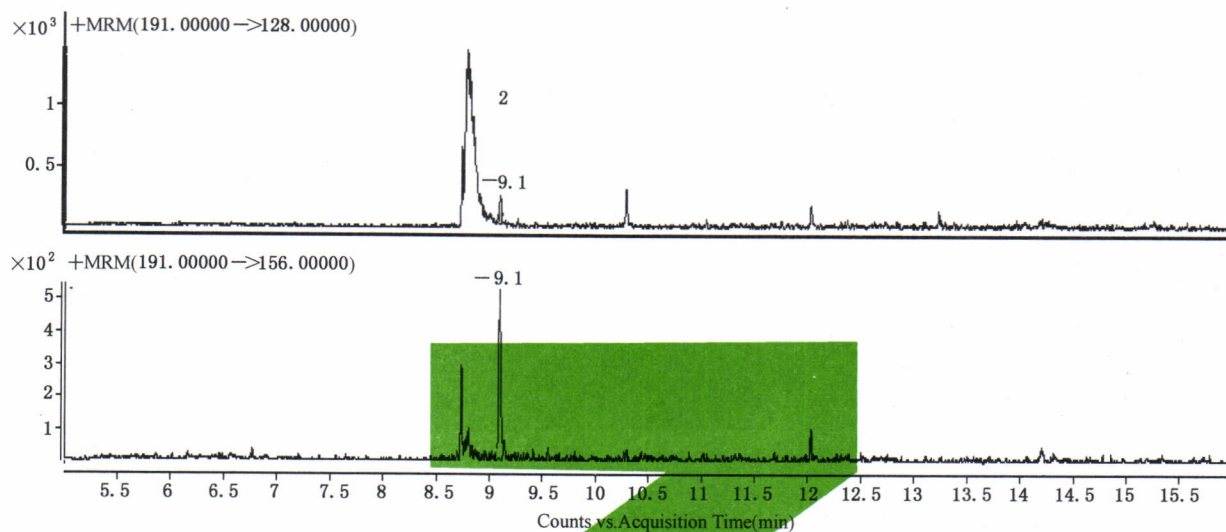


Figure A.2 Mass spectrogram of clopidol standard derivatization



2—clopidol standard derivatization

Figure A.3 MRM chromatogram of clopidol standard derivatization(20 ng/mL)

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