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中华人民共和国出入境检验检疫行业标准

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进出口食品中茚虫威残留量的检测方法 气相色谱法和液相色谱-质谱/质谱法

Determination of indoxacarb residues in food for import and export—GC and LC-MS/MS methods

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前　言

本标准的附录A、附录B、附录C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。

本标准起草单位：中华人民共和国福建出入境检验检疫局、中国检验检疫科学研究院、中华人民共和国江苏出入境检验检疫局、食品安全分析与检测技术教育部重点实验室(福州大学)。

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本标准系首次发布的出入境检验检疫行业标准。

进出口食品中茚虫威残留量的检测方法 气相色谱法和液相色谱-质谱/质谱法

第一法 气相色谱法

1 范围

本标准规定了食品中茚虫威残留量检测的制样和气相色谱检测方法。

本标准适用于毛豆、青梗菜、柚子、生姜、木耳、笋、茶叶、猪肉、鱼肉、鸡肉中茚虫威残留量的测定。

2 方法提要

试样中残留的茚虫威采用丙酮-正己烷混合溶剂振荡提取，ENVI-CARB 固相萃取柱净化植物源性产品、Florisil 固相萃取柱净化动物源性产品，供带有电子捕获检测器的气相色谱仪测定，外标法定量。

3 试剂和材料

除非另有规定，所用试剂均为分析纯，水为重蒸水。

3.1 正己烷：色谱纯。

3.2 丙酮：色谱纯。

3.3 乙腈：色谱纯。

3.4 氯化钠。

3.5 中性氧化铝：层析用，100 目～200 目。

3.6 饱和氯化钠溶液：称取过量氯化钠溶于水中。

3.7 丙酮＋正己烷(1＋2)混合溶剂：量取 100 mL 丙酮和 200 mL 正己烷，混匀。

3.8 茚虫威标准物质：Indoxacarb，分子式 $C_{22}H_{17}ClF_3N_3O_7$，CAS 174060-41-4，分子量 527.84；纯度大于等于 98%。

3.9 茚虫威标准储备液(100 mg/L)：准确称取适量茚虫威标准物质，用丙酮溶解并定容至 100 mL，该标准储备液置于 4℃冰箱中保存。

3.10 茚虫威标准工作液：根据需要取适量标准贮备液，以正己烷稀释成适当浓度的标准工作液。标准工作液需现配现用。

3.11 ENVI-Carb 固相萃取柱：250 mg，3 mL，用前以 3 mL 丙酮＋正己烷(1＋2)活化，保持柱体湿润。

3.12 Florisil 固相萃取柱：500 mg，3 mL。

4 仪器和设备

4.1 气相色谱仪，配电子捕获检测器。

4.2 离心机：4 000 r/min。

4.3 粉碎机。

4.4 组织捣碎机。

4.5 涡旋混合器。

4.6 超声波清洗器。

4.7 无油真空泵。

4.8 固相萃取装置。

4.9 氮吹仪。

5 试样制备和保存

5.1 试样制备

5.1.1 水果蔬菜类

取有代表性样品 500 g，将可食部分切碎后（不可水洗），用组织捣碎机将样品加工成浆状，混匀，装入洁净容器内，密封并标识。

5.1.2 茶叶、食用菌（干）类

取有代表性样品 300 g，用粉碎机粉碎并通过 2.0 mm 圆孔筛，混匀，装入洁净容器内，密封并标识。

5.1.3 肉及肉制品

取有代表性样品 500 g，切碎后用组织捣碎机将样品加工成浆状，混匀，装入洁净容器内，密封并标识。

5.2 试样保存

茶叶、食用菌（干）类等试样在 0℃～4℃保存；水果、蔬菜及肉和肉制品等试样在－18℃保存。

在制样过程中，应防止样品受到污染或发生残留物含量的变化。

6 测定步骤

6.1 提取

6.1.1 茶叶、食用菌（干）的样品：称取 1.00 g 均匀试样，置于 50 mL 具塞塑料离心管中，加入 2 mL 饱和氯化钠溶液（3.6），加入 3 mL 丙酮＋正己烷混合溶剂（3.7），涡旋振荡器混匀 30 s，超声提取 20 min，以 2 500 r/min 离心 3 min，取上层有机相于另一试管中。残渣再分别用 3 mL 丙酮＋正己烷混合溶剂（3.7）重复提取两次，合并提取液，于 35℃水浴氮吹浓缩至约 1 mL。

6.1.2 蔬菜与水果样品：称取 10.00 g 均匀试样，置于 50 mL 具塞塑料离心管中，加 5 g 氯化钠，再加入 10 mL 丙酮＋正己烷混合溶剂（3.7），超声提取 20 min，2 500 r/min 离心 3 min，取上层有机相于另一试管中，残渣再分别用 10 mL 丙酮＋正己烷混合溶剂重复提取两次，合并提取液，于 35℃水浴氮吹浓缩至约 1 mL。

6.1.3 肉及肉制品：称取 10.00 g 均匀试样置于 50 mL 具塞塑料离心管中，加 5 g 氯化钠，再加入 10 mL 丙酮＋正己烷混合溶剂（3.7），超声提取 20 min，2 500 r/min 离心 3 min，取上层有机相于另一试管中，残渣再分别用 10 mL 丙酮＋正己烷混合溶剂（3.7）重复提取两次，合并提取液，浓缩至约 1 mL，用 3×2 mL 乙腈提取两次，合并乙腈相，于 35℃水浴氮吹浓缩至约 1 mL。

6.2 净化

6.2.1 对于按 6.1.1 与 6.1.2 步骤提取的样品：将样品提取液过 ENVI-CARB 固相萃取柱，保持流速约为 1 mL/min，当样液液面接近固相萃取柱填料层时，用 6 mL 丙酮＋正己烷混合溶剂（3.7）洗脱，收集全部洗脱液，于 35℃下水浴氮吹至近干，用丙酮定容 1 mL，供气相色谱分析。

