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中华人民共和国出入境检验检疫行业标准

SN/T 1753—2006

进出口浓缩果汁中噻菌灵、多菌灵残留量检测方法 高效液相色谱法

Determination of thiabendazole and carbendazim residues in concentrated fruit juice for import and export—High performance liquid chromatographic method

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前　言

本标准的附录A、附录B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。

本标准负责起草单位：中华人民共和国广西出入境检验检疫局。

本标准参加起草单位：中华人民共和国北京出入境检验检疫局。

本标准主要起草人：刘晓松、高欣、郑玲、李丽华、徐超一、罗兆飞。

本标准系首次发布的出入境检验检疫行业标准。

进出口浓缩果汁中噻菌灵、多菌灵残留量检测方法 高效液相色谱法

1 范围

本标准规定了浓缩果汁中噻菌灵、多菌灵检验的制样和高效液相色谱测定方法。

本标准适用于浓缩苹果汁、浓缩菠萝汁、浓缩芒果汁、浓缩橙汁、浓缩梨汁和浓缩刺梨汁中噻菌灵、多菌灵残留量的检测。

2 制样

2.1 样品的制备

取适量浓缩果汁样品，混合均匀后取样。冷冻状态下的浓缩果汁样品，应自然解冻至室温状态，使呈流体状后混合均匀取样。

2.2 样品的保存

需要短期保存的样品，应密封完好，置 4℃冰箱冷藏室中储存备用；需要长期保存的样品，应置于—20℃以下冰箱中储存。

在样品的保存和制样过程中，应防止样品受到污染或发生噻菌灵和多菌灵含量的变化。

3 测定方法

3.1 方法提要

样品用水按一定比例稀释后，经过调 pH、离心、过滤，用混合相固相萃取小柱(Mixed-mode SPE)进行提取、净化，用配有二级管阵列检测器(DAD)的液相色谱仪检测，外标法定量。

3.2 试剂和材料

3.2.1 水为超纯水，其余试剂除非特别说明，均为分析纯。

3.2.2 甲醇：色谱纯。

3.2.3 乙腈：色谱纯。

3.2.4 磷酸二氢钠。

3.2.5 磷酸氢二钠。

3.2.6 氨水(30%，7.5 mol/L)。

3.2.7 氨水(0.15 mol/L)：2 mL 氨水溶于 100 mL 水中。

3.2.8 氨水(0.15 mol/L)＋甲醇溶液(7＋3)：混合 30 mL 甲醇、2 mL 7.5 mol/L 氨水，以水定容至 100 mL。

3.2.9 氨水(0.3 mol/L)＋甲醇溶液：4 mL 7.5 mol/L 氨水溶于 100 mL 甲醇中。

3.2.10 盐酸(0.1 mol/L)溶液：量取 9 mL 盐酸，加适量水并稀释到 1 000 mL。

3.2.11 氢氧化钠(2 mol/L)溶液：吸取 11 mL 澄清的氢氧化钠饱和溶液，加适量新煮沸过的冷水至 100 mL，摇匀。

3.2.12 磷酸盐缓冲液(0.02 mol/L，pH6.8)：1.38 g 磷酸二氢钠和 1.41 g 磷酸氢二钠溶于 900 mL 水中，用稀氢氧化钠或稀磷酸调 pH＝6.8，定容至 1 000 mL。

3.2.13 混合相固相萃取小柱(Mixed-mode SPE)：Oasis MCX 6 mL，150 mg；或相当者。使用前预先

顺序用 2 mL 甲醇、3 mL 0.15 mol/L 氨水(3.2.7)进行活化。

3.2.14 聚丙烯塑料离心管:50 mL。

3.2.15 两相滤膜:0.45 μm。

3.2.16 噻菌灵标准品:纯度大于 99%。

3.2.17 多菌灵标准品:纯度大于 99%。

3.2.18 噻菌灵、多菌灵标准储备液:称取噻菌灵、多菌灵标准品各 10.0 mg,用甲醇溶解并定容到 100 mL,浓度为 100 μg/mL(标准储备液在−20℃冷冻条件下可以储存 4 个月)。

3.2.19 噻菌灵、多菌灵标准工作液:用上述标准储备液(3.2.18),根据需要用流动相配制成适当浓度的标准工作液。所有标准工作液均需在冷藏条件下储存(每周配制)。

