SN

中华人民共和国出入境检验检疫行业标准

SN/T 1133—2002

进出口肉及肉制品中持久霉素残留量检验方法 打孔法

Method for the determination of enradine residues in meat and meat products for import and export—Piercing method

2002-08-02 发布　　2003-01-01 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

本标准中的测定方法等效采用了日本厚生省《畜、水产食品中的残留物质检验方法》中持久霉素残留量检验方法，技术内容与原方法相同，经验证后按规定格式要求作了编辑性修改。

本标准的附录A是规范性附录，附录B是资料性附录。

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：中华人民共和国天津出入境检验检疫局。

本标准主要起草人：袁而森、陈子红、唐丹舟、林安清、孙俐。

本标准系首次发布的出入境检验检疫行业标准。

进出口肉及肉制品中持久霉素残留量检验方法 打孔法

1 范围

本标准规定了进出口肉及肉制品中持久霉素残留量检验的抽样、制样和打孔测定方法。

本标准适用于进出口鸡肉中持久霉素残留量的检验。

2 抽样和制样

2.1 检验批

以不超过2 500件为一检验批。

同一检验批的商品应具有相同特征，如包装、标记、产地、规格和等级等。

2.2 抽样数量

批量/件	最低抽样数/件
1～25	1
26～100	5
101～250	10
251～500	15
501～1 000	17
1 001～2 500	20

2.3 抽样方法

按2.2规定的抽样件数随机抽取，逐件开启，每件至少取一袋作为原始样品。如每件中无小包装或有小包装，但每袋重量超过2 kg者，则可用灭菌刀具在抽出的包件只能割取不少于100 g样品，作为混合原始样。原始样的总量不少于2 kg。混合后，置于清洁容器内，标明标记，及时送交实验室。

2.4 试样制备

从原始样品中取出部分有代表性的样品，将可食部分用绞碎机绞碎，充分混匀。用四分法缩分出不少于500 g，作为试样。装入清洁容器内，加封并表明标记。

2.5 试样保存

将试样于－18℃以下冷冻保存。

注：在抽样及制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

将试样用水均质后，再以丙酮提取试样中持久霉素残留，提取液经浓缩后，加入正丁醇和稀盐酸净化，用氢氧化钠调整pH值，减压浓缩至干，最后再用甲醇-磷酸盐缓冲液溶解作为试液，利用试液中持久霉素残留与枯草杆菌作用产生抑菌圈，根据抑菌圈大小用标准曲线法定量。

3.2 试剂和材料

除另有规定外，所用试剂均为分析纯，水为蒸馏水或去离子水。标准品及标准溶液均以效价计。

3.2.1 丙酮。

3.2.2 丙酮-水溶液：60%(*V*/*V*)。

3.2.3 正丁醇。

3.2.4 盐酸溶液：4 mol/L、1 mol/L、0.005 mol/L。

3.2.5 氢氧化钠溶液：1 mol/L。

3.2.6 甲醇-磷酸盐缓冲液(pH8.0±0.1)：称取 13.3 g 磷酸二氢钾(精确至 0.1 g)，溶解于 900 mL 水中，另取 6.2 g 氢氧化钾溶解于 100 mL 水中，两种混合均匀。取其 300 mL 与 700 mL 甲醇混合即可。

3.2.7 甲醇水溶液：甲醇＋水(4＋5)。

3.2.8 三羟甲基氨基甲烷缓冲液：称取 6.06 g 三羟甲基氨基甲烷，溶解于 40 mL 1 mol/L 盐酸加水定容至 1 000 mL，115℃高压灭菌 15 min，灭菌后调整 pH 值为 7.0±0.1。

3.2.9 持久霉素标准品。

3.2.10 持久霉素标准贮备液：准确称取一定量的本标准品(精确至 0.000 1 g)，用甲醇-水溶液(3.2.7)配制成 500 μg/mL 的标准贮备液，于 5℃冰箱中保存，可使用 8 天。

3.2.11 持久霉素标准工作液：吸取一定量的持久霉素贮备液，用三羟甲基氨基甲烷缓冲液(3.2.8)稀释成(0.15、0.30、0.60、1.20、2.40) μg/mL 浓度的标准工作液备用。该溶液均须当日配制。

3.2.12 实验菌种：枯草芽孢杆菌(Bacillus subtilis)。

3.2.13 培养基Ⅰ：见附录 A 中 A.1。

3.2.14 培养基Ⅱ：见附录 A 中 A.2。

3.2.15 培养基Ⅲ：见附录 A 中 A.3。

3.3 仪器和设备

3.3.1 培养皿：内径 90 mm，底部平整光滑的玻璃或塑料皿，具备陶瓦盖。

3.3.2 打孔器：内径 8 mm±1 mm 的不锈钢制品。

3.3.3 游标卡尺：测量范围 0 mm～200 mm，精度 0.02 mm 或抑菌圈测量仪。

3.3.4 均质器：不低于 10 000 r/min。

3.3.5 离心机：不低于 8 000 r/min。

3.3.6 恒温培养箱：36℃±1℃，隔板保持水平。

3.3.7 旋转蒸发器。

3.4 测定步骤

3.4.1 试液制备

称取 10 g 试样(精确至 0.1 g)于均质杯内，加入 30 mL 水，以 8 000 r/min 均质 5 min，煮沸 5 min。待冷却后加入 60 mL 丙酮，用 4 mol 盐酸调整 pH3.5～4.0，充分搅拌 5 min。移入离心管中离心 10 min(10 000 r/min)，取出上清液，用 100 mL 的丙酮-水溶液(3.2.2)洗涤沉淀物二次，将洗涤液移入上清液中，减压浓缩至约 40 mL。将浓缩液用 1 mol/L 氢氧化钠调整 pH8.0 后，加入 80 mL 正丁醇(3.2.3)振摇混匀，收集正丁醇层。水相中再加入 20 mL 正丁醇振摇混匀，弃去水相，合并正丁醇层。加入 100 mL 0.005 mol/L 盐酸溶液，振摇混匀，分取水层。再加入 100 mL 0.005 mol/mL 盐酸于正丁醇层中，振摇混匀，弃去正丁醇层。合并水层，用 1 mol/L 的氢氧化钠调整 pH8.0 后减压浓缩至干。用 5 mL 甲醇-磷酸盐缓冲液(3.2.6)溶解残留物，作为试验样液。

3.4.2 菌悬液的制备

将菌种以无菌操作接种于培养基Ⅱ(3.2.14)内，于 36℃±1℃培养 24 h±1 h。再转种至培养基Ⅰ(3.2.13)斜面上，继续培养 7 d，检查芽孢，如达到 80%时，用适当的生理水洗下菌苔，使之均匀悬浮，以 3 000 r/min 离心 30 min，弃去上清液，加适量生理盐水振荡间隔 24 h，以 65℃、20 min 加热两次后，置入灭菌试管中备用。

3.4.3 检定用平板的制备

将适量的试验菌液加入溶化的培养基Ⅲ(3.2.15)冷却至 50℃～55℃，混匀。于每个消毒平皿中注

入 20 mL，保持水平，待其凝固后备用。在测定前先用 0.15 μg/mL 和 0.60 μg/mL 的标准工作液，对不同浓度的菌液进行测试，经培养后呈现的抑菌圈直径分别在 10 mm～15 mm 和 15 mm～20 mm，即为最宜菌量。

3.4.4 标准曲线的制备

用(0.15、0.30、0.60、1.20、2.40) μg/mL 标准工作液做曲线，以 0.60 μg/mL 浓度的标准工作液作为参考浓度。标准曲线上的每个标准工作液浓度各取 3 个检定平板为一组。在每个平板上，用已消毒的打孔器打 6 个孔，在半径为 2.8 cm 的圆面上成 60°角间距。其中 3 个孔内加 0.1 mL 参考浓度溶液，另 3 个孔内加 0.1 mL 其他一种浓度的标准工作液。参考浓度溶液与标准工作液要间隔放置。5 个浓度标准工作液共用 15 个检定平板。

将陶瓦盖盖好，在 36℃±1℃培养 17 h±1 h。保温后，取出平板，精确地测量所产生的抑菌圈的直径(精确到 0.1 mm)。求出每组 3 个检定平板上 0.60 μg/mL 浓度的抑菌圈直径读数与其他浓度标准工作液的抑菌圈直径读数的平均值。再求出参考浓度 0.60 μg/mL 的所有 45 个抑菌圈直径数值的平均值，用此平均值与每组中 9 个参考浓度抑菌圈直径平均值之差来校正其他各浓度标准工作液的抑菌圈读数的平均值。

将校正后的值，用式(1)、式(2)算出 L 和 H。在半对数坐标纸上，以抑菌圈直径(mm)为纵坐标(算术级)，以浓度(μg/mL)为横坐标(对数级)，标出 L 和 H 点。通过 L 和 H 点连一直线即为标准曲线。

$$L = \frac{3a + 2b + c - e}{5} \quad \cdots\cdots(1)$$

$$H = \frac{3e + 2d + c - a}{5} \quad \cdots\cdots(2)$$

式中：

L——标准曲线上的最低浓度(0.15 μg/mL)抑菌圈直径，单位为毫米(mm)；

H——标准曲线上的最高浓度(2.40 μg/mL)抑菌圈直径，单位为毫米(mm)；

c——所有参考浓度(0.60 μg/mL)抑菌圈直径的平均值，单位为毫米(mm)；

a、b、d、e——分别表示标准曲线中其他浓度标准工作液(0.15、0.30、1.20、2.40) μg/mL 的抑菌圈直径经校正后的平均值，单位为毫米(mm)。

3.4.5 试液的测定

每份试液用三个检定用平板。不需定量时，用已消毒的打孔器，在每个平板上打四个对称的孔，其中两个对角孔中，各滴入 0.1 mL 试液，其他两个孔中分别滴入 0.1 mL 的 0.15 μg/mL 和 0.60 μg/mL 的标准工作液。需定量时，在每个平板上打 6 个孔，间隔分别加入 0.1 mL 试液和 0.60 μg/mL 浓度的标准工作液。于 36℃±1℃温箱中，培养 17 h±1 h。

3.5 结果计算和表述

如试液呈现出的清晰圈，直径平均值小于 12 mm 者为“阴性”。

如试液呈现出的抑菌圈直径平均大于等于 12 mm 时，经校正后，从标准曲线上查出相应的持久霉素的浓度。再用式(3)计算试样中持久霉素残留含量。

$$X = \frac{c}{m} \quad \cdots\cdots(3)$$

式中：

X——试样中持久霉素残留的含量，单位为毫克每千克(mg/kg)；

c——从标准曲线上查出的试液中持久霉素浓度，单位为微克每毫升(μg/mL)；

m——每毫升最终试液所代表的试样量，单位为克(g)。

注：如测定结果呈阳性，即所显示的抑菌圈平均直径大于等于 12 mm，必要时，尚需进行确证试验(可用微生物显像法附录 B)，以证明抑菌圈物确系持久霉素。

4 测定低限和回收率

4.1 测定低限

本方法的测定低限为 0.075 mg/kg。

4.2 回收率

持久霉素的添加浓度及其回收率的实验数据：

0.075 mg/kg 时，回收率为 77.9%；

0.15 mg/kg 时，回收率为 84.9%；

0.3 mg/kg 时，回收率为 90.1%。

附 录 A
（规范性附录）
培养基成分及制备方法

A.1 培养基Ⅰ

蛋白胨	10.0 g
牛肉浸膏	5.0 g
氯化钠	2.5 g
琼 脂	15.0 g
蒸馏水	1 000 mL

将上述各成分于水中加热溶解，调节 pH 使其灭菌后 pH 值为 6.5±0.1，分装于试管中，于 121℃灭菌 15 min。制成斜面备用。

A.2 培养基Ⅱ

蛋白胨	10.0 g
牛肉浸膏	5.0 g
氯化钠	2.5 g
蒸馏水	1 000 mL

将上述各成分于水中加热溶解，调节 pH 使其灭菌后 pH 值为 7.0±0.1，分装于试管中，于 121℃灭菌 15 min。

A.3 培养基Ⅲ

蛋白胨	5.0 g
牛肉浸膏	5.0 g
氯化钠	5.0 g
磷酸氢二钠	2.0 g
琼 脂	15.0 g
蒸馏水	1 000 mL

将上述各成分于水中加热溶解，调节 pH 使其灭菌后 pH 值为 8.0±0.1，分装于试管中，于 121℃灭菌 15 min。

附 录 B
（资料性附录）
确 证 试 验

确证试验采用生物自显影法，用标准品作对照，求 R_f 值。

微量注射器：50 μL。

薄层板：硅胶，Q/YT257-85SG，5 cm×20 cm，使用前 110℃活化 2 h。

展开剂：三氯甲烷＋甲醇＋醋酸(86＋12＋2)。

展开槽：240 mm×57 mm×32 mm。

点样量：20 μL。

长方形培养皿：20 cm×10 cm×5 cm。

展开：在饱和槽内进行。

检测：取经活化的薄层板，在其下端 2 cm 处划一条基线，然后在该线上分别取 20 μL 试液和 0.30 μg/mL浓度的持久霉素标准溶液，两点相距 2.5 mm，置展开槽中，用展开剂进行展开，直至溶剂前沿线距板顶端 1.5 cm 处为止。取出薄层板，待风干后放入经高压消毒的长方形培养皿中，将已熔化并冷却至 50℃～55℃的培养基Ⅱ(3.2.13)均匀地喷在表面，然后用 10 mL 上述已适量接种枯草芽胞菌悬液的培养基，铺满整个薄层板，保持水平，待其凝固，于 36℃±1℃培养 17 h±1 h。

经培养后，在薄层上与持久霉素标准液产生抑菌圈的 R_f 值相同位置上，显现抑菌圈者即为阳性。

Foreword

In this standard, the testing method for enradine residues in the publication "Methods for the determination of residues in animal and aquatic foods" approved by the Public Health Department of Japan is adopted as the determination method of enradine residues, which is thus technically same as that of the original method, only minor editorial modification required for the stipulated format was made after going through verification.

Annex A of this standard is on normative annex.

Annex B of this standard is on informative annex.

This standard was proposed and administrated by National Regulatory Commission for Certification and Accreditation.

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

The main drafters of this standard Yuan Ersen, Chen Zihong, Tang Danzhou, Lin Anqing, Sun Li.

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

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

Method for the determination of enradine residues in meats and meat products for import and export —piercing method

1 Scope

This standard specifies the method of sampling, sample preparation and determination by Piercing method of enradine residues in meats and meat products for import and export.

This standard is applicable to the determination of enradine residues in chicken meat for import and export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 2 500 packages.

The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification and grade, should be the same.

2.2 Quantity of sample taken

Number of Packages in Each inspection lot	Minimum number of packages to be taken
1 ~ 25	1
26 ~ 100	5
101 ~ 250	10
251 ~ 500	15
501 ~ 1 000	17
1 001 ~ 2 500	20

2.3 Sampling procedure

A number of packages specified in 2.2 are taken at random and opened one by one. From each at least one bag shall be taken as primary sample. In case there are no small bags or if there are small bags inside the package, but the content of a bag exceeds 2 kg, cut out a part form the meat in each package of not less than 100 g as primary sample with a sterile knife. Mix the parts of the meat as primary sample. The total weight of all primary samples should not be less than 2 kg which shall be mixed, placed in a clean container sealed, labeled and sent to the laboratory in time.

2.4 Preparation of sample

Parts of the representitive sample are taken form the primary sample and the edible potions are homogenized by grinding in a grinder. The homogenized sample is thoroughly mixed and reduced to at least 500 g by quartering as the test sample. The test sample is placed in a clean container, which is then sealed and labeled.

2.5 Storage of test sample

The test sample should be stored below -18℃

Note: 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.

3 Method of determination

3.1 Principle

Homogene the sample with water, the extract enradine in sample with actone, and concentrate to a exact volume. Add n-butanol and diluted. Hydrochloric in order to cleanup the sample, adjust the pH value of the solution with sodium hydroxide solution, evaporate off the solvent, dissolve the reside with methanol-phoshateacid buffer solution. Use Bacillus subtilis as the indicating bacterium. Quantitation is made by measuring the size of inhibition zone, using standard curve method.

3.2 Reagents and materials

Unless otherwise specified, all reagents should be analytically pure, "water" is distilled or deionized water. Standard and standard solution is calculated by potency.

3.2.1 Acetone

3.2.2 Acetone solution: 60% (*V/V*).

3.2.3 n - Butanol.

3.2.4 Hydrochloric acid: 4 mol/L, 1 mol/L, 0.005 mol/L.

3.2.5 Sodium hydroxide: 1 mol/L.

3.2.6 Methanol - Phosphate buffer solution (pH8.0 ± 0.1): Dissolve 13.3 g (accurate to 0.1 g) potassium dihyrogen phosphate in 900 mL of water, and 6.2 g potassium hydroxide in 100 mL of water, mix well. Take - out the solution 300 mL mingle in 700 mL methanol.

3.2.7 Methanol solution: Methanol + water(4 + 5).

3.2.8 Tris (Hydroxymethyl) aminomethane buffer solution: Dissolve 6.06 g Tris (Hydroxymethyl) aminoethane in 1 mol/L 40 mL hydrochloric acid addition 100 mL water and autoclave at 115℃ for 15 min, after sterilization, pH(7.0 ± 0.1).

3.2.9 Enradine standard.

3.2.10 Enradine standard stock solution: Accurately weigh a proper quantity of enranycan standard (accurate to 0.000 1 g), dissolve in methanol solution (3.2.7) to make a standard stock solution of 500 μg/mL in concentration, and store in refrigerator at 5℃. It could be used within 8 days.

3.2.11 Enradine standard working solution: Pipet a serious amounts of enradine standard stocks solution, dilute separately with Tris (Hydroxymethly) aminomethane buffer solution (3.2.8) to make a serious enradine standard working solution of (0.15、0.30、0.60、1.20、2.40) μg/mL in concentrations. All the above solutions should be prepared daily.

3.2.12 Test Strain: Bacillus subtilis.

3.2.13 Medium Ⅰ (See A1 in annex A).

3.2.14 Medium Ⅱ (See A2 in annex A).

3.2.15 Medium Ⅲ (See A3 in annex A).

3.3 Apparatus and equipment

3.3.1 Culture dish: 90 mm(id) dish or plastic with smooth and flat bottom, and with clay cover.

3.3.2 Piercer: Inner diameter of 8 mm ± 1 mm, made of stainless steel.

3.3.3 Vernier caliper: Measuring range 0 mm ~ 200 mm, precision 0.02 mm or inhibrtion zone measurer.

3.3.4 Homogenizer: No less than 10 000 r/min.

3.3.5 Centrifuger: No less than 8 000 r/min.

3.3.6 Incubtor: 36℃ ± 1℃, with horizontal board.

3.3.7 Rotary evaporator.

3.4 Procedure

3.4.1 Preparation of test solution

Weigh 10 g(accurate to 0.1 g)of the test sample into a homogenizer cup. Add 30 mL of water and homogenize at 8 000 r/min for 5 min, then boil for 5 min. After cooling, add 60 mL of acetone, adjust the pH value to 3.5 ~ 4.0 with 4 mol/L hydrochloric acid solution. Centrifuge for 10 min (1 000

r/min), take out the upper clear solution. Wash the residue with 100 mL of 60% acetone (solution) (3.2.2) twice, Combine the washing solution to the upper clear solution, concentrate under low pressure to 40 mL. Adjust the pH value of the concentrated solution to 8.0 with 1 mol/L sodium hydroxide solution. Then add 80 mL of n – butanol (3.2.3), shake mixing, combine the n – butanol. Further add 20 mL n – butanol, mix discard water layer. Combine the n – butanol. Add 100 mL of 0.005 mol/L hydrochloric acid, shake Mixins, collect the water phase, repeat add 100 mL of 0.005 mol/L hydrochloric acid to eh n – butanol, shake thoroughly. Combine the water layer. adjust the pH value with 1 mol/L. Sodium hydroxide solution to 8.0, the nevaporate off the solvent. Dissolve the residue with 10 mL methand. Phophote buffer (3.2.6) as the sample solution.

3.4.2 Preparation of organison suspension

Incubate the strain to the enrichment medium Ⅱ (3.2.14) and incubate at (36 ± 1)℃ (24 ± 1) h, transfer to the preservative slant medium Ⅰ (3.2.13), continue incubate a week. When sporulation hs occurred about 80%, add proper saline to make spore suspension, centrifuge 3 000 r/min for 30 min, than discard top of the suspension, add proper saline, after heat twice at intervals of 24 h on the 65℃ 20 min, Take out supernate into sterilized bube for use.

3.4.3 Preparation of the plates

Take off melted medium Ⅲ (3.2.15) and cool to 50℃ ~ 55℃, add a suitable amount of the test in strain suspension then mix thoroughly. Pepit 20 mL of the medium bottle each sterilized dish. Keep horizonal and solidified for later use. Before determination, The concentration of the testing bacteria in the strain medium should be pre – testcd using 0.15 μg/mL, and 0.60 μg/mL standard working solution. After incubating, when the diameter of inhibit zone caused by standard working solution should be 10 mm ~ 15 mm and 15 mm ~ 20 mm, it is suitable for determination.

3.4.4 Preparation of standard curve

Use 0.60 μg/mL standard working solution as the reference concentration solution. Take three plates as a set for each concentration of the standard working solution. Use a sterilized picrcer to make six boles. On each plate at 60° intervals on the circle of 2.8 cm radius. Fill three holes with 0.1 mL reference concentration solution and the other three holes with one 0.1 mL of the other concentrations of the standard working solution, alternating. Use three plates for each concentration of the curve, for a total of 15 plates. This will give 45 determinations for 0.60 μg/mL and 9 determinations for each of the other concentrations on the curve.

Place the clay covers on the plates and incubate the plates for 17 h ± 1 h at 36℃ ± 1℃. After incubation, take the plates measure the diameter of each zone of inhibition as accurately as possible (to the nearest 0.1 mm). Average the readings of the 0.60 μg/mL concentration and the readings of the other concentration on each set of three plates. Also average all 45 readings of the 0.60 μg/mL concentration as the correction point for the curve. The difference between the total (45 readings) average of 0.60 μg/mL reference concentration and average of the reference concentration of each set is the correction value for each set.

By these corrected values. Calculate the values of point L and point H by the formula(1) and (2), and plot the point L and point H on the semilog graph paper, using logarithmic scale for concentration (μg/mL) as abscissa and arithmetic scale for average zone diameters (mm) as ordinate.

Construct a straight line through the point L and H as the standard curve.

$$L = \frac{3a + 2b + c - e}{5} \quad \cdots\cdots(1)$$

$$H = \frac{3e + 2d + c - a}{5} \quad \cdots\cdots(2)$$

Where

L—the zone diameter of the lowest concentration (0.15 μg/mL) of the standard curve, mm;

H—the zone diameter of the highest concentration (2.40 μg/mL) of the standard curve, mm;

c—the average of 45 zone diameters for the reference concentration (0.60 μg/mL), mm;

a、b、d、e—the corrected average zone diameter for each of the other concentrations (0.15、0.30、1.20、2.40) μg/mL used for the standard curve, mm.

3.4.5 Determination of test solution.

Using three testing plates for each test solution. If quantitive determination is not required, take a sterilized piercer to make four symmetry holes, among the two symmetrical holes, put in 0.1 mL test solution, the other two holes, add 0.1 mL of 0.15, 0.60 μg/mL concentration standard working solution.

If quantitative assay is required to make six symmetry holes on plates. Alternate put in 0.1 mL test solution and 0.6 μg/mL concentration standard working solution, incubate at 36℃ ± 1℃ for 17 h ± 1 h.

3.5 Calculation and expressing of result

If the diameter of inhibition zones caused by the sample solution is < 12 mm report as "negative". If the diameter of inhibition zone ≥ 12 mm after correction, obtain the corresponding concentration of enradine from the standard curve. Then, calculate the content of enradine residues in the test sample according to formula (1).

$$X = \frac{c}{m} \quad \cdots\cdots(3)$$

Where

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

c—the concentration of enradine in sample solution obtained from the standard curve, μg/mL;

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

Note: If the result of determination is "positive", ie the average diameter of inhibition zones is equal or larger than 12 mm, confirmation test (bioautographic method may be caused, ef. Annex B) may be carried out, to confirm that the inhibiting substance is actually enradine.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.075 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of enradine in chicken meat and its corresponding recoveries are.

0.075 mg/kg, recovery 77.9%;

0.15 mg/kg, recovery 84.9%;

0.30 mg/kg, recovery 90.1%.

Annex A
(normative)
Mediums

A.1 Medium Ⅰ

Peptone	10.0 g
Beff extract	5.0 g
NaCl	2.5 g
Agar	15.0 g
Water	1 000 mL

Dissolve each ingredients in water. After autoclaving, pH 6.5 ± 0.1. Dispense into test tubes, autoclave at 121℃ for 15 min. As required, prepare the slant. Store for later use.

A.2 Medium Ⅱ

Peptone	10.0 g
Beef extract	5.0 g
NaCl	2.5 g
Water	1 000 mL

Dissolve each ingredients in water. After autoclaving pH 7.0 ± 0.1. Dispense into test tubes, autoclave at 121℃ for 15 min.

A.3 Medium Ⅲ

Peptone	5.0 g
Beef extract	5.0 g
NaCl	5.0 g
Na_2HPO_4	2.0 g
Agar	15.0 g
Water	1 000 mL

Dissolve each ingredients in water. After autoclaving pH is 8.0 ± 0.1. Dispense into conical flasks. Autoclave at 121℃ for 15 min.

Annex B
(informative)
Confirmation test

Bioautographic method is used for confirmation. Measure the Rf value and compare with that of the standard to confirm.

Micro – syringe: 50 μL.

Thin – layer plate: Silica gel Q/YT257-85SG, 5 cm × 20 cm, activate at 110℃ for 2 h before use.

Developing agent: Trchloromethahe Methanol Acetic acied (86 + 12 + 2).

Developing tank: 240 mm × 57 mm × 32 mm

Rectangular culture dish: 20 cm × 10 cm × 5 cm.

Development: In saturation developing tank.

Determination:

Taking a TL plate is activated. On plate, at first scribe a baseline across the plate 2 cm from the bottom, then spot 20 μL sample extract and enradine standard solution (0.30 μg/mL) on the baseline, the sample spot shall be 2.5 cm away from the standard spot. Develop the TL plate in the tank use developing solution, until the front margin of the solvent is 1.5 cm from the top of plate. Remove the plate, after the plate is air – dried place the thin – layer plate into a sterilized rectangular culture dish horizontally. Aseptically spray the melted and cooled to (50 ~ 55)℃ medium (3.2.13) onto the surface of the plate then pour 10 mL of the above medium inoculated with Bacillus subtilis spores suspension over the surface of the plate. Allow the plate to keep on surface until the agar solidifies and incubate at 37℃ ± 1℃ for 17 h ± 1 h.

After incubation, the test is considered positive if the sample shows inhibit zone on the plate with the same Rf value, by comparing with the inhibit of the enradine standrd solution.