6.2.2 对于按 6.1.3 步骤提取的样品：在 Florisil 固相萃取柱中加入 0.5 g 中性氧化铝，先用 3 mL 丙酮＋正己烷混合溶剂（3.7）预淋洗，弃去全部流出液。将样品提取液过 Florisil 固相萃取柱，保持流速约为 1 mL/min，当样液液面接近固相萃取柱填料层时，用 6 mL 丙酮＋正己烷混合溶剂（3.7）洗脱，收集全部洗脱液，于 35℃下水浴氮吹至近干，用丙酮定容 1 mL，供气相色谱分析。

6.3 测定

6.3.1 色谱条件

a) 色谱柱：HP-5 石英毛细管柱，30 m×0.32 mm（内径），0.25 μm，或相当者；

b) 色谱柱温度：初始温度 80℃，以 20℃/min 升至 180℃，保持 3 min，以 20℃/min 升至 240℃，保持 3 min，再以 20℃/min 升至 270℃，保持 13 min；

c) 进样口温度:220℃;

d) 检测器温度:280℃;

e) 载气:氮气,纯度大于等于99.995%,流速1 mL/min;

f) 进样量:2 μL;

g) 进样方式:不分流进样,1 min后开阀。

6.3.2 色谱测定

根据试样中被测物的含量情况,选取响应值适宜的标准工作液进行色谱分析,对标准工作液和样液等体积参插进样。标准工作液和待测样液中茚虫威的响应值均应在仪器线性响应范围内。在本方法条件下,茚虫威的参考保留时间为24.8 min。标准品色谱图参见附录A中图A.1。

6.4 结果计算和表述

用数据处理软件或按式(1)计算试样中茚虫威药物的残留量:

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

式中:

X——试样中茚虫威残留量的数值,单位为毫克每千克(mg/kg);

A——样液中茚虫威的峰面积;

A_S——标准工作液中茚虫威的峰面积;

c_S——标准工作液中茚虫威的浓度,单位为毫克每升(mg/L);

V——样品最终定容体积,单位为毫升(mL);

m——试样量,单位为克(g)。

7 测定低限、回收率

7.1 测定低限

本方法的测定低限为0.005 mg/kg。

7.2 回收率

茚虫威添加回收率数据见表1。

表1 茚虫威药物残留的添加回收率数据

样品名称	添加浓度/(mg/kg)	回收率/%
青梗菜	0.005	73.2～91.4
	0.010	87.4～105.3
	0.020	75.2～88.4
	0.040	81.8～108.5
生姜	0.005	81.3～99.6
	0.010	87.3～112.4
	0.020	78.9～85.9
	0.040	91.9～102.7
茶叶	0.005	78.4～116.1
	0.010	96.4～105.5
	0.020	96.3～108.8
	0.040	99.2～107.5

表 1(续)

样品名称	添加浓度/(mg/kg)	回收率/%
笋	0.005	73.4～84.4
	0.010	81.1～93.3
	0.020	81.6～86.8
	0.040	79.9～88.3
柚子	0.005	72.4～100.9
	0.010	96.8～106.4
	0.020	88.9～103.7
	0.040	93.2～101.9
毛豆	0.005	64.3～90.5
	0.010	86.6～100.5
	0.020	83.5～99.9
	0.040	81.5～89.9
木耳	0.005	84.6～107.6
	0.010	92.6～105.4
	0.020	78.6～83.6
	0.040	89.5～95.9
猪肉	0.005	73.6～95.7
	0.010	95.7～114.1
	0.020	88.9～97.9
	0.040	93.0～108.5
鸡肉	0.005	73.4～91.9
	0.010	86.4～95.3
	0.020	81.0～94.8
	0.040	92.5～105.2
鱼肉	0.005	67.7～79.6
	0.010	67.7～79.6
	0.020	92.5～105.2
	0.040	86.1～94.1

第二法　液相色谱-质谱/质谱法

8　范围

本标准规定了食品中茚虫威残留量检测的制样和液相色谱-质谱/质谱检测方法。

本标准适用于毛豆、青梗菜、柚子、生姜、木耳、笋、茶叶、猪肉、鱼肉、鸡肉中茚虫威残留量的测定和确证。

9 方法提要

试样中残留的茚虫威采用丙酮-正己烷混合溶剂振荡提取，ENVI-CARB 固相萃取柱净化植物源性产品、Florisil 固相萃取柱净化动物源性产品，液相色谱-质谱/质谱仪测定和确证，外标法定量。

10 试剂和材料

除非另有规定，所用试剂均为分析纯，水为重蒸水。

10.1 正己烷：色谱纯。

10.2 丙酮：色谱纯。

10.3 乙腈：色谱纯。

10.4 氯化钠。

10.5 中性氧化铝：层析用，100 目～200 目。

10.6 饱和氯化钠溶液：称取过量氯化钠溶于水中。

10.7 丙酮＋正己烷(1＋2)混合溶剂：量取 100 mL 丙酮和 200 mL 正己烷，混匀。

10.8 茚虫威标准品：Indoxacarb，分子式 $C_{22}H_{17}ClF_3N_3O_7$，CAS 174060-41-4，分子量 527.84；纯度大于等于 98%。

