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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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进出口食品中环己基氨基磺酸钠的检测方法 液相色谱-质谱/质谱法

Determination of sodium cyclamate in foods for import and export—
HPLC-MS/MS method

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前 言

本标准的附录 A 和附录 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。

本标准起草单位：中华人民共和国吉林出入境检验检疫局、中华人民共和国湖南出入境检验检疫局、中华人民共和国云南出入境检验检疫局。

本标准主要起草人：王明泰、牟峻、戴华、马晓刚、张代辉、周晓、李爱军、常东华。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中环己基氨基磺酸钠的检测方法

液相色谱-质谱/质谱法

1 范围

本标准规定了食品中环己基氨基磺酸钠(甜蜜素)检验的制样和液相色谱-质谱/质谱法。

本标准适用于水果罐头、浓缩山葡萄汁、白酒、糕点、糖果、甜面酱、酱菜中环己基氨基磺酸钠的检测和确证。

2 方法提要

试样用水超声提取,离心后,上清液供液相色谱-质谱/质谱仪检测,外标法定量。

3 试剂和材料

除另有规定外,所有试剂均为分析纯,水为超纯水。

3.1 乙酸。

3.2 乙腈:液相色谱纯。

3.3 0.1%乙酸水溶液(体积分数):量取乙酸4 mL,用水稀释至4 000 mL。

3.4 环己基氨基磺酸钠标准品(Sodium Cyclamate, $C_6H_{12}NNaO_3S$, CAS号:139-05-9):纯度大于等于99%。

3.5 环己基氨基磺酸钠标准储备液:准确称取适量环己基氨基磺酸钠标准品,用水配制成浓度为500 $\mu\text{g}/\text{mL}$ 的标准储备溶液。该溶液在 $0^{\circ}\text{C}\sim 4^{\circ}\text{C}$ 冰箱中保存。

3.6 环己基氨基磺酸钠标准中间液:准确移取一定体积的环己基氨基磺酸钠标准储备液,用水配制成浓度为1 $\mu\text{g}/\text{mL}$ 的标准中间液,该溶液在 $0^{\circ}\text{C}\sim 4^{\circ}\text{C}$ 冰箱中保存。

3.7 环己基氨基磺酸钠标准工作溶液:准确移取一定体积的标准中间液,可根据需要用水稀释成适用浓度的标准工作液。该溶液在 $0^{\circ}\text{C}\sim 4^{\circ}\text{C}$ 冰箱中保存。

3.8 0.45 μm 滤膜(水相)。

4 仪器和设备

4.1 液相色谱串联质谱仪:配有电喷雾(ESI)离子源。

4.2 混匀器。

4.3 超声波提取器。

4.4 离心机。

5 试样制备与保存

5.1 试样制备

5.1.1 水果罐头类

将所取原始样品在室温下开罐,将罐内液体和固体物分开并分别称量。按照两者比例取1 kg代表性样品,经组织捣碎机捣碎,再将混匀样品分装置洁净容器内密封,作为试样,并标明标记。

5.1.2 酒类、果汁类

将所取原始样品1 kg在瓷混样桶内充分混匀,再将混匀样品分装置洁净容器内密封,作为试样,标

明标记。

5.1.3 糕点类、糖果类

取有代表性样品 1 kg,将样品按四分法缩至 200 g,在玻璃研钵中研碎,再将混匀样品分装置洁净容器内密封,作为试样,并标明标记。

5.1.4 甜面酱类、酱菜类

取有代表性样品 1 kg,将样品按四分法缩至 500 g,所取样品经组织捣碎机捣碎,再将混匀样品分装置洁净容器内密封,作为试样,并标明标记。

5.2 试样保存

试样于 0℃~4℃ 保存。在抽样及制样的操作过程中,应防止样品受到污染或发生含量的变化。

6 测定步骤

6.1 提取

6.1.1 水果罐头、糕点、糖果、甜面酱、酱菜、果汁类样品

称取试样约 1 g(精确至 0.01 g)置于 25 mL 离心管中,加入 8.0 mL 水,混匀后,于超声波清洗器中超声 20 min 后,于 4 000 r/min 离心 5 min,过滤并用水定容至 10 mL 容量瓶中,取部分过 0.45 μm 滤膜,供液相色谱-质谱/质谱仪测定。