3.3 仪器和设备

3.3.1 高效液相色谱仪:配有二级管阵列检测器(DAD)。

3.3.2 pH 计。

3.3.3 固相萃取装置。

3.3.4 离心机:最大转速 10 000 r/min。

3.3.5 微孔膜过滤器。

3.3.6 氮吹仪。

3.4 测定步骤

3.4.1 提取

称取 10.0 g 浓缩果汁,加水稀释,振荡混匀并定容至 100 mL。将该稀释液转移到 250 mL 锥型瓶中,用 2 mol/L 氢氧化钠调 pH=10 后,倒入 50 mL 聚丙烯塑料离心管中以 8 000 r/min 速度离心 10 min,吸取上清液备用。(如果样液中组份浓度超出线性范围,则做适当稀释。)

3.4.2 净化

准确吸取 10.0 mL 离心上清液上混合相固相萃取小柱(3.2.13)。依次用 2 mL 0.15 mol/L 氨水(3.2.7)、2 mL 氨水(0.15 mol/L)-甲醇溶液(3.2.8)、2 mL 0.1 mol/L 盐酸(3.2.10)、3 mL 甲醇淋洗小柱,弃去淋洗流出液,整个淋洗过程的流速控制在 3 mL/min 以内。最后用 3 mL 氨水(0.3 mol/L)-甲醇溶液(3.2.9)洗脱柱子,收集洗脱液,于 45℃水浴用微弱的氮气吹干。用 1.0 mL 流动相[3.4.3.1 中的 b)]溶解残渣,经 0.45 μm 滤膜过滤后,供液相色谱测定用。

3.4.3 测定

3.4.3.1 液相色谱条件

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

b) 流动相:磷酸盐缓冲液(3.2.12)+乙腈(72.5+27.5),使用前经 0.45 μm 滤膜过滤;

c) 流速:1.0 mL/min;

d) 检测波长:288 nm;

e) 柱温:室温;

f) 进样量:20 μL。

3.4.3.2 色谱测定

分别取净化后的样液和标准溶液进行 HPLC 分析,以标准溶液峰的保留时间和该标准溶液峰在二级管阵列检测器(DAD)上呈现的紫外光谱特征(参见附录 A)为依据进行定性,以其峰高求出样液中被测物质的含量,供计算。在上述色谱条件下,多菌灵的保留时间为 5.0 min,噻菌灵的保留时间为 6.4 min,多菌灵、噻菌灵标准溶液的高效液相色谱图参见附录 B。

3.4.3.3 空白试验

除不加试样外,按上述测定步骤进行。

3.5 结果计算和表述

按式(1)计算试样中噻菌灵或多菌灵含量，计算结果应扣除空白值：

$$X = \frac{h \cdot c_S \cdot V_3}{h_S \cdot m \cdot (V_2/V_1)} \quad \cdots\cdots(1)$$

式中：

X——试样中噻菌灵或多菌灵的含量，单位为毫克每千克(mg/kg)；

h——样液中噻菌灵或多菌灵的峰高；

c_S——标准溶液中噻菌灵或多菌灵的浓度，单位为微克每毫升(μg/mL)；

V_3——试样净化液最后定容体积；

h_S——标准溶液中噻菌灵或多菌灵的峰高；

m——称取的试样量，单位为克(g)；

V_2——净化用提取液体积，单位为毫升(mL)；

V_1——试样提取液总体积，单位为毫升(mL)。

4 测定低限、回收率

4.1 测定低限：本方法对噻菌灵、多菌灵的测定低限为 0.020 mg/kg。

4.2 回收率：噻菌灵标准添加浓度在 0.02 mg/kg～0.20 mg/kg 范围内，回收率为 75.7%～93.3%；多菌灵标准添加浓度在 0.02 mg/kg～0.20 mg/kg 范围内，回收率为 80.8%～99.2%。