10.9 标准储备液(100 mg/L)：准确称取适量茚虫威标准品，用丙酮溶解并定容至 100 mL，该标准储备液置于 4℃冰箱中保存。

10.10 标准工作液：根据需要取适量标准贮备液，以乙腈＋水(5＋5)，稀释成适当浓度的标准工作液。标准工作液需现配现用。

10.11 ENVI-Carb 固相萃取柱：250 mg，3 mL，用前以 3 mL 丙酮＋正己烷(1＋2)活化，保持柱体湿润。

10.12 Florisil 固相萃取柱：500 mg，3 mL。

11 仪器和设备

11.1 液相色谱-质谱/质谱联用仪，配电喷雾(ESI)源。

11.2 离心机：4 000 r/min。

11.3 粉碎机。

11.4 组织捣碎机。

11.5 涡旋混合器。

11.6 超声波清洗器。

11.7 无油真空泵。

11.8 固相萃取装置。

11.9 氮吹仪。

12 试样制备和保存

12.1 试样制备

12.1.1 水果蔬菜类

取有代表性样品 500 g，将可食部分切碎后(不可水洗)，用组织捣碎机将样品加工成浆状，混匀，装入洁净容器内，密封并标识。

12.1.2 茶叶、食用菌(干)类

取有代表性样品 300 g，用粉碎机粉碎并通过 2.0 mm 圆孔筛，混匀，装入洁净容器内，密封并标识。

12.1.3 肉及肉制品

取有代表性样品 500 g，切碎后用组织捣碎机将样品加工成浆状，混匀，装入洁净容器内，密封并标识。

12.2 试样保存

茶叶、食用菌(干)类等试样在 0℃～4℃保存；水果、蔬菜及肉和肉制品等试样在－18℃保存。

在制样过程中，应防止样品受到污染或发生残留物含量的变化。

13 测定步骤

13.1 提取

13.1.1 茶叶、食用菌(干)的样品：称取 1.00 g 均匀试样，置于 50 mL 具塞塑料离心管中，加入 2 mL 饱和氯化钠溶液(10.6)，加入 3 mL 丙酮＋正己烷混合溶剂(10.7)，涡旋振荡器混匀 30 s，超声提取 20 min，以 2 500 r/min 离心 3 min，取上层有机相于另一试管中。残渣再分别用 3 mL 丙酮＋正己烷混合溶剂(10.7)重复提取两次，合并提取液，于 35℃水浴氮吹浓缩至约 1 mL。

13.1.2 蔬菜与水果样品：称取 10.00 g 均匀试样，置于 50 mL 具塞塑料离心管中，加 5 g 氯化钠，再加入 10 mL 丙酮＋正己烷混合溶剂(10.7)，超声提取 20 min，2 500 r/min 离心 3 min，取上层有机相于另一试管中，残渣再分别用 10 mL 丙酮＋正己烷混合溶剂重复提取两次，合并提取液，于 35℃水浴氮吹浓缩至约 1 mL。

13.1.3 肉及肉制品：称取 10.00 g 均匀试样置于 50 mL 具塞塑料离心管中，加 5 g 氯化钠，再加入 10 mL 丙酮＋正己烷混合溶剂(10.7)，超声提取 20 min，2 500 r/min 离心 3 min，取上层有机相于另一试管中，残渣再分别用 10 mL 丙酮＋正己烷混合溶剂(10.7)重复提取两次，合并提取液，浓缩至约 1 mL，用 3×2 mL 乙腈提取两次，合并乙腈相，于 35℃水浴氮吹浓缩至约 1 mL。

13.2 净化

13.2.1 对于按 13.1.1 与 13.1.2 步骤提取的样品：将样品提取液过 ENVI-CARB 固相萃取柱，保持流速约为 1 mL/min，当样液液面接近固相萃取柱填料层时，用 6 mL 丙酮＋正己烷混合溶剂(10.7)洗脱，收集全部洗脱液，于 35℃下水浴氮吹至近干，用乙腈＋水(5＋5)定容 5 mL，供液相色谱-质谱/质谱分析。

13.2.2 对于按 13.1.3 步骤提取的样品：在 Florisil 固相萃取柱中加入 0.5 g 中性氧化铝，先用 3 mL 丙酮＋正己烷混合溶剂(10.7)预淋洗，弃去全部流出液。将样品提取液过 Florisil 固相萃取柱，保持流速约为 1 mL/min，当样液液面接近固相萃取柱填料层时，用 6 mL 丙酮＋正己烷混合溶剂(10.7)洗脱，收集全部洗脱液，于 35℃下水浴氮吹至近干，用乙腈＋水(5＋5)定容 5 mL，供液相色谱-质谱/质谱分析。

13.3 测定

13.3.1 色谱条件

a) 色谱柱：Acquity UPLC BEH C_{18} 柱，55 mm×2.1 mm×1.7 μm，或相当者；

b) 柱温：30℃；

c) 流动相：乙腈＋水(7＋3)；

d) 流速：300 μL/min；

e) 进样量：10 μL。

13.3.2 质谱条件

a) 离子源：电喷雾源(ESI)，正离子模式；

b) 扫描方式：多反应监测(MRM)。

其他参考质谱条件参见附录 B 中表 B.1。

13.3.3 液相色谱-质谱/质谱测定

根据试样中被测物的含量情况，选取响应值适宜的标准工作液进行色谱分析，对标准工作液和样液

等体积参插进样。标准工作液和待测样液中茚虫威的响应值均应在仪器线性响应范围内。在本方法条件下，茚虫威的参考保留时间为 1.9 min，标准品的多反应监测(MRM)色谱图参见附录 C 中图 C.1。

13.4 定性标准

13.4.1 保留时间

待测样品中化合物色谱峰的保留时间与标准溶液相比变化范围应在±5%之内。

13.4.2 信噪比

待测化合物的定性离子的重构离子色谱峰的信噪比应大于等于 3(S/N≥3)，定量离子的重构离子色谱峰的信噪比应大于等于 10(S/N≥10)。

13.4.3 定量离子、定性离子及子离子丰度比

定性确证时相对离子丰度的最大允许偏差见表 2。

表 2 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/%	±20	±25	±30	±50

每种化合物的质谱定性离子应出现至少应包括一个母离子和两个子离子，而且同一检测批次，对同一化合物，样品中目标化合物的两个子离子的相对丰度比与浓度相当的标准溶液相比，其允许偏差不超过表 3 规定的范围。

13.5 结果计算和表述

用数据处理软件或按式(2)计算试样中茚虫威药物的残留量。

$$X=\frac{A\times c_S\times V}{A_S\times m} \quad \cdots\cdots(2)$$

式中：

X——试样中茚虫威残留量的数值，单位为毫克每千克(mg/kg)；

A——样液中茚虫威的峰面积；

A_S——标准工作液中茚虫威的峰面积；

c_S——标准工作液中茚虫威的浓度，单位为毫克每升(mg/L)；

V——样品最终定容体积，单位为毫升(mL)；

m——最终样液中所代表的试样量，单位为克(g)。

14 测定低限、回收率

14.1 测定低限

本方法的测定低限为 0.005 mg/kg。

14.2 回收率

茚虫威回收率数据见表 3。

表 3 茚虫威药物残留的添加回收率数据

样品名称	添加浓度/(mg/kg)	回收率/%
青梗菜	0.005	103.0～117.6
	0.010	91.4～117.4
	0.020	96.8～108.5
生姜	0.005	99.2～117.6
	0.010	94.8～118.1
	0.020	93.3～103.2