6.1.2 酒类样品

准确移取 2 mL 试样,过 0.45 μm 滤膜,供液相色谱-质谱/质谱仪测定。

6.2 测定

6.2.1 液相色谱条件

- a) 色谱柱:Extend-C₁₈柱,250 mm×4.6 mm(内径),粒度 5 μm 或相当者;
- b) 0.1%乙酸水溶液+甲醇(4+6,体积比);
- c) 流速:0.6 mL/min;
- d) 进样量:5 μL;
- e) 柱温:室温。

6.2.2 质谱条件

- a) 离子源:电喷雾离子源;
- b) 扫描方式:负离子模式;
- c) 检测方式:多反应监测 MRM;
- d) 雾化气、气帘气、辅助加热气、碰撞气均为高纯氮气;使用前应调节各气体流量以使质谱灵敏度达到检测要求,详细条件参见附录 A 中第 A.1 章。
- e) 喷雾电压(IS)、雾化气压力(GS1)、气帘气压力(CUR)、辅助气流速(GS2)、去簇电压(DP)、碰撞气能量(CE)等值应优化至最优灵敏度,参考条件和定性离子对、定量离子参见第 A.1 章。

6.2.3 液相色谱-质谱/质谱测定和确证

6.2.3.1 按照确定的液相色谱-串联质谱条件测定样品和标准工作溶液,响应值均应在仪器检测的线性范围内,以色谱峰面积外标法定量,负离子模式扫描。在上述色谱条件下,环己基氨基磺酸钠的参考保留时间约为 5 min。环己基氨基磺酸钠标准溶液的选择性离子流图参见附录 B 中图 B.1。MS2 碎片质谱图参见附录 B 中图 B.2。

6.2.3.2 如果样液与标准工作溶液的选择性离子流色谱图中,在相同保留时间有色谱峰出现,可用正、负离子模式同时扫描(正离子模式选 202.2/122.0;负离子模式选 178.2/79.8),根据产生的两组离子对之间的丰度比,对其进行确证。负离子模式和正离子模式下的液相色谱-质谱/质谱仪参数见附录 A。

6.2.3.3 在相同实验条件下,试样中待测物质的保留时间与标准工作溶液中对应的保留时间偏差在 ±2.5% 之内;且试样谱图中各组分定性离子的相对丰度与标准工作溶液中定性离子的相对丰度,其允

许偏差不得超过表 1 规定的范围时,则可确定为样品中存在这种待测物。

表 1 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

6.3 空白试验

除不加试样外,均按上述操作步骤进行。

7 结果计算和表述

用色谱数据处理机或按式(1)计算试样中环己基氨基磺酸钠含量,计算结果应扣除空白值。

$$X = \frac{A \cdot c \cdot V}{A_s \cdot m} \dots\dots\dots(1)$$

式中:

X——试样中环己基氨基磺酸钠含量,单位为毫克每千克(mg/kg);

A——样液中环己基氨基磺酸钠的色谱峰面积;

A_s——标准工作液中环己基氨基磺酸钠的色谱峰面积;

c——标准工作液中环己基氨基磺酸钠的浓度,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);

m——最终样液所代表的试样质量,单位为克(g)。

8 测定低限、回收率

8.1 测定低限和确证低限

测定低限和确证低限为 0.10 mg/kg。

8.2 添加浓度范围及回收率

本方法的添加浓度范围及回收率试验数据见表 2。

表 2 本方法添加浓度及回收率范围

样品名称	添加浓度范围/(mg/kg)	回收率范围/%
浓缩葡萄汁	0.10~1.00	81.2~101.0
糕点	0.10~1.00	80.2~99.4
甜面酱	0.10~1.00	81.5~99.9
酱菜	0.10~1.00	82.6~101.3
糖果	0.10~1.00	80.1~100.0
白酒	0.10~1.00	84.9~100.0
罐头	0.10~1.00	82.1~102.0

附录 A
(资料性附录)

负离子模式和正离子模式下的液相色谱-质谱/质谱参数参考条件¹⁾

A.1 负离子模式下液相色谱-质谱/质谱参数参考条件

监测离子对及电压参数:

- a) 电喷雾电压(IS): -4 500 V;
- b) 雾化气压力(GS1): 10 Psi;
- c) 气帘气压力(CUR): 10 Psi;
- d) 辅助气压力(GS2): 30 Psi;
- e) 离子源温度(TEM): 500 °C;
- f) 碰撞气(CAD): 10 mL/min;
- g) 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量(CE)、碰撞池出口电压(CXP)、碰撞池入口电压(EP)见表 A.1。

表 A.1 环己基氨基磺酸钠定性、定量离子对、碰撞气能量(CE)、去簇电压(DP)、碰撞池出口电压(CXP)、碰撞池入口电压(EP)

分析物	Q1/Q3 (m/z)	驻流时间/ ms	解簇电压 DP/V	碰撞池入口电压 EP/V	碰撞气能量 CE/V	碰撞池出口电压 CXP/V
环己基氨基磺酸钠	178.2/79.8	200	-60	-9	-37	-8

A.2 正离子模式下液相色谱-质谱/质谱参数参考条件

监测离子对及电压参数:

- a) 电喷雾电压(IS): 5 000 V;
- b) 雾化气压力(GS1): 40 Psi;
- c) 气帘气压力(CUR): 20 Psi;
- d) 辅助气压力(GS2): 45 Psi;
- e) 离子源温度(TEM): 500 °C;
- f) 碰撞气(CAD): 7 mL/min;
- g) 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量(CE)、碰撞池出口电压(CXP)、碰撞池入口电压(EP)见表 A.2。

表 A.2 环己基氨基磺酸钠定性、定量离子对、碰撞气能量(CE)、去簇电压(DP)、碰撞池入口电压(EP)、碰撞池出口电压(CXP)

分析物	Q1/Q3 (m/z)	驻流时间/ ms	解簇电压 DP/V	碰撞池入口电压 EP/V	碰撞气能量 CE/V	碰撞池出口电压 CXP/V
环己基氨基磺酸钠	202.2/122.0	500	48.08	9.14	12.50	6.30

1) 非商业性声明: 所列参数是在 API4000 质谱仪上完成的, 此处列出试验用仪器型号仅是为了提供参考, 并不涉及商业目的, 鼓励标准使用者尝试采用不同厂家或型号的仪器。

附录 B
(资料性附录)

环己基氨基磺酸钠标准品的液相色谱-串联质谱选择离子色谱图和液相色谱-串联质谱图

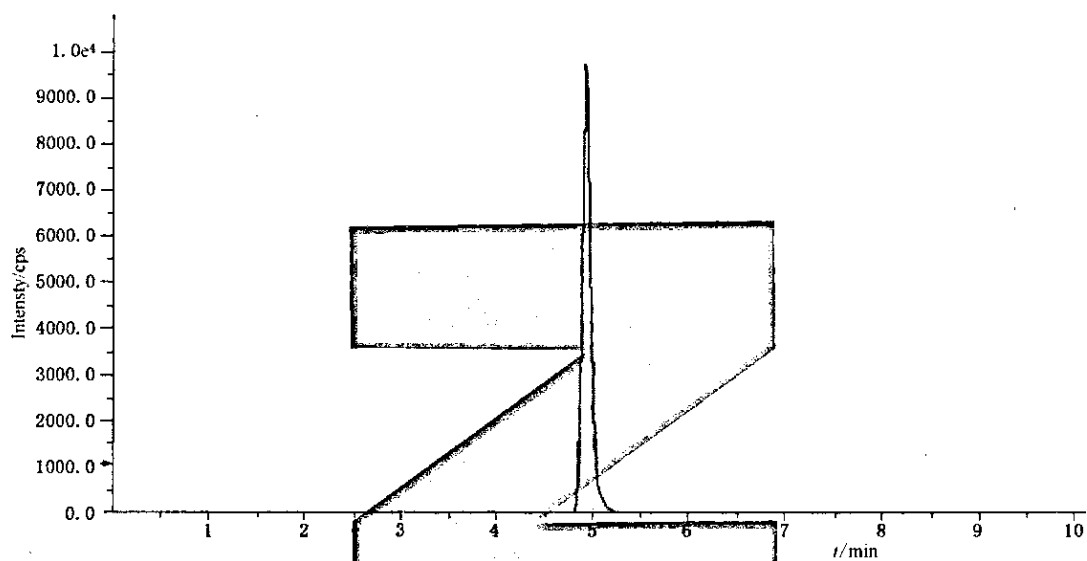


图 B.1 环己基氨基磺酸钠