SN

中华人民共和国出入境检验检疫行业标准

SN/T 0122—2011
代替 SN 0122—1992

进出口肉及肉制品中甲萘威残留量检验方法　液相色谱-柱后衍生荧光检测法

Determination of carbaryl residues in meat and meat products for import and export—HPLC-fluoresce detector with post column derivation

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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 0122—1992《出口肉及肉制品中甲萘威残留量检验方法》。

本标准与 SN 0122—1992 相比，主要技术变化如下：

——样品净化方法采用全自动凝胶渗透色谱净化方法替代原有的液液分配法；

——测定采用柱后衍生液相色谱-荧光检测法。

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：葛宝坤、赵孔祥、陈其勇、陈旭艳。

本标准所代替标准的历次版本发布情况为：

——SN 0122—1992。

进出口肉及肉制品中甲萘威残留量检验 方法　液相色谱-柱后衍生荧光检测法

1　范围

本标准规定了进出口肉及肉制品中甲萘威残留量的液相色谱-柱后衍生荧光检测法。

本标准适用于进出口牛肉、鸡肉、虾肉、鱼肉及火腿罐头中甲萘威残留量的测定。

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　制样

3.1　肉

将所取全部样品，充分搅碎混匀，取有代表性的样品，总量不少于500 g，装入清洁容器内，密封并标明标记。

3.2　罐头

将所取全部样品整罐倒出，充分搅碎混匀，取有代表性的样品，总量不少于500 g，装入清洁容器密封并标明标记。

4　试样保存

试样应于－18 ℃以下保存。在抽样和制样的操作中，应防止样品受到污染或发生含量的变化，以保证实验样品能代表总体样本。

5　原理

用丙酮-石油醚混合溶液提取样品中的甲萘威残留物，经凝胶层析柱净化后，浓缩，高效液相色谱分离，经柱后衍生后，用荧光检测器检测，外标法定量。

6　试剂和材料

本标准所用试剂和水在没有注明其他要求时，均指分析纯试剂，有机试剂为色谱纯和GB/T 6682中规定的三级水。

6.1　乙腈。

6.2　乙酸乙酯。

6.3 环己烷。
6.4 丙酮。
6.5 石油醚:沸程 30 ℃～60 ℃。
6.6 无水硫酸钠:650 ℃灼烧 4 h,在干燥器内冷却至室温,贮于密封瓶中备用。
6.7 氯化钠。
6.8 柱后衍生试剂。
6.8.1 氢氧化钠溶液(0.2%,质量浓度)。
6.8.2 邻苯二甲醛(O-Phthaladehyde,OPA)。
6.8.3 邻苯二甲醛稀释液:硼砂溶液(0.4%,质量浓度)。
6.8.4 巯基乙醇(Thiofluor)。
6.8.5 邻苯二甲醛试液的配制:溶剂储罐中注入 945 mL OPA 稀释剂,用惰性气体(氮气)吹扫至少 10 min,100 mg OPA 固体溶解于约 10 mL 的色谱纯甲醇中,将 OPA 溶液加入除氧的 OPA 稀释剂中,溶解 2 g 巯基乙醇固体于 5 mL OPA 稀释剂中,加入到储罐中,盖上瓶盖,打开气流,再不断的吹扫几分钟,关闭排气阀,轻轻地搅动溶剂以使其完全混合。
6.9 乙酸乙酯-环己烷混合溶液(1+1,体积比)。
6.10 0.45 μm 尼龙滤膜。
6.11 甲萘威:分子式 $C_{12}H_{11}NO_2$,CAS 编号 63-25-2,纯度大于 99.5%。
6.12 甲萘威标准储备液:准确称取适量的甲奈威标准品,以乙腈溶解,4 ℃冰箱保存,有效期为 6 个月。
6.13 甲萘威标准工作液:取一定量的标准储备液,用乙腈稀释至适当浓度,4 ℃冰箱保存,有效期为 1 周。

7 仪器和设备

7.1 液相色谱仪:配有荧光检测器和柱后衍生单元。
7.2 全自动凝胶色谱仪(配有馏分收集浓缩器)。
7.3 旋转蒸发装置。
7.4 氮吹仪。
7.5 组织匀浆机。
7.6 振荡器。