表 3(续)

样品名称	添加浓度/(mg/kg)	回收率/%
茶叶	0.005	77.8～98.6
	0.010	82.3～112.3
	0.020	82.9～95.0
笋	0.005	80.2～112.6
	0.010	108.3～118.9
	0.020	93.9～105.8
柚子	0.005	105.0～119.8
	0.010	86.0～113.8
	0.020	90.7～100.7
毛豆	0.005	86.0～113.6
	0.010	103.0～119.2
	0.020	98.1～118.0
木耳	0.005	67.4～93.8
	0.010	72.3～112.3
	0.020	72.5～92.1
猪肉	0.005	87.4～100.2
	0.010	82.3～112.3
	0.020	93.7～106.5
鸡肉	0.005	86.6～115.8
	0.010	71.2～102.9
	0.020	86.3～100.4
鱼肉	0.005	66.2～88.4
	0.010	94.1～104.1
	0.020	85.9～102.1

附 录 A
（资料性附录）
茚虫威的气相色谱图

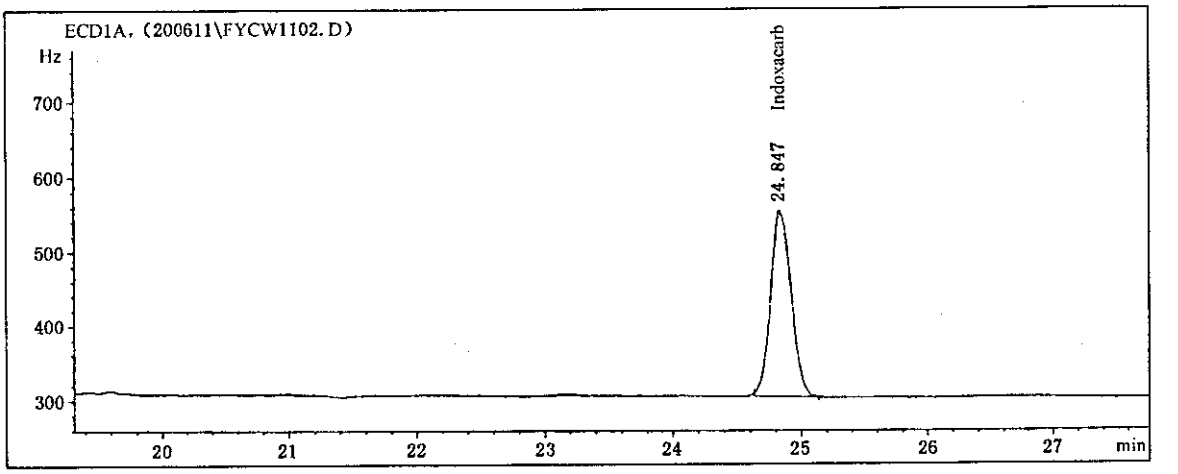

图 A.1 茚虫威的气相色谱图

附 录 B
（资料性附录）
参考质谱条件[1)]

参考质谱条件：

a） 毛细管电压：0.5 kV；

b） 源温度：120℃；

c） 去溶剂温度：450℃；

d） 锥孔气流（氮气）：45 L/h；

e） 去溶剂气流（氮气）：900 L/h；

f） 碰撞气压（氩气）：2.20×10⁻³ Pa；

g） 其他质谱参数见表 B.1。

表 B.1 茚虫威的主要参考质谱参数

化合物	母离子 (m/z)	子离子 (m/z)	驻留时间/ s	锥孔电压/ V	碰撞能量/ eV
茚虫威	528.2	293.1*	0.2	30	13
		249.1	0.2	30	16
注：表中带 * 的离子为定量离子。对于不同质谱仪器，仪器参数可能存在差异，测定前应将质谱参数优化到最佳。					

1） 非商业性声明：附录 B 所列参考质谱条件是在 Waters UPLC/Priemer 型液质联用仪上完成的，此处列出试验用仪器型号仅为提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家或型号的仪器。

附 录 C
（资料性附录）
茚虫威的多反应监测(MRM)离子色谱图

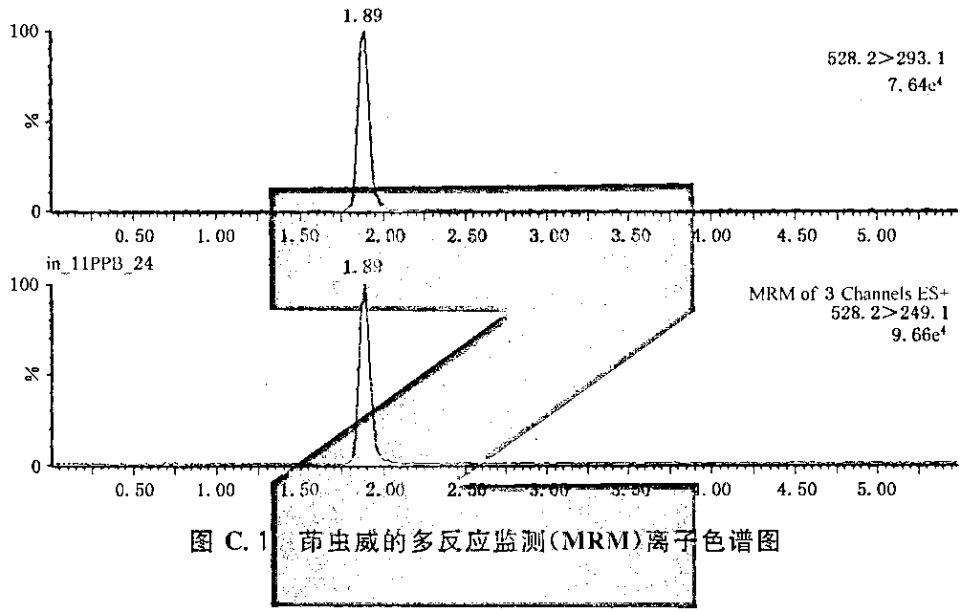

图 C.1 茚虫威的多反应监测(MRM)离子色谱图

Foreword

Annex A, Annex B, Annex C of this standard are informative annexes.

This standard is proposed by and is under the jurisdiction of the Certification and Accreditation administration of the People's Republic of China.

This standard is drafted by Fujian Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Chinese Academy of Inspection and Quarantine, Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Key Laboratory of Analysis & Detection Technology for Food Safety (Fuzhou University) Ministry of Education.

Main drafters of this standard are Yang Fang, Lu Shenyu, Xu Dunmin, Li Shujuan, Sheng Chongyu, Yu Kongjie, Lin Yonghui, Lin Zhengcai, Chen Guonan.

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

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

Determination of indoxacarb residues in food for import and export —GC and LC-MS/MS methods

The first method Gas chromatography method

1 Scope

The standard specifies the method of determination of indoxacarb residue in foodstuffs for import and export by gas chromatography.

The method is applicable to determine indoxacarb residues in green soybeans, qing-gen-cai, grapefruit, ginger, fungi, bamboo shoot, tea, pork, fish and chicken for import and export.

2 Principle

Extraction of the indoxacarb residues with acetone and *n*-hexane mixed solvents, and then purification with a ENVI-CARB solid phase extraction (SPE) cartridge for plant origin food, Florsil solid phase extraction (SPE) cartridge for animal origin food, followed by gas chromatography analysis.

3 Reagents and Materials

Unless otherwise specified, all regents used are A. R. , and pure "water" is redistilled water.

3.1 *n*-Hexane: HPLC grade.

3.2 Acetone: HPLC grade.

3.3 Acetonitrile: HPLC grade.

3.4 Sodium chloride.

3.5 Neutral aluminum oxide: 100 mesh~200 mesh.

3.6 Saturated sodium chloride solution: Add sodium chloride in water to the solution is saturated.

3.7 Acetone and *n*-hexane mixed solvent: Pipet 100 mL actone and 200 mL *n*-hexane and mix well.

3.8 Standard of indoxacarb: Molecular formula $C_{22}H_{17}ClF_3N_3O_7$, CAS 174060-41-4, molecular weight 527.84, purity≥98%.

3.9 Stock solutions of indoxacarb (100 mg/L): Accurately weigh indoxacarb standard material, dissolve with methanol to a volume of 100 mL, and store at approximately 4℃ for a maximum period.

3.10 Calibration solutions of indoxacarb for GC: Dilute appropriate volume of stock solutions to a intended concentration with *n*-hexane and mixed well. These solutions should be prepared before use.

3.11 ENVI-CARB solid phase extraction cartridge: 250 mg, 3 mL, the extraction cartridge was conditioned using 3 mL methanol and *n*-hexane mixed solvent before use, prevent the columns from runing dry.

3.12 Florisil solid phase extraction catridge: 500 mg, 3 mL.

4 Apparatus and equipment

4.1 Gas Chromatography: Equipped with lectron capture detector.

4.2 Centrifuge: 4 000 r/min.

4.3 Grinder.

4.4 Tissues homogenizer.

4.5 Vortex mixer.

4.6 Ultrasonic machine.

4.7 Vacuum pump.

4.8 Solid phase extraction equipment.

4.9 Pressured gas blowing concentrator.

5 Sample preparation and storage

5.1 Sample preparation

5.1.1 Furits and vegetables

Collect ca 500 g the representative samples the edible portions are cut up (without washing by water) and mixed well with a tissure homogenizer, then sealed in clean containers and marked.

5.1.2 Tea and fungi

Collect ca 300 g the representative samples and crush with a grinder, let them pass through 2.0 mm sieve, and then sealed in clean containers and marked.

5.1.3 Meats and Meat products

Collect ca 500 g the representative samples and the edible portions are mixed well with a tissure homogenizer, and then sealed in clean containers and marked.

5.2 Sample storage

Samples may be stored at −18℃, fresh or frozen tissues may be stored at 0℃～4℃ for 72 h.

Precaution measures should be taken to avoid contamination or other factors may cause the change of residues concentration in samples.

6 Method of Determination

6.1 Extraction

6.1.1 For tea and fungi: Weigh 1.00 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube, then add 2 mL saturated sodium chloride solution (3.6) and 3 mL acetone and *n*-hexane mixed solvent (3.7), stopper the tubes and votrtex for 30 s and extract with ultersonic machine for 20 min, and then centrifuge at 2 500 r/min for 3 min. transfer the supernatant to another clean tube and repeat the extraction procedure again. The supernatants are collected in a 3 mL tube and evaporat to ca 1 mL at 35℃ under a stream of nitrogen.

6.1.2 For vegetables and furits: weigh 10.00 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube, add ca 5 g sodium chloride and 10 mL acetone and *n*-hexane mixed solvent (3.7), extract with ultersonic machine for 20 min, and then centrifuge at 2 500 r/min for 3 min, transfer the supernatant to another clean tube and repeat the extraction procedure again. The supernatants are collected in a 10 mL tube and evaporat to ca 1 mL at 35℃ under a stream of nitrogen.

6.1.3 For meat and meat products: weigh 10.00 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube, add ca 5 g sodium chloride and 10mL acetone and *n*-hexane mixed solvent (3.7), extract with ultersonic machine for 20 min, and then centrifuge at 2 500 r/min for 3 min. transfer the supernatant to another clean tube and repeat the extraction procedure again. The supernatants are collected in a 10 mL tube and evaporated to ca 1 mL at 35℃ under a stream of nitrogen, and re-extract the analyte with 3×2 mL acetonitrile, collect the acetonitrile phase and evaporat to ca 1 mL at 35℃ under a stream of nitrogen.

6.2 Clean-up

6.2.1 For samples extracted according to 6.1.1 and 6.1.2: Pass extracts through ENVI-CARB cartridges conditioned at a flow rate ca 1 mL/min, discard elutes, then the cartridges are dried under vacuum, and the indoxacarb residues are eluted with 6 mL acetone and *n*-hexane mixed solvent (3.7). The elutes are collected and evaporated to dryness at 35℃ under a stream of nitrogen and redissolved in 1 mL acetone for GC-ECD analysis.