附 录 A
（资料性附录）
多菌灵、噻菌灵的光谱特征参考图

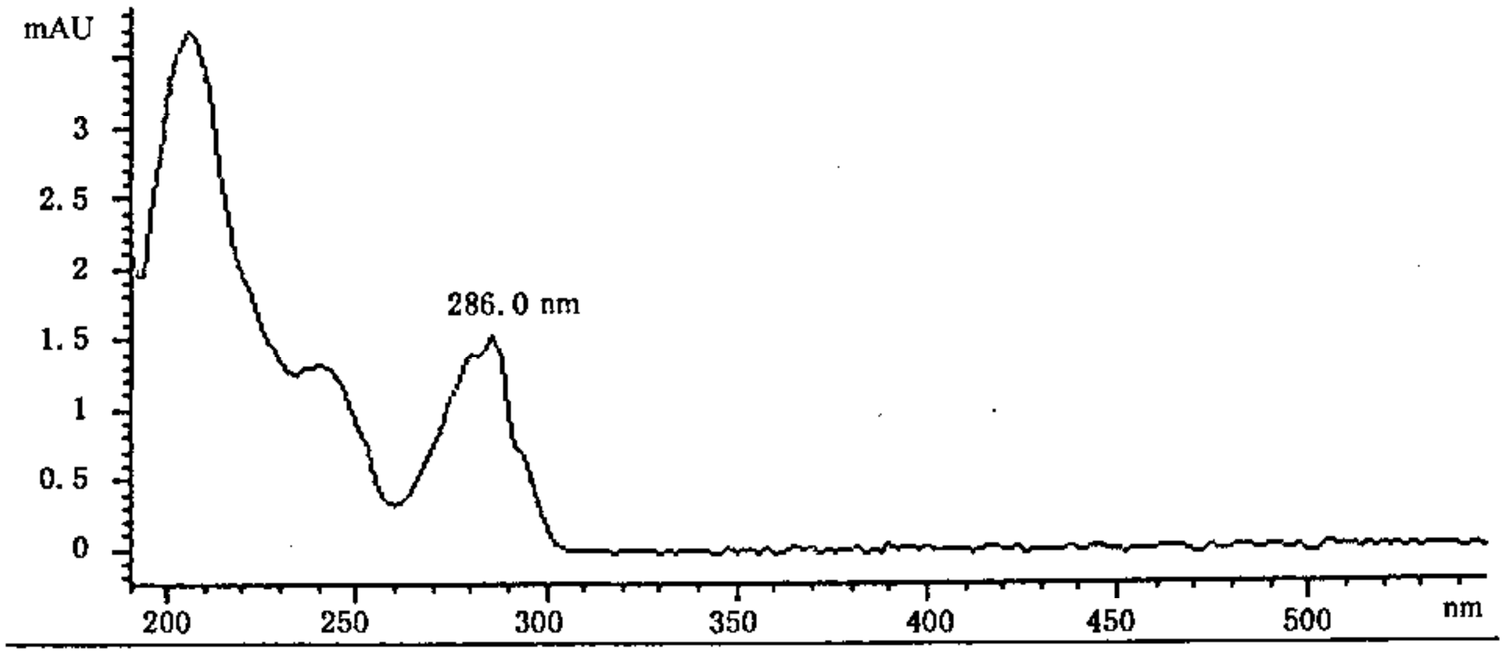


图 A.1 多菌灵(Carbendazim)紫外光谱特征图

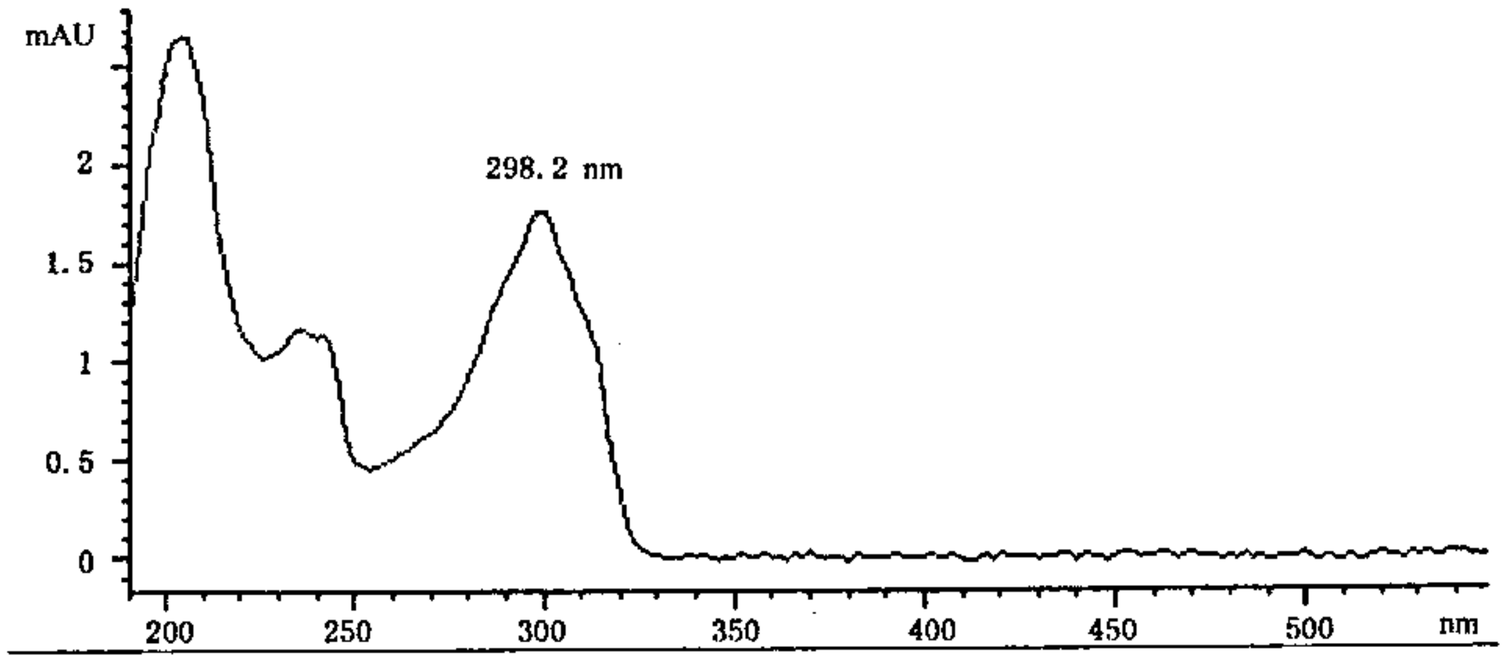
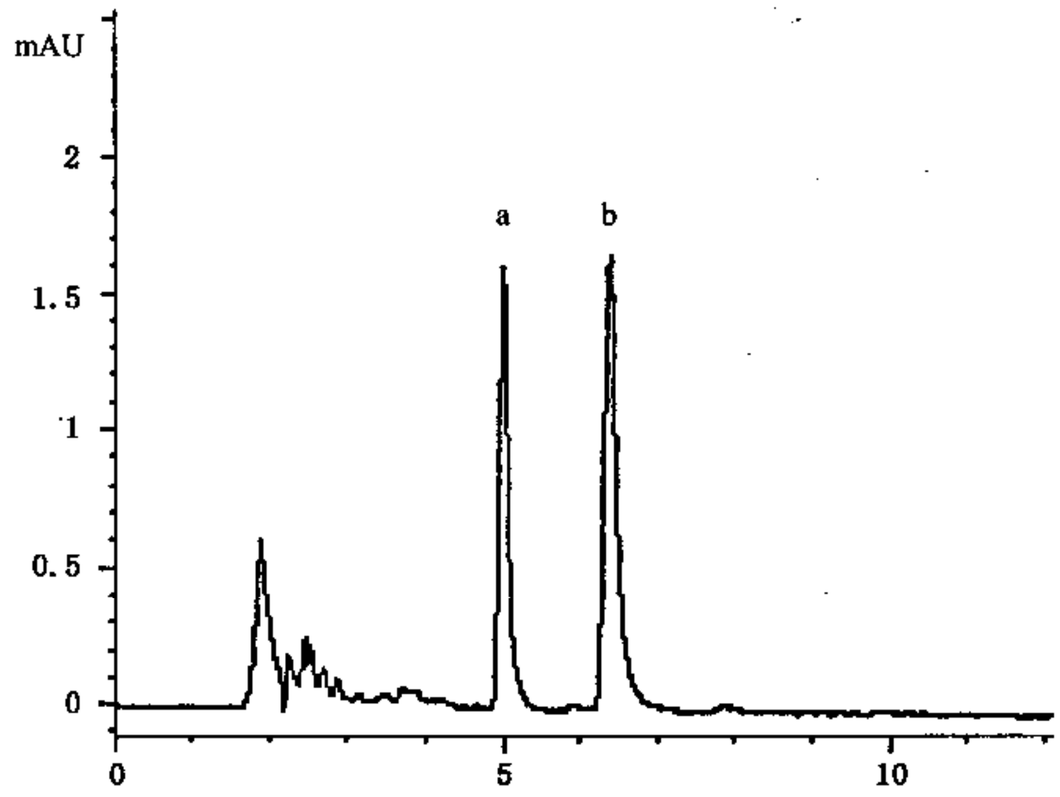


图 A.2 噻菌灵(Thiabendazole)紫外光谱特征图

附 录 B
（资料性附录）
多菌灵、噻菌灵标准溶液高效液相色谱图



a——多菌灵；
b——噻菌灵。

图 B.1 多菌灵(Carbendazim)及噻菌灵(Thiabendazole)标准溶液色谱图

Foreword

Annex A and Annex B of this standard is for information only.