8 分析步骤

8.1 提取及净化

8.1.1 样品的提取

称取试样 20 g(精确到 0.01 g),于 100 mL 具塞三角瓶中,加水 6 mL(视样品水分含量加水使总水量约 20 g,肉通常在 70%左右,加水 6 mL),加 40 mL 丙酮,匀浆 1 min,加氯化钠 6 g,充分摇匀,再加 30 mL 石油醚,振摇 30 min。取 35 mL 有机层上清液,经无水硫酸钠滤于旋转蒸发瓶中,浓缩至约 1 mL,加 2 mL 乙酸乙酯-环己烷(6.9)溶液再浓缩,如此重复 3 次。乙酸乙酯-环己烷(6.9)定容为 5 mL,0.45 μm 滤膜过滤,待净化。

8.1.2 样品的凝胶色谱(GPC)净化

8.1.2.1 凝胶色谱净化条件

8.1.2.1.1 净化柱:400 mm×30 mm,Bio Beads S-X3,或相当者。

8.1.2.1.2 流动相:乙酸乙酯-环己烷(6.9)。
8.1.2.1.3 流速:5.0 mL/min。
8.1.2.1.4 样品定量环:5 mL。
8.1.2.1.5 馏分收集段:10.0 min～15.0 min。
8.1.2.1.6 净化柱平衡时间:5 min。

8.1.2.2 凝胶色谱浓缩条件

8.1.2.2.1 样品浓缩条件见表1。

表1 馏分浓缩条件

浓缩时间段	浓缩杯区域	温度 ℃	真空度 Torr
样品浓缩过程	1区	50	280
	2区	52	220
	3区	54	210
浓缩终点到达过程	1区	52	220
	2区	53	220

8.1.2.2.2 浓缩终点判定:液位传感模式。
8.1.2.2.3 溶剂替换:2 mL 乙腈,重复两次。
8.1.2.2.4 定容体积:1 mL。

8.1.2.3 凝胶色谱净化步骤

将5 mL待净化液按8.1.2.1和8.1.2.2规定的条件进行净化与浓缩,最后得到1 mL净化液待测定。

8.2 测定

8.2.1 液相色谱条件

8.2.1.1 色谱柱:C_{18},250 mm×4.6 mm×5 μm,或相当者。
8.2.1.2 柱温:42 ℃。
8.2.1.3 荧光检测器:λ_{ex}330 nm,λ_{em}465 nm。
8.2.1.4 流动相:乙腈+水(40+60,体积比)。
8.2.1.5 流速:1.0 mL/min。
8.2.1.6 进样量:20 μL。

8.2.2 柱后衍生

8.2.2.1 衍生试剂1:水解试剂,0.4%的氢氧化钠溶液,流速0.4 mL/min。
8.2.2.2 衍生试剂2:OPA试剂,流速0.4 mL/min。
8.2.2.3 反应器温度:水解温度为100 ℃,衍生温度为室温。

8.2.3 色谱测定

根据样液中被测甲萘威的含量情况,选定浓度相近的标准工作液,其响应值应在方法检测的线性范围内。在上述液相色谱条件下,甲萘威保留时间为11.1 min,色谱图参见图A.1。

8.2.4 空白试验

除不加试样外,均按上述步骤进行。

8.2.5 结果计算

按式(1)计算试样中甲萘威的含量：

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

式中：

X ——试样中甲萘威的含量，单位为毫克每千克(mg/kg)；

A ——试样中甲萘威的色谱峰面积；

c_s ——标准工作溶液中甲萘威的浓度，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——标准工作溶液中甲萘威的色谱峰面积；