6.2.2 For samples extracted according to 6.1.3: Connect Florisil PR cartridges to a solid phase extraction equipment, fill 0.5 g neutral aluminum oxide in the top of the cartridegs, and condition the SPE cartridegs with 3×1 mL acetone and *n*-hexane mixed solvent (3.7) at a flow rate ca 1 mL/min. Pass extracts through Florisil PR cartridges and elute with 6 mL acetone and *n*-hexane mixed solvent. The elutes are collected and evaporated to dryness at 35℃ under a stream of nitrogen and redissolved in 1 mL acetone for GC-ECD analysis.

6.3 Determination

6.3.1 GC operation conditions

a) Column: HP-5 fused quartz capillary column, 30 m×0.32 mm(i.d.), film thickness 0.25 μm or equivalent columns;

b) Column temperature: Initial temperature 80℃, ramp at 20℃/min to 180℃, hold for 3 min, ramp at 20℃/min to 240℃, hold for 3 min, and then ramp at 20℃/min to 270℃, hold for 13 min;

c) Injection port temperature: 220℃;

d) Temperature of detector: 280℃;

e) carrier gas: Helium, purity≥99.995%, flow rate is 1 mL/min;

f) Injection volume: 2 μL;

g) Injection mode: Splitless, purge after 1 min.

6.3.2 GC/ECD detection

Prepare standard solutions containing indoxacarb at appropriate concentrations according to the analyte in sample extracts. The standard solutions should be injected in between the injections of sample

solution of equal volume. The responses of standard solutions and sample solutions should be within the linear range. The referenced retention time of indoxacarb is 24.8 min. Annex A is the chromatogram of the standard solution.

6.4 Calculation and expression of the result

Calculate the concentration of the indoxacarb residues in sample according to the formula (1):

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

where

X—indoxacarb content in the test sample, mg/kg;

A—the peak area of indoxacarb aliquot of sample solution injected into GC/ECD system;

A_S—the peak area of indoxacarb of stand solution injected into GC/ECD system;

c_S—concentration of indoxacarb in the standard solution, mg/L;

V—final volume of sample extract, mL;

m—mass of sample, g.

7 Limit of determination and recovery

7.1 Limit of determination

The limit of determination of indoxacarb is 0.005 mg/kg.

7.2 Recovery

The recovery data of indoxacarb residues is listed in table 1.

Table 1—Recovery for indoxacarb residues

Matrix	Spike level/(mg/kg)	Recovery/%
qing-gen-cai	0.005	73.2~91.4
	0.010	87.4~105.3
	0.020	75.2~88.4
	0.040	81.8~108.5
ginger	0.005	81.3~99.6
	0.010	87.3~112.4
	0.020	78.9~85.9
	0.040	91.9~102.7
tea	0.005	78.4~116.1
	0.010	96.4~105.5

Table 1 (continued)

Matrix	Spike level/(mg/kg)	Recovery/%
tea	0.020	96.3～108.8
	0.040	99.2～107.5
bamboo shoot	0.005	73.4～84.4
	0.010	81.1～93.3
	0.020	81.6～86.8
	0.040	79.9～88.3
grapefruit	0.005	72.4～100.9
	0.010	96.8～106.4
	0.020	88.9～103.7
	0.040	93.2～101.9
green soybeans	0.005	64.3～90.5
	0.010	86.6～100.5
	0.020	83.5～99.9
	0.040	81.5～89.9
fungi	0.005	84.6～107.6
	0.010	92.6～105.4
	0.020	78.6～83.6
	0.040	89.5～95.9
pork	0.005	73.6～95.7
	0.010	95.7～114.1
	0.020	88.9～97.9
	0.040	93.0～108.5
chicken	0.005	73.4～91.9
	0.010	86.4～95.3
	0.020	81.0～94.8
	0.040	92.5～105.2
fish	0.005	67.7～79.6
	0.010	67.7～79.6
	0.020	92.5～105.2
	0.040	86.1～94.1

The two method Liquid chromatography-tandem mass spectrometry method

8 Scope

The standard specifies the method of determination of indoxacarb residue in foodstuffs for import and export by liquid chromatography-tandem mass spectrometry method.

The method is applicable to determine and confirm indoxacarb residues in green soybeans, qing-gen-cai, grapefruit, ginger, fungi, bamboo shoot, tea, pork, fish and chicken for import and export.

9 Principle

Extraction of the indoxacarb residues with acetone and *n*-hexane mixed solvents, and then purification with a ENVI-CARB solid phase extraction (SPE) cartridge for plant origin food, Florsil solid phase extraction (SPE) cartridge for animal origin food, followed by liquid chromatography-tandem mass spectrometry determination and confirmation.

10 Reagents and Materials

Unless otherwise specified, all regents used are A.R., and pure "water" is redistilled water.

10.1 *n*-Hexane: HPLC grade.

10.2 Acetone: HPLC grade.

10.3 Acetonitrile: HPLC grade.

10.4 Sodium chloride.

10.5 Neutral aluminum oxide: 100 mesh~200 mesh.

10.6 Saturated sodium chloride solution: Add sodium chloride in water to the solution is saturated.

10.7 Acetone and *n*-hexane mixed solvent: Pipet 100 mL acetone and 200 mL *n*-hexane and mix well.

10.8 Standard of indoxacarb: Molecular formula $C_{22}H_{17}ClF_3N_3O_7$, CAS 174060-41-4, molecular weight 527.84, purity≥98%.

10.9 Stock solutions of indoxacarb (100 mg/L): Accurately weigh indoxacarb standard material, dissolve with methanol to a volume of 100 mL, and store at approximately 4℃ for a maximum period.

10.10 Calibration solutions of indoxacarb for GC: Dilute appropriate volume of stock solutions to a intended concentration with *n*-hexane and mixed well. These solutions should be prepared before use.

10.11 ENVI-CARB solid phase extraction cartridge: 250 mg, 3 mL, the extraction cartridge was con-

ditioned using 3 mL methanol and *n*-hexane mixed solvent before use, prevent the columns from runing dry.

10.12 Florisil solid phase extraction catridge: 500 mg, 3 mL.

11 Apparatus and equipment

11.1 High performance liquid chromatography-mass spectrometer equipment: Equipped with electrospray (ESI) LC interface.

11.2 Centrifuge: 4 000 r/min.

11.3 Grinder.

11.4 Tissues homogenizer.

11.5 Vortex mixer.

11.6 Ultrasonic machine.

11.7 Vacuum pump.

11.8 Solid phase extraction equipment.

11.9 Pressured gas blowing concentrator.

12 Sample preparation and storage

12.1 Sample preparation

12.1.1 Furits and vegetables

Collect ca 500 g the representative samples the edible portions are cut up (without washing by water) and mixed well with a tissure homogenizer, then sealed in clean containers and marked.

12.1.2 Tea and fungi

Collect ca 300 g the representative samples and crush with a grinder, let them pass through 2.0 mm sieve, and then sealed in clean containers and marked.

12.1.3 Meats and Meat products

Collect ca 500 g the representative samples and the edible portions are mixed well with a tissure homogenizer, and then sealed in clean containers and marked.

12.2 Sample storage

Samples may be stored at −18℃, fresh or frozen tissues may be stored at 0℃～4℃.

Precaution measures should be taken to avoid contamination or other factors may cause the change of residues concentration in samples.

13 Method of Determination

13.1 Extraction

13.1.1 For tea and fungi: weigh 1.00 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube, then add 2 mL saturated sodium chloride solution (10.6) and 3 mL acetone and *n*-hexane mixed solvent (10.7), stopper the tubes and votrtex for 30 s and extract with ultersonic machine for 20 min, and then centrifuge at 2 500 r/min for 3 min, transfer the supernatant to another clean tube and repeat the extraction procedure again. The supernatants are collected in a 3 mL tube and evaporat to ca 1 mL at 35℃ under a stream of nitrogen.

13.1.2 For vegetables and furits: weigh 10.00 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube, add ca 5 g sodium chloride and 10 mL acetone and *n*-hexane mixed solvent (10.7), extract with ultersonic machine for 20 min, and then centrifuge at 2 500 r/min for 3 min, transfer the supernatant to another clean tube and repeat the extraction procedure again. The supernatants are collected in a 10 mL tube and evaporat to ca 1 mL at 35℃ under a stream of nitrogen.

13.1.3 For meat and meat products: weigh 10.00 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube, add ca 5 g sodium chloride and 10 mL acetone and *n*-hexane mixed solvent (10.7), extract with ultersonic machine for 20 min, and then centrifuge at 2 500 r/min for 3 min, transfer the supernatant to another clean tube and repeat the extraction procedure again. The supernatants are collected in a 10 mL tube and evaporated to ca 1 mL at 35℃ under a stream of nitrogen, and re-extract the analyte with 3×2 mL acetonitrile, collect the acetonitrile phase and evaporat to ca 1 mL at 35℃ under a stream of nitrogen.

13.2 Clean-up

13.2.1 For samples extracted according to 13.1.1 and 13.1.2: Pass extracts through ENVI-Carb cartridges conditioned at a flow rate ca 1 mL/min, discard elutes, then the cartridges are dried under vacuum, and the indoxacarb residues are eluted with 6 mL acetone and *n*-hexane mixed solvent

(10.7). The elutes are collected and evaporated to dryness at 35℃ under a stream of nitrogen and redissolved in 5 mL acetonitrile-water mixed solvent (5+5,v/v) for LC-MS detection.

13.2.2 For samples extracted according to 13.1.3:Connect Florisil PR cartridges to a solid phase extraction equipment,fill 0.5 g neutral aluminum oxide in the top of the cartridegs,and condition the SPE cartridegs with 3×1 mL acetone and *n*-hexane mixed solvent (10.7) at a flow rate ca 1 mL/min. Pass extracts through Florisil PR cartridges and elute with 6 mL acetone and *n*-hexane mixed solvent. The elutes are collected and evaporated to dryness at 35℃ under a stream of nitrogen and redissolved in 5 mL acetonitrile-water mixed solvent (5+5,v/v) for LC-MS detection.

13.3 Determination

13.3.1 LC operation conditions

a) Column:Acquity UPLC BEH C_{18} column (55 mm×2.1 mm×1.7 μm) or equivalent;

b) Column temperature:30℃;

c) Mobile phase:acetonitrile-water (7+3);

d) Flow rate:300 μL/min;

e) Injection volume:10 μL.

13.3.2 MS operation conditions

a) Ion source:ESI,positive ionisation mode;

b) Scan mode:multiple reaction monitoring (MRM) mode.

Other reference mass operating conditions are listed in annex B.

13.3.3 LC-MS detection

Prepare standard solutions containing indoxacarb at appropriate concentrations according to the analyte in sample extracts. The referenced retention time of indoxacarb is 1.9 min. Annex C is the reconstituted ion chromatogram of indoxacarb standard solution.

13.4 Confirmation test

13.4.1 Retention time

The variation range of the retention time of the analyte in the test portion and in the standard work-

ing solution shall be within the range of ±5%.

13.4.2 Signal-to-noise ratio

The signal-to-noise ratio for each diagnostic ion shall be≥3 : 1, the signal-to-noise ration for quantitative ion shall be≥10 : 1.

13.4.3 Relative intensities

The qualification ions of the analyte must be found, and at least include one precursor ion and two daughter ions. For the same analysis batch and the same analyte, the variation range of the ion ratio between the two daughter ions for the unknown samples and the standard working solutions at the similar concentration can not be out of range of table 2 under the same determination conditions.

Table 2—Maximum permitted tolerances for relative ion intensities

Relative intensity (base peak)/%	>50	>20~50	>10~20	≤10
Maximum permitted tolerances for relative ion intensities/%	±20	±25	±30	±50

13.5 Calculation and expression of the result

Calculate the concentration of the indoxacarb residues in sample according to the formula (2):

$$X = \frac{A \times c_s \times V}{A_s \times m} \quad \cdots\cdots (2)$$

where

X—indoxacarb content in the test sample, mg/kg;

A—the peak area of indoxacarb aliquot of sample solution injected into LC/MS system;

A_s—the peak area of indoxacarb of stand solution injected into LC/MS system;

c_s—concentration of indoxacarb in the standard solution, mg/L;

V—final volume of sample extract, mL;

m—mass of sample, g.

14 Limit of determination and recovery

14.1 Limit of determination

The limit of determination of indoxacarb is 0.005 mg/kg.

14.2 Recovery

The recovery data of indoxacarb residues is listed in table 3.

Table 3—Recovery for indoxacarb residues

Matrix	Spike level/(mg/kg)	Recovery/%
qing-gen-cai	0.005	103.0～117.6
	0.010	91.4～117.4
	0.020	96.8～108.5
ginger	0.005	99.2～117.6
	0.010	94.8～118.1
	0.020	93.3～103.2
tea	0.005	77.8～98.6
	0.010	82.3～112.3
	0.020	82.9～95.0
bamboo shot	0.005	80.2～112.6
	0.010	108.3～118.9
	0.020	93.9～105.8
grapefruit	0.005	105.0～119.8
	0.010	86.0～113.8
	0.020	90.7～100.7
green soybean	0.005	86.0～113.6
	0.010	103.0～119.2
	0.020	98.1～118.0
fungi	0.005	67.4～93.8
	0.010	72.3～112.3
	0.020	72.5～92.1
pork	0.005	87.4～100.2
	0.010	82.3～112.3
	0.020	93.7～106.5
chicken	0.005	86.6～115.8
	0.010	71.2～102.9
	0.020	86.3～100.4
fish	0.005	66.2～88.4
	0.010	94.1～104.1
	0.020	85.9～102.1

Annex A
(Informative)
Gas chromatogram of indoxacarb standard solution

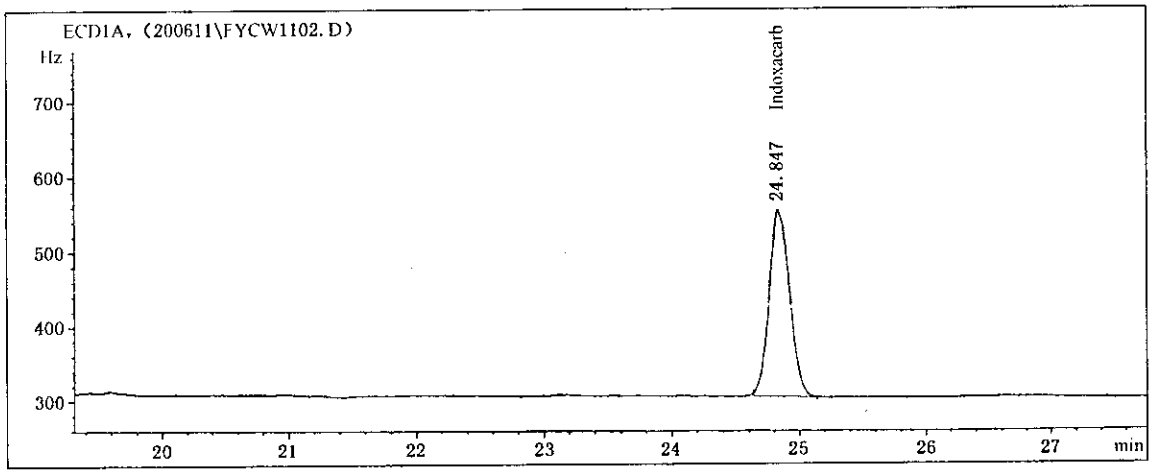

Figure A.1—Gas chromatogram of indoxacarb standard solution

Annex B
(Informative)
Reference mass conditions[1)]

Reference mass conditions:

a) Capillary:0.5 kV;

b) Source Temperature:120℃;

c) Desolvation Temperature:450℃;

d) Cone Gas Flow (Nitrogen):45 L/h;

e) Desolvation Gas Flow (Nitrogen):900 L/h;

f) Collision Pressure (Argon):2.20×10^{-6} Pa;

g) Other mass operating conditions are listed in table B.1.

Table B.1—Main Mass parameters of indoxacarb

Compound	Precursor (m/z)	Moniter ions (m/z)	Dwell time/s	Cone/V	CE/eV
indoxacarb	528.2	293.1	0.2	30	13
		249.1*	0.2	30	16
Note: The daughter ion with "*" mark is the quantification ion. For the different MS equipment, the parameters may be different, and the MS parameters should be optimized to the best before analysis.					

1) Non-commercial statement: the reference mass parameters in Annex A are accomplished by waters UPLC/Priemer LC-MS/MS, the equipment and its type involved in the standard method is only for reference and not related to any commercial aim, and the analysts are encouraged to use equipments of different corporation or different type.

Annex C
(Informative)
Multiple reaction monitoring chromatogram of indoxacarb standard solution

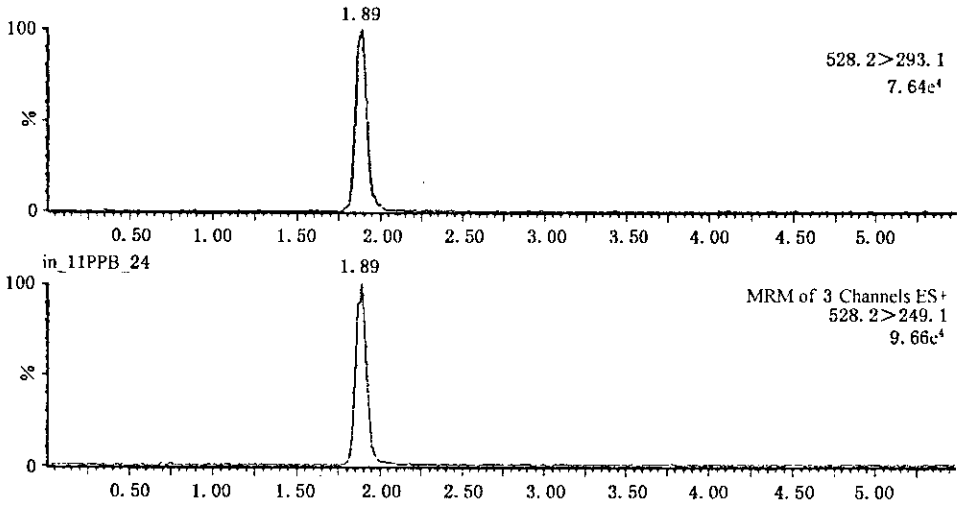

Figure C.1—Multiple reaction monitoring chromatogram of indoxacarb standard solution

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