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

This standard was drafted by Guangxi and Beijing Entry—Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Liu Xiaosong, Gao Xin, Zheng Ling, Li Lihua, Xu Chaoyi, Luo Zhaofei.

This standard is a professional standard promulgated for the first time.

Determination of thiabendazole and carbendazim residues in concentrated fruit juice for import and export — High performance liquid chromatographic method

1 Scope

This standard specifies the method of sample preparation and the methods of determination by high-performance liquid chromatography(HPLC) of Thiabendazole and Carbendazim residues in concentrated fruit juice.

This standard is applicable to the determination of Thiabendazole and Carbendazim residues in concentrated apple、pineapple、mango、orange、pear and cili juices.

2 Sampling and sample preparation

2.1 Preparation of test sample

Take a potion of well-sealed concentrated juice sample, sampling after mixing thoroughly. If samples are refrigerated, they must be equilibrated to room temperature and well mixed before testing

2.2 Storage of test sample

Seal the sample well. The sample for short time must be kept at 4℃, and for the long-term at −20℃.

In the course of sampling and sample preparation precaution must be taken to avoid contamination or any factors which may cause the change of Thiabendazole and Carbendazim residue content.

3 Method of determination

3.1 Principle

Dilute the sample with an amount of distilled water. After centrifuging, filtering and adjusting the pH-value, extract and clean up the sample with Mixed-mode SPE, and determinate the amount of Thiabendazole and Carbendazim residues by high-performance liquid chromatography (HPLC) with diode array detector (DAD) using external standard.

3.2 Reagents and materials

3.2.1 Water is super pure-water, the other reagents are analytical grade except for special expla-

nation.

3.2.2 Methanol: HPLC grade.

3.2.3 Acetonitrile: HPLC grade.

3.2.4 Sodium phosphate monobasic monohydrate.

3.2.5 Sodium phosphate dibasic.

3.2.6 NH_4OH (30%, 7.5 mol/L).

3.2.7 NH_4OH (0.15 mol/L): Dilute 2 mL 7.5 mol/L NH_4OH with water to 100 mL.

3.2.8 NH_4OH (0.15 mol/L) in methanol-water (30+70): Mix 30 mL methanol and 2 mL 7.5 mol/L NH_4OH with water to make 100 mL solution.

3.2.9 NH_4OH (0.3 mol/L): Dilute 4 mL 7.5mol/L NH_4OH with water to 100 mL.

3.2.10 HCl (0.1 mol/L): Dilute 9 mL Hydrochloric acid to 100 mL with water.

3.2.11 NaOH (2 mol/L): Draw 11 mL clear saturated NaOH solution, dilute to 100 mL with boiled water and shake up.

3.2.12 Phosphate buffer (0.02 mol/L, pH 6.8): Dissolve 1.38 g sodium phosphate monobasic monohydrate and 1.41 g sodium phosphate dibasic in 900 mL water, and adjust to pH 6.8 with dilute NaOH or H_3PO_4 as necessary. Then adjust volume to exactly 1 000 mL.

3.2.13 Mixed-mode SPE cartridge: Oasis MCX 6 mL, 150 mg, or the similar. The SPE cartridge is conditioned sequentially with 2 mL of methanol, 3 mL of 0.15 mol/L aqueous ammonia before using.

3.2.14 Polypropylene plastic centrifuge tube: 50 mL.

3.2.15 Filter film: 0.45 μm.

3.2.16 Thiabendazole standard: Purity above 99%.

3.2.17 Carbendazim standard: Purity above 99%.

3.2.18 Thiabendazole and Carbendazim standard stock solution: Accurately weigh 10.0 mg of Thiabendazole and Carbendazim standard, dissolve in methanol and prepare a solution in a 100 mL volumetric flask, and the concentration is 100 μg/mL (The standard stock solution can store 4 months under the condition of −20℃ refrigeration).

3.2.19 Thiabendazole and Carbendazim standard working solution: Use the above-mentioned standard stock solution, according to the requirement, prepare a standard working solution of appropriate concentration. All standards working solution need to be store under refrigerate condition. (Prepared every week).

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatography(HPLC): With the diode array detector(DAD).

3.3.2 pH meter.

3.3.3 SPE instrument.

3.3.4 Centrifuge: Max. 10 000 r/pm.

3.3.5 Microporous membrane filter.

3.3.6 Nitrogen evaporator

3.4 Procedure

3.4.1 Extraction

Weigh 10.0 g of the concentrated juice, and add 10 mL of water. Dilute to 100 mL with water and mix well. Transfer the diluent into the 250 mL erlenmeyer flask and adjust pH = 10 with 2 mol/L NaOH. Transfer the sample solution into a 50 mL polypropylene plastic tube and centrifuge for 10 min at 8 000 r/min. Decant the supernatant liquid. (If the contents of Thiabendazole or Carbendazim in test sample are above linear range, dilution should be adopted.)

3.4.2 Cleanup

Load 10 mL of the supertanant liquid and sequentially wash the SPE cartridge with 2 mL of 0.15 mol/L aqueous ammonia, 2 mL of methanol/0.15 mol/L aqueous ammonia (30:70), 2 mL of 0.1 N HCl and 3 mL of methanol; Maintain at the flow rate of below 3 mL/min for all steps. Discard all of filtrate. Elute the SPE cartridge with 3 mL of methanol/ 0.3 mol/L aqueous ammonia. The eluate is evaporated to dryness with a weak stream of nitrogen gas at 45 ℃ ,and the residue is dissolved in 1.0 mL mobile phase. Filter with 0.45 μm filter membrane, the solution is ready for liquid chromatographic determination.

3.4.3 Determination

3.4.3.1 LC operating conditions

a) Chromatographic column: Agilent Zorbax C_{18} column, 250 mm × 4.6 mm(i.d.),5 μm, or the similar.
b) Mobile phase: Phosphate buffer (3.2.12) + acetonitrile (72.5 + 27.5), filtered with 0.45 μm filter membrane.
c) Flow rate of mobile phase: 1.0 mL/min.
d) Wave length of UV detector:288 nm.
e) Column temperature: Room temperature.
f) Injection volume :20 μL.

3.4.3.2 LC determination

LC determination is performed by separately injecting 20 μL of the standard working solution and the sample solution into the LC system. Identify by comparing peak retention time of the sample with corresponding standard peak retention time and by comparing peak violet-spectrum characteristics (See annex A) displaying on DAD. Calculate the result by comparing peak height of the sample with corresponding standard peak height, using external standard method.
Under the above LC conditions, the retention time of Carbendazim is about 5.0 min, the retention time of Thiabendazole is about 6.4 min. The chromatogram of Thiabendazole and Carbendazim standard solution see annex B.

3.4.3.3 Blank test

Perform the blank test with the same procedures as that described in the method of determination , but without addition of test sample.

3.4.3.4 Calculation and expression of results

The calculation of results is according to formula(1), the blank value shall be subtracted from the above result of calculation:

$$X = \frac{h \cdot c_S \cdot V_3}{h_S \cdot m \cdot (V_2 / V_1)} \quad \cdots\cdots(1)$$

Where

X——the residue content of Thiabendazole or Carbendazim, mg/kg;

h——the peak height of Thiabendazole or Carbendazim of the sample solution;

c_S——the concentration of Thiabendazole or Carbendazim in the standard working solution, μg/mL;

V_3——the final volume of sample solution;

h_S——the peak height of Thiabendazole or Carbendazim of the standard working solution;

m—— the mass of test sample, g;

V_2—— the volume of purified extraction, mL;

V_1——the total volume of extraction, mL;

4 Limit of determination and recovery

4.1 Limit of determination: According to the experimental data, the limit of determination of Thiabendazole and Carbendazim is 0.020 mg/kg respectively.

4.2 Recovery: The recovery of Thiabendazole are 75.7%～93.3% when the fortifying concentrations of Thiabendazole are between 0.02 mg/kg～0.20 mg/kg. The recovery of Carbendazim are 80.8%～99.2% when the fortifying concentrations of Carbendazim are between 0.02 mg/kg～0.20 mg/kg.

Annex A
(informative annex)
The references of violet-spectrum characteristics of Thiabendazole and Carbendazim

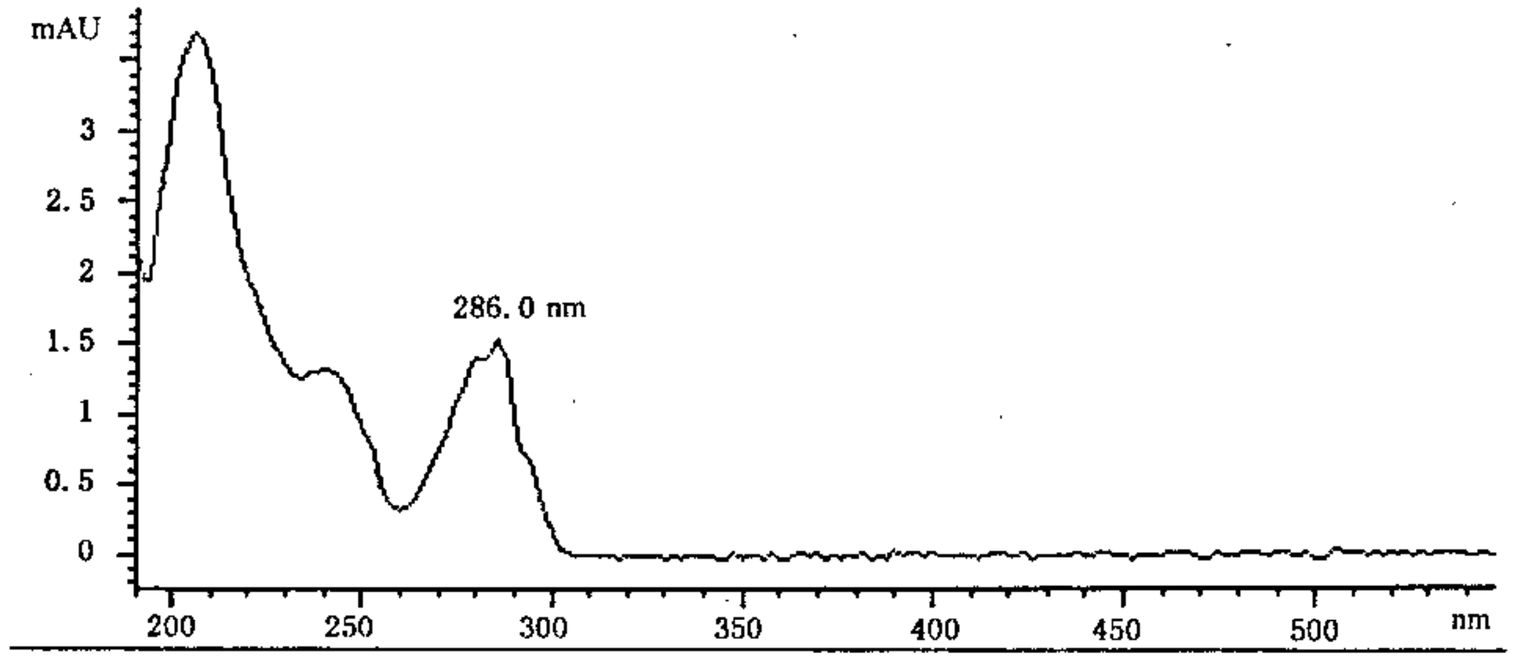


Figure A.1 The violet-spectrum characteristics of Carbendazim

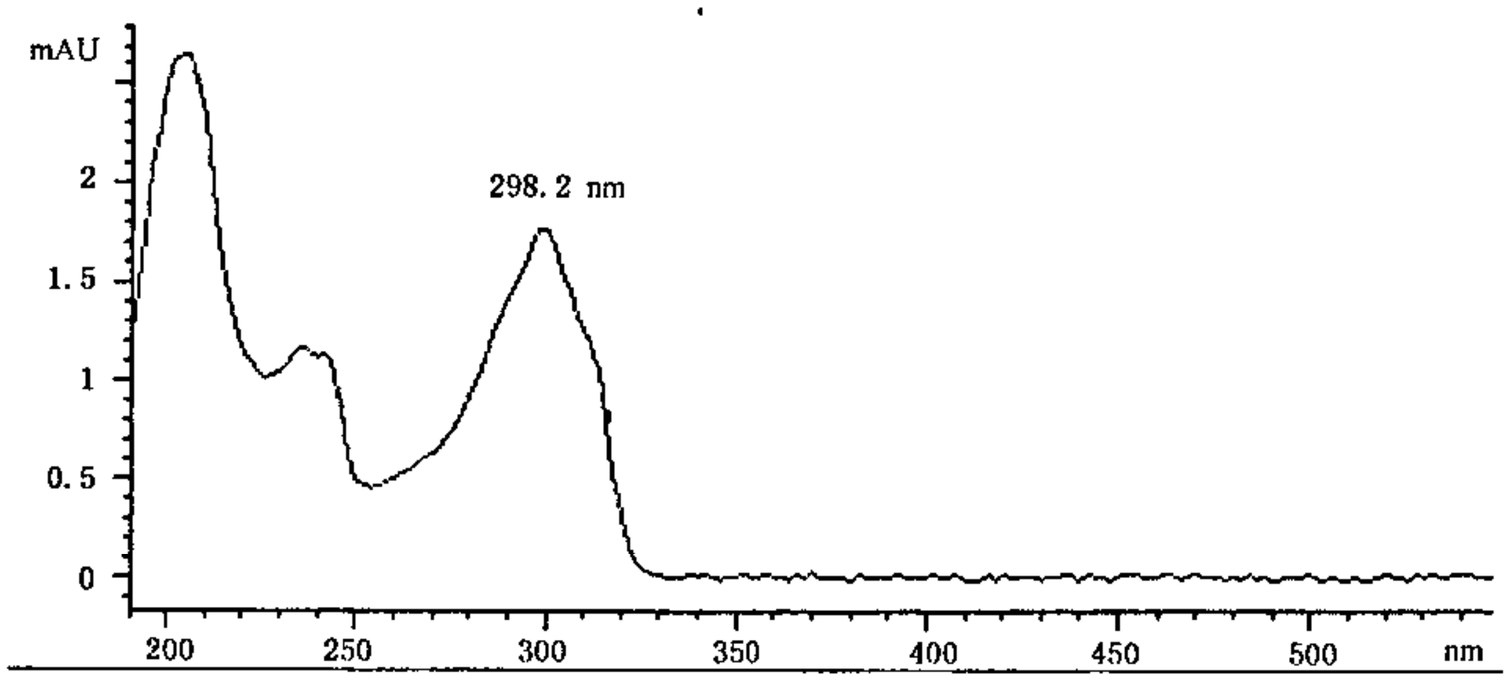


Figure A.2 The violet-spectrum characteristics of Thiabendazole

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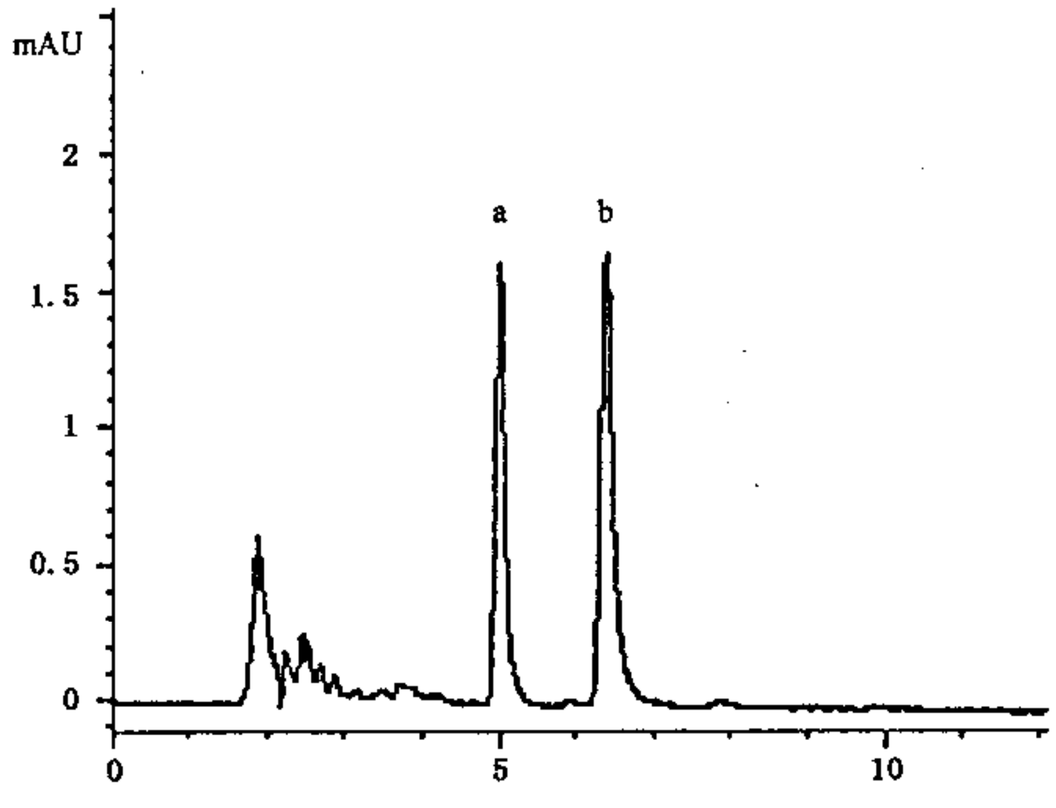
Annex B
(informative annex)
Chromatogram of Thiabendazole and Carbendazim standard solution



a——Carbendazim;

b——Thiabendazole.

Figure B. 1 The HPLC Chromatogram of Carbendazim and Thiabendazole standard

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