m ——最终样液所代表的量，单位为克(g)。

9 测定低限、回收率

9.1 测定低限

本方法的测定低限：0.005 mg/kg。

9.2 回收率

肉及肉制品添加回收率见表2。

表2 肉及肉制品添加回收率

样品	添加水平 mg/kg	回收率范围 %	样品	添加水平 mg/kg	回收率范围 %
鱼肉	0.005	82.0～110.0	火腿	0.005	90.0～96.0
	0.010	89.0～107.0		0.010	100.0～114.0
	0.020	94.0～112.5		0.020	97.0～115.5
	0.050	90.0～105.6		0.050	101.6～113.6
	0.100	92.0～111.2		0.100	92.5～107.3
牛肉	0.005	82.0～98.0	虾肉	0.005	86.0～100.0
	0.010	86.0～99.0		0.010	85.0～96.0
	0.020	84.5～109.5		0.020	87.0～105.0
	0.050	90.6～98.0		0.050	88.6～94.6
	0.100	88.4～98.0		0.100	86.7～95.1
鸡肉	0.005	86.0～96.0			
	0.010	89.0～97.0			
	0.020	95.5～112.5			
	0.050	92.0～97.2			
	0.100	85.9～95.0			
	0.500	87.7～102.5			

附 录 A
（资料性附录）
甲萘威标准色谱图

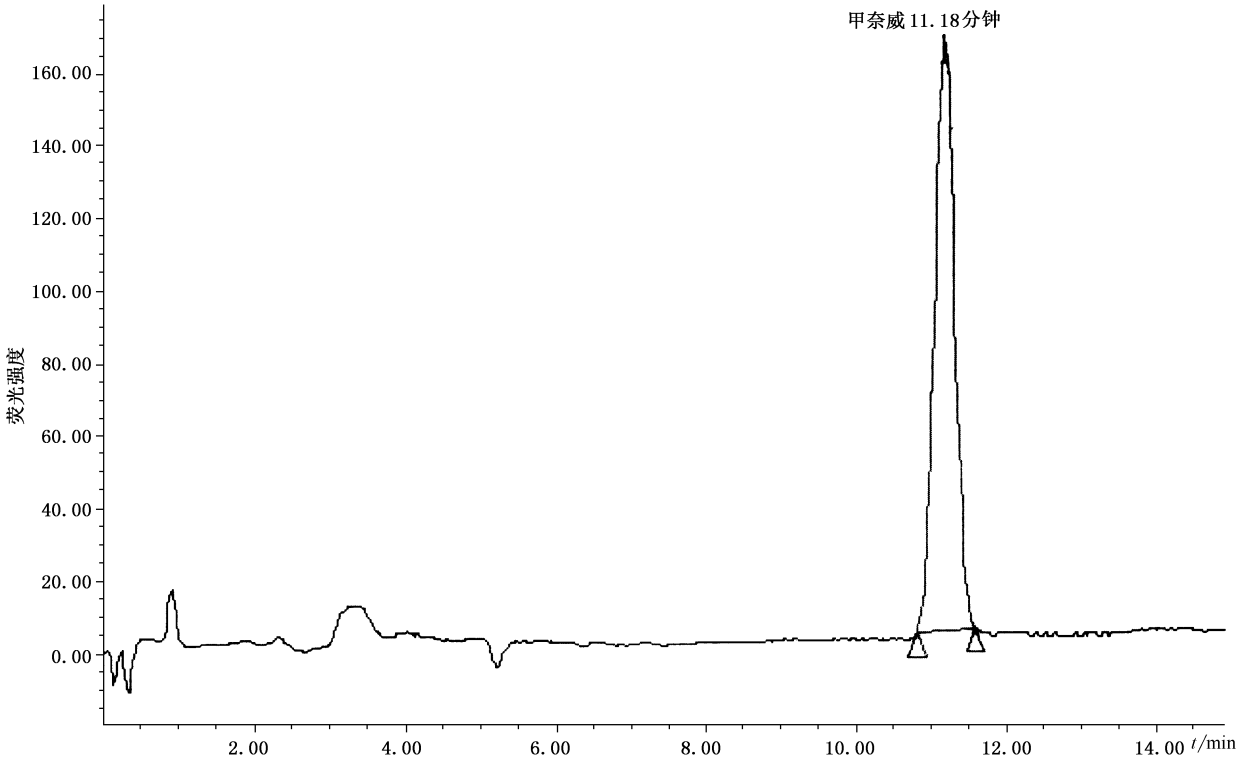

图 A.1 甲萘威标准的液相色谱图(0.1 μg/mL)

Foreword

This standard was drafted in accordance with the GB/T 1.1—2009.

