SN

中华人民共和国出入境检验检疫行业标准

SN/T 0645—2014
代替 SN 0645—1997

出口肉及肉制品中敌草隆残留量的测定 液相色谱法

Determination of diuron residues in meat and meat products for export—Liquid chromatography method

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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准代替 SN 0645—1997《出口肉及肉制品中敌草隆残留量检验方法》。

与 SN 0645—1997 相比，主要技术变化如下：

——将原标准名称《出口肉及肉制品中敌草隆残留量检验方法》修订为《出口肉及肉制品中敌草隆残留量的测定　液相色谱法》；

——取消了原标准中的抽样和制样部分；

——将原标准的适用范围修订为：牛肉、午餐肉罐头、鸡肉丸子、羊肉、驴肉；

——对样品前处理条件进行了修订；

——对液相色谱条件进行了修订。

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：中华人民共和国山东出入境检验检疫局。

本标准主要起草人：李军民、白兴华、贺同欣、黄化成、耿金培、闫秋成。

本标准所代替标准的历次版本发布情况为：

——SN 0645—1997。

出口肉及肉制品中敌草隆残留量的测定 液相色谱法

1 范围

本标准规定了出口肉及肉制品中敌草隆残留量的液相色谱测定方法。

本标准适用于牛肉、午餐肉罐头、鸡肉丸子、羊肉、驴肉中敌草隆残留量的测定。

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 方法提要

试样中残留的敌草隆采用甲醇-乙腈溶液(1+1，体积比)提取，经液液分配和中性氧化铝柱净化后，用配有紫外检测器的高效液相色谱仪测定，外标法定量。

4 试剂和材料

除非另有规定，所用试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1 甲醇：液相色谱级。

4.2 乙腈：液相色谱级。

4.3 三氯甲烷：经全玻璃装置重蒸馏。

4.4 石油醚：沸程 60 ℃～90 ℃，经全玻璃装置重蒸馏。

4.5 无水硫酸钠：经 650 ℃灼烧 4 h，贮干燥器中备用。

4.6 氯化钠。

4.7 甲醇-乙腈溶液(1+1，体积比)：量取等体积的甲醇和乙腈，充分混合后备用。

4.8 甲醇-乙腈-水溶液(70+5+25，体积比)：按照体积比 70∶5∶25 量取甲醇、乙腈和水，充分混合，并脱气、过滤后备用。

4.9 饱和氯化钠溶液：在水中加入过量的氯化钠，加热并煮沸 10 min，直至氯化钠不再溶解为止，冷却至室温，冷却后的溶液用滤纸过滤，即为饱和氯化钠溶液。

4.10 敌草隆(diuron)标准品纯度大于或等于 98.0%。

4.11 标准储备液(100 mg/L)：精确称取 10.0 mg 敌草隆标准品，溶于甲醇-乙腈溶液(4.7)中，移入 100 mL 容量瓶，用甲醇-乙腈溶液(4.7)定容至刻度，混匀，0 ℃～4 ℃保存。

4.12 标准工作液：根据需要，吸取一定量的标准储备液(4.11)，用甲醇-乙腈-水溶液(4.8)稀释成系列标准工作液。

4.13 中性氧化铝柱：6 mL，1 000 mg，或相当者。使用前用 5 mL 甲醇-乙腈-水溶液(4.8)预淋洗。

4.14 无水硫酸钠柱：3 g 无水硫酸钠置于玻璃柱管中。

4.15 微孔滤膜：0.45 μm，有机相型。

5 仪器和设备

5.1 高效液相色谱仪：配有紫外检测器。
5.2 组织捣碎机。
5.3 研钵。
5.4 振荡器。
5.5 布氏漏斗。
5.6 分液漏斗：250 mL。
5.7 旋转蒸发器。
5.8 玻璃注射器：5 mL。

6 试样制备和保存

6.1 取有代表性样品 500 g，切碎后用组织捣碎机捣碎、混匀，分成两份，装入洁净容器内，密封并标识。
6.2 试样在－18 ℃下避光保存。在制样和保存过程中，应防止样品受到污染或发生残留物含量的变化。

7 测定步骤

7.1 提取

称取 10 g(精确至 0.01 g)试样于研钵中，再加入 10 g 无水硫酸钠及 5 mL 甲醇-乙腈溶液(4.7)。经充分研磨后，转移至 250 mL 具塞锥形瓶中，研钵用 50 mL 甲醇-乙腈溶液(4.7)分数次洗涤，洗涤液合并至同一具塞锥形瓶中。置于振荡器上振荡 20 min 后，提取液用布氏漏斗过滤至 250 mL 分液漏斗中。再用 15 mL 甲醇-乙腈溶液(4.7)分数次洗涤，合并滤液及洗液。分液漏斗中按照 35 mL、20 mL、20 mL 分次加入石油醚脱脂，每次振摇 1 min 后，静置分层，弃去石油醚层，然后往分液漏斗中加 22 mL 水及 3 mL 饱和氯化钠溶液，混匀。再分次加入 35 mL、20 mL、20 mL 三氯甲烷振摇提取，合并三氯甲烷提取液，并通过无水硫酸钠柱(4.14)脱水，再用 5 mL 三氯甲烷洗涤无水硫酸钠柱。合并三氯甲烷提取液和洗涤液于旋转蒸发瓶中，于 45 ℃水浴下旋转蒸发至近干，再用氮气吹干。残渣用 5 mL 甲醇-乙腈-水溶液(4.8)溶解。

7.2 净化

将玻璃注射器与中性氧化铝柱(4.13)的顶部相连，加 5 mL 甲醇-乙腈-水溶液(4.8)到注射器中，以流速 2 mL/min 通过中性氧化铝柱，弃去流出液。转入全部待净化液(7.1)于注射器中，通过中性氧化铝柱，收集流出液。再用 4 mL 甲醇-乙腈-水溶液(4.8)分数次旋转蒸发瓶并转入注射器中，合并流出液。用甲醇-乙腈-水溶液(4.8)定容到 10 mL，混匀，微孔滤膜(4.15)过滤后，供液相色谱分析。

7.3 液相色谱测定

7.3.1 液相色谱条件

液相色谱条件如下：

a) 色谱柱：Lichrospher C_{18}柱，25 cm×4.6 mm(内径)，填充物粒度 5 μm，或相当者；

b) 流动相：甲醇-乙腈-水溶液(4.8)；
c) 流速：0.7 mL/min；
d) 检测波长：235 nm；
e) 进样量：10 μL。

7.3.2 色谱测定

根据试样中待测物含量，选定峰面积相近的标准工作液，标准工作液和待测样液中敌草隆的响应值均应在仪器检测线性范围内。将系列标准工作液依次按上述色谱条件上机测定，记录色谱峰面积。以峰面积为纵坐标，浓度为横坐标，绘制标准曲线。根据保留时间定性，外标法定量。在上述色谱条件下，敌草隆的保留时间约为 3.87 min。标准品色谱图参见附录 A 中图 A.1。

7.4 空白试验

除不称取试样外，均按上述测定步骤进行。

8 结果计算和表述

用色谱数据处理软件或按式(1)分别计算试样中敌草隆残留含量，计算结果需扣除空白值：

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

式中：

X ——试样中敌草隆的含量，单位为毫克每千克(mg/kg)；

c ——标准曲线上查出试样溶液中敌草隆的浓度，单位为毫克每升(mg/L)；

V ——样液溶液最终定容体积，单位为毫升(mL)；

m ——最终样液所代表的试样量，单位为克(g)。

9 测定低限、回收率

9.1 定量限

本方法的定量限为 0.04 mg/kg。

9.2 回收率

冻牛肉、午餐肉罐头、鸡肉丸子、冻羊肉、冻驴肉中敌草隆的回收率数据见表 1。

表 1 敌草隆的添加回收率试验数据

样 品	添加浓度/(mg/kg)	回收率/%
冻牛肉	0.04	77.8～95.2
	0.05	80.4～97.4
	0.10	84.9～99.5
午餐肉罐头	0.04	80.0～98.5
	0.05	82.0～96.0
	0.10	89.1～100.1

表 1（续）

样　品	添加浓度/(mg/kg)	回收率/%
鸡肉丸子	0.04	77.8～99.0
	0.05	84.4～101.2
	0.10	87.9～99.9
冻羊肉	0.04	77.8～97.2
	0.05	80.8～97.4
	0.10	83.9～98.8
冻驴肉	0.04	77.2～96.5
	0.05	80.4～99.2
	0.10	87.2～95.5

附 录 A
（资料性附录）
敌草隆的液相色谱图

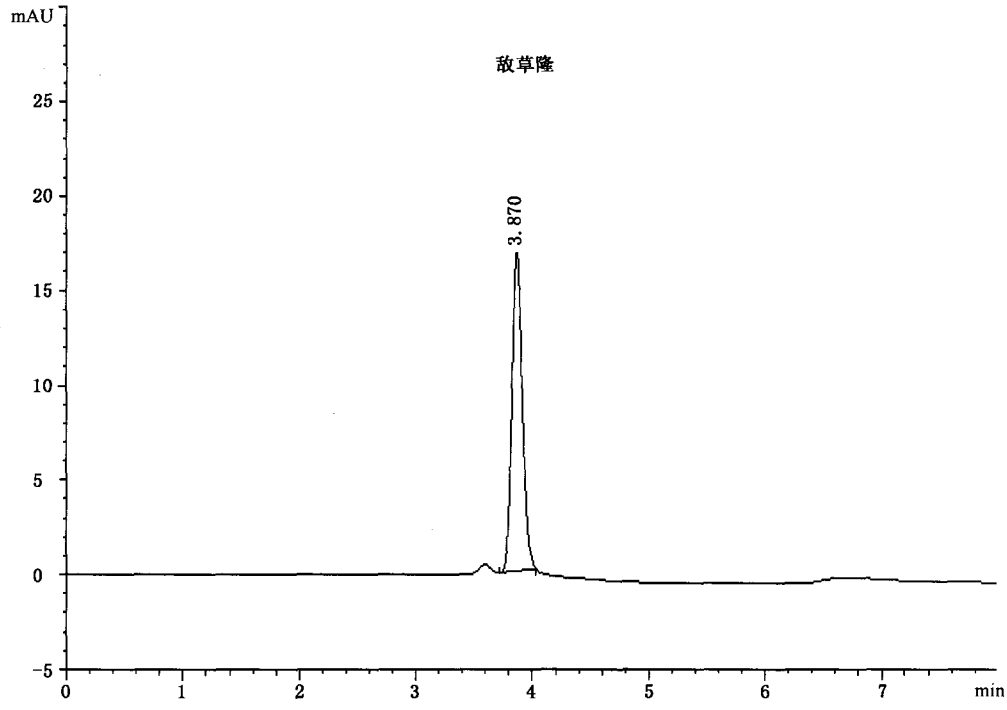


图 A.1 敌草隆标准溶液(试验浓度)的液相色谱图

Foreword

This standard was drafted according to GB/T 1.1—2009.

This standard replaces SN 0645—1997《Method for the determination of diuron residues in meats and meat products for export》.

Compared with SN 0645—1997, the main changes are as follows:

—The title is amended from "Method for the determination of diurons residues in meat and meat products for export" to be "Determination of diurons residues in meat and meat products for export—Liquid chromatography method";

—The part of sampling and sample preparation is deleted;

—The applicable scope of this standard are amended as "beef, canned pork, chicken ball, mutton meat and donkey meat";

—The sample pretreatment condition is modified;

—The LC operating condition is amend.

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

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

The main drafters of this standard are Li junmin, Bai Xinghua, He Tongxin, Huang Huacheng, Geng Jinpei and Yan Qiucheng.

This standard replaces the previous version of the release of the standard as follows:

—SN 0645—1997.

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

Determination of diuron residues in meat and meat products for export—Liquid chromatography method

1 Scope

This standard specifies the method of sampling, sample preparation and determination by liquid chromatography of diuron residues in meat and meat products .

This standard is applicable to the determination of diuron residues in frozen beef, canned pork, chicken ball, mutton, rabbit meat and donkey meat.

2 Normative references

The following documents are necessary for this standard. For dated references, only dated editions shall apply to this standard. For undated references, the latest edition of the normative document (including subsequent amendments) referred to applies.

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

3 Principle

Extracted the residues of diuron with methanol-acetontirile (1 + 1, *V*/*V*). After liquid-liquid distribution and cleaned up with an neutral aluminum oxide column, the residue is then detected by HPLC with UV-detector and quantified by external standard method.

4 Reagents and materials

Unless specified, all reagents should be analytical grade; "water" is the first grade prescribed by GB/T 6682.

4.1 Methanol: UV-grade.

4.2 Acetonitrile: UV-grade.

4.3 Chloroform: Redistilled in all-glass distilling system.

4.4 Petroleum ether: With boiling range of 60 ℃ ~90 ℃ , redistilled in all-glass distilling system.

4.5 Anhydrous sodium sulfate: Ignite at 650 ℃ for 4 h, and keep in a desiccator.

4.6 Sodium chloride.

4.7 Methanol-acetontirile (1 + 1, *V*/*V*): Prepare the same volume of methanol and acetontirile, mix well.

4.8 Methanol-acetontirile-water (70 + 5 + 25, *V*/*V*): Prepare 70 mL methanol, 5 mL acetontirile and 25 mL water, mix well, and filtrate.

4.9 Saturated sodium chloride solution: Add excessive sodium chloride to the water, by heating and boring for 10 minutes until the sodium chloride can not dissolve, cool down to room temperature, and filtrate. This is the saturated sodium chloride solution.

4.10 Diuron standard: Purity≥98.0%.

4.11 Diuron standard stock solution(100 mg/L): Accurately weigh 10.0 mg of diuron standard, dissolve in methonol-acetonitrile (4.7), prepare a solution of 100 mL as the standard stock solution, and store in 0 ℃～4 ℃.

4.12 Diuron standard working solution: According to the requirement, a standard working solution is prepared from the standard stock solution(4.11) with methanol-acetontirile-water(4.8).

4.13 Neutral aluminum oxide column: 6 mL, 1 000 mg. Before use, to elute the column with 5 mL methanol-acetontirile-water(4.8).

4.14 Anhydrous sodium sulphate column: Prepare 3 g anhydrous sodium sulphate in a glass column.

4.15 Filter membrane: 0.45 μm, nylon syringe filter.

5 Apparatus and equipment

5.1 High performance liquid chromatograph equipped with UV-detector.

5.2 High speed blender.

5.3 Mortar.

5.4 Shaker.

5.5 Busher funnel.

5.6 Separator funnel: 250 mL.

5.7 Rotary evaporator.

5.8 Glass injector: 5 mL.

6 Sample prepartion and storage

6.1 Collect ca 500 g the representative samples, the edible are cut up and mixed well with a tissure homogenizer, dived the prepared samples, seal in two clean containers and label.

6.2 The test sample should be stored below −18 ℃. 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.

7 Procedure of determination

7.1 Extraction

Weigh ca 10 g (accurately, 0.01 g) of the sample into a mortar, adding 10 g anhydrous sodium sulfate and 5 mL methanol-acetonitrile (1+1, *V/V*) (4.7), grind thoroughly, then transfer completely into a 250 mL conical flask (with stopper) with 50 mL of methanol- acetonitrile (1+1, *V/V*) (4.7) for several times. Shake for 20 min on a shaker, filtering the extract with Buchner funnel into 250 mL separatory funnel. Wash the flask and the Buchner funnel for several times with 15 mL in total of methanol-acetonitrle(1+1, *V/V*) (4.7). Combine the filtrate and the washing in the separatory funnel. Defat with 35 mL, 20 mL, 20 mL of petroleum ether respectively. Each time shake vigorously, and let stand for separating compensately, discard the petroleum ether phase. To the separatory funnel, add 22 mL of water and 3 mL of saturated sodium chloride solution, then extract with 35 mL, 20 mL, 20 mL of chloroform respectively. Collect the chloroform extract and let it pass through a column of anhydrous sodium sulfate (4. 14), then wash the column with 5 mL of chloroform, collect the chloroform extract again. Evaporate the dehydrated chloroform extract to nearly dryness with rotary evaporator in a water-bath below 45 ℃, blow the concentrate to dryness under nitrogen flow. Dissolve the residues by adding 5 mL of methanol-acetonitrile-water (70+5+25, *V/V*) (4.8).

7.2 Cleanup

Connect glass injector with the top of neutral aluminum oxide column(4.13), add 5 mL methanol-acetonitrile-water (70+5+25, *V/V*) (4.8) into the injector tube, make it pass through the column at the flow rate of 2 mL/min, discard all the effluents when it drains out. Then pass the above solution through the injector tube and the neutral aluminum oxide column as same as that described above, collect the effluents. Wash the flask and the column for several times with 4 mL in total of methanol-acetonitrile-water (70+5+25, *V/V*) (4.8). Combine the effluents and make up exactly to 10 mL with methanol-acetonitrile-water (70+5+25, *V/V*) (4.8). Mix well, and 0.45 μm filtrate (4.8) through a membrane filter for HPLC analysis.

7.3 Determination

7.3.1 HPLC condition

HPLC condition as follows:

a) Column: Lichrosphers C_{18} column or equivalent, 25 cm × 4.6 mm (i.d.). The mesh size of pack is 5 μm;

b) Mobile phase: Methanol-acetonitrile-water (4.8);

c) Flow rate: 0.7 mL/min;

d) Detection wavelength: 235 nm;

e) Injection volume: 10 μL.

7.3.2 HPLC determination

According to the estimated approximate concentration of herbicides in the sample solution, select the standard working solution with similar peak area to that of sample solution. The responses of each herbicide in the standard working solution and the sample solution should be within the linear range of the instrumental detection. Detect the series of standard working solution, record the peak area. Make the peak area as ordinate, and concentration as abscissa, plot the standard curve. Qualitative analysis according to the retention time, and quantified by external standard method. Under the above chromatographic condition, the retention time of diuron about 3.87 min. The chromatogram of the standard working solution is shown in chromatograms A.1 for Annex A.

7.4 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

Calculation the content of each herbicide residue in the test sample by HPLC data processor or according to formula (1):

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

Where:

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

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

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

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

9 Limit of determination and recovery

9.1 Limit of determination

The limit of quantification of this method is 0.04 mg/kg.

9.2 Recovery

According to the experimental data, the fortifying concentration of each herbicide in test sample and its corresponding recoveries are listed in table 1.

Table 1 The fortifying concentration of diuron in samples and its corresponding recoveries

Sample	Spiked level/(mg/kg)	Recovery/%
Frozen beef	0.04	77.8~95.2
	0.05	80.4~97.4
	0.10	84.9~99.5
Canned pork	0.04	80.0~98.5
	0.05	82.0~96.0
	0.10	89.1~100.1
Chicken ball	0.04	77.8~99.0
	0.05	84.4~101.2
	0.10	87.9~99.9
Lamb	0.04	77.8~97.2
	0.05	80.8~97.4
	0.10	83.9~98.8
Donkey meat	0.04	77.2~96.5
	0.05	80.4~99.2
	0.10	87.2~95.5

Annex A
(Informative)
Chromatogram of the standard

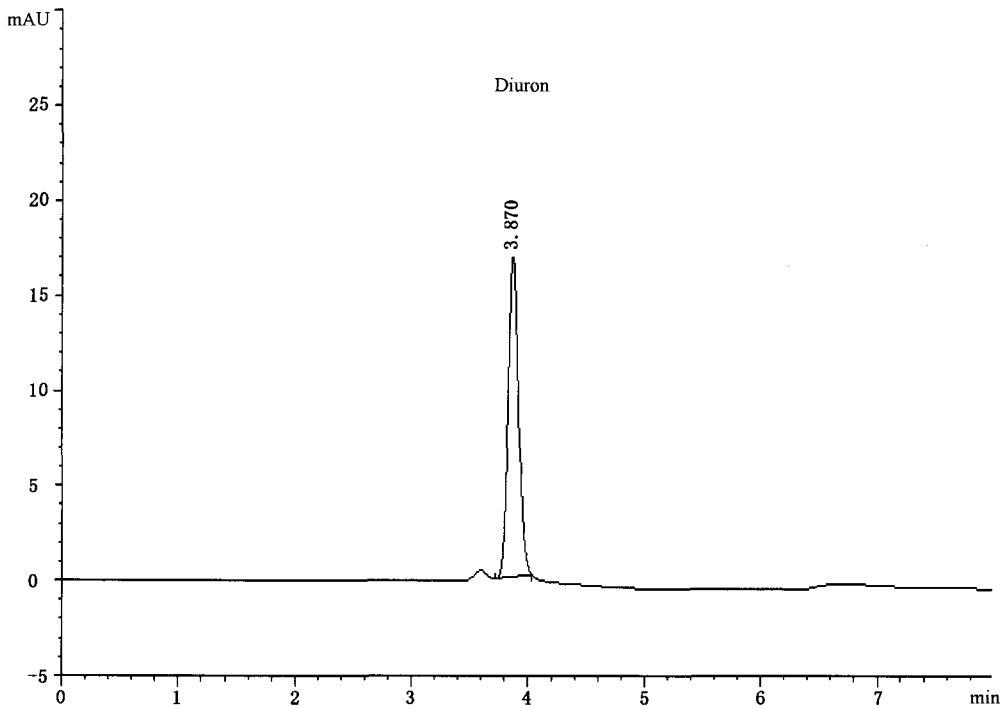


Figure A. 1 Liquid chromatogram of the diuron woking standard