This standard replaced SN 0122—1992 *Method for determination of carbaryl residues in meat for export*.

Compared with SN 0122—1992, this standard is of major modifications as follows:

—Modified the sample purification mothod for automatic gel chromatography;

—Changed the detection for liquid chromatography-fluresence detector with post column derivation.

This standard was proposed by and was under the charge of Certification and Acrreditation Adiministation of People's Republic of China.

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

This standard was mainly drafted by Ge Baokun, Zhao Kongxiang, Chen Qiyong, Chen Xuyan.

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

—SN 0122—1992.

Determination of carbaryl residues in meat and meat products for import and export—HPLC-fluoresce detector with post column derivation

1 Scope

The standard provides a determination method of cabaryl residues in meat and meat products for import and export by HPLC-fluoresce detector with post column derivation.

The standard is used to determine cabaryl residues in chicken, fish, beef, thrimp and ham for import and export.

2 Normative references

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

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

3 Sample

3.1 Meat

All samples should be mixing minced, the total amount of the representative sample should be not less than 500 g. The samplemust be placed into a suitable clean, sealed and marked container.

3.2 Canned Foods

All samples should be poured out the entire tank and mixing minced, the total amount of the representative sample should be not less than 500 g. The sample must be placed into a suitable clean, sealed and marked container.

4 Sample preservation

The specimen should be preserved below −18 ℃ and should not be contaminated. There should be no change in content of sample to ensure that experimental samples can represent the entire sample.

5 Principle

The carbaryl residues of the sample were extrated by acetone-petroleum and purified by gel permeation chromatography. After concentrated，the residues were determined by high performance liquid chromatography with fluorescence detector and the set of post-column derivatizaion.

6 Reagents and materials

Unless specified，all the reagents used should be analytical grade，and “water” is the third grade water which is defiened in GB/T 6682—2008.

6.1 Acetonitril.

6.2 Ethyl acetate.

6.3 Cyclohexane.

6.4 Acetone.

6.5 Petroleum ether，boiling point ranges from 30 ℃～60 ℃.

6.6 Anhydrous sodium sulfate. Heat at 650 ℃ for 4 h，cool in desiccator and store in an amber bottle.

6.7 Sodium chloride.

6.8 Post-Column Derivatization reagent.

6.8.1 Sodium hydroxide solutions（0.2%，m/V）.

6.8.2 O-Phthaladehyde（OPA）.

6.8.3 O-phthalaldehyde diluent (Borax Solution, 0.4%, m/V).

6.8.4 Thiofluor.

6.8.5 Solution of OPA: 945 mL OPA dilution was poured into the solvent tank, then nitrogen purging for 10 min at least. 100 mg OPA was dissolved in about 10 mL chromatographic grade methanol, then turn off the gas and strip the bottle cap. The OPA solution was added into the OPA dilution. 2 g mercaptoethanol was dissolved in 5 mL OPA dilution and added into the solution tank, close the bottle cap and turn on the gas, then nitrogen purging for several minutes and turn off the vent valve. Stir slowly to mix the solution completely.

6.9 Cyclohexane-ethyl acetate (1+1, V/V).

6.10 Nylon fliter, 0.45 μm.

6.11 Carbaryl: Molecular formula $C_{12}H_{11}NO_2$, CAS No. 63-25-2, purity of greater than 99.5%.

6.12 Storage standard solution: Accurate weigh the right amount of carbaryl standard, dissolve it in acetonitrile, and storage in 4 ℃ refrigerator. The solution is valid for 6 months.

6.13 Work standard solution. Dilute the storage solution with acetonitrile to Appropriate concentration. This solution is valid in one week.

7 Instrucments

7.1 Liquid chromatography: Fluorescence detector and the unit of post-column derivation.

7.2 Automated gel permeation chromatography (with fraction collection with concentrator).

7.3 Rotary evaporation.

7.4 Nitrogen blowing Instrument.

7.5 Homogenate machine.

7.6 Electronic oscillator.

8 Experimention

8.1 Extraction and purification performance

8.1.1 Extraction

A 20 g sample of meat product was weighted out exactly into a 100 mL conical flask with stopper,

6 mL water and 40 mL acetone were added. After homogenized for 1 min ,6 g NaCl was added,the mixture was shaken vigorously with 30 mL petroleum ether for 30 min. 35 mL organic layer was filtered through anhydrous sodium sulfate. The filtrate was collected in a flask and evaporated to approximately 1 mL with a rotary evaporator. Then 2 mL ethyl acetate-cyclohexane (1+1, V/V) was added into the flask and evaporated,the performance was repeated for 3 times. The residue obtained was dissolved in 5 mL ethyl acetate-cyclohexane (1+1, V/V),and then filtered through a 0.45 μm filter before GPC purification.

8.1.2 GPC purification

8.1.2.1 GPC purification conditions

8.1.2.1.1 Purification column:400 mm×30 mm,Bio Beads S-X3,or the comparative.

8.1.2.1.2 Mobile phase:Ethyl acetate-cyclohexane (1+1, V/V).

8.1.2.1.3 Flow:5.0 mL/min.

8.1.2.1.4 Sample loop:5 mL.

8.1.2.1.5 Collection:10.0 min～15.0 min.

8.1.2.1.6 Equilibrium time:5 min.

8.1.2.2 GPC concentration conditions

8.1.2.2.1 GPC concentration conditions was shown in table 1.

Table 1—Evaporation conditions

Evaporation session	Zone	Heating rate ℃	Vacuum Torr
evaporation	1	50	280
	2	52	220
	3	54	210
endpoint	1	52	220
	2	53	220

8.1.2.2.2 Endpoint judgment: Level sensor mode.

8.1.2.2.3 Solution exchange: 2 mL acetonitrile, repeated for 2 times.

8.1.2.2.4 Constant volume: 1 mL.

8.1.2.3 Procedure of GPC purification

5 mL residue obtained from the extraction procedure was purified and concentrated through the method mentioned in 8.1.2.1 and 8.1.2.2, then 1 mL purified solution was obtained for further HPLC analysis.

8.2 HPLC Analysis

8.2.1 HPLC conditions

8.2.1.1 Column: C_{18}, 250 mm × 4.6 mm × 5 μm, or the comparative.

8.2.1.2 Column temperature: 42 ℃.

8.2.1.3 FLD: λ_{ex} 330 nm, λ_{em} 465 nm.

8.2.1.4 Mobile phase: Acetonitrile-water (40 + 60, *V*/*V*).

8.2.1.5 Flow: 1.0 mL/min.

8.2.1.6 Volume of injection: 20 μL.

8.2.2 Post-column derivatization

8.2.2.1 Derivative Reagent 1: Hydrolytic reagent, 0.4% NaOH, Flow 0.4 mL/min.

8.2.2.2 Derivative Reagent 2: OPA reagent, Flow: 0.4 mL/min.

8.2.2.3 Reactor temperature: Hydrolysis temperature, 100 ℃; Derivatization temperature, Room temperature.

8.2.3 HPLC quantification

Choose appropriate standard solution according to the amount of carbaryl in sample. Retention time of carbaryl was about 11.1 min under the conditions mentioned above. Chromatogram of carbaryl was shown in annex A Figure A.1.

8.2.4 Blank trial

Performed through the above procedure with blank sample.

8.2.5 Calculation

The amount of carbaryl in sample was calculated through formula (1):

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

Where

X —Amount of carbaryl in sample, mg/kg;

A —Peak area of carbaryl;

c_s —Concentration of carbaryl in the standard curves, μg/mL;

V —Final volume of the carbaryl residue, mL;

A_s—Peak area of carbaryl in the standard solution;

m —Amount of the sample, g.

9 Limit of detection and rocoveries

9.1 Limit of detection (LOD)

LOD of the method was 0.005 mg/kg.

9.2 Recoveries

Recoveries of carbaryl residues in meat and meat products see table 2.

Table 2—Recoveries of carbaryl residues in meat and meat products

Sample	Fortified level mg/kg	Recoveries %	Sample	Fortified level mg/kg	Recoveries %
fish	0.005	82.0～110.0	ham	0.005	90.0～96.0
	0.010	89.0～107.0		0.010	100.0～114.0
	0.020	94.0～112.5		0.020	97.0～115.5
	0.050	90.0～105.6		0.050	101.6～113.6
	0.100	92.0～111.2		0.100	92.5～107.3
beef	0.005	82.0～98.0	shrimp	0.005	86.0～100.0
	0.010	86.0～99.0		0.010	85.0～96.0
	0.020	84.5～109.5		0.020	87.0～105.0
	0.050	90.6～98.0		0.050	88.6～94.6
	0.100	88.4～98.0		0.100	86.7～95.1
chicken	0.005	86.0～96.0			
	0.010	89.0～97.0			
	0.020	95.5～112.5			
	0.050	92.0～97.2			
	0.100	85.9～95.0			
	0.500	87.7～102.5			

Annex A
（Informative）
Chromatogram of carbaryl standard

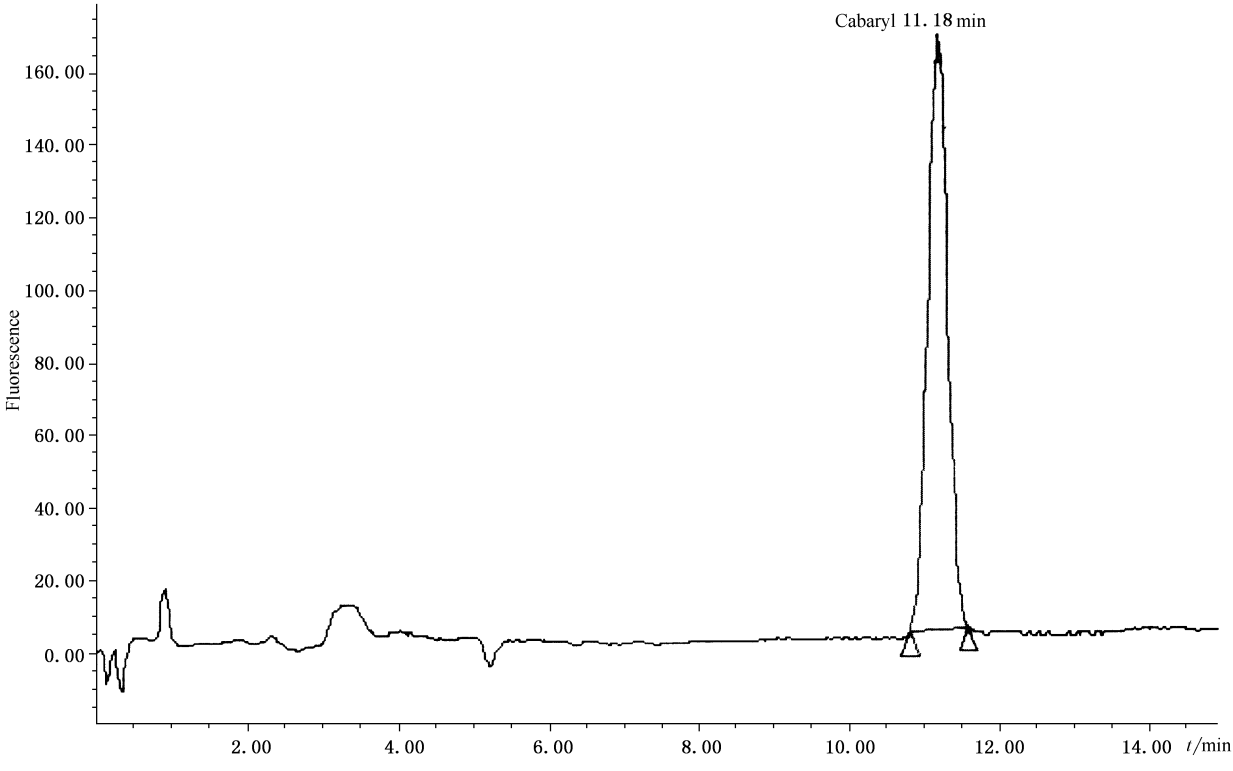

Figure A.1—Chromatogram of carbaryl standard（0.1 μg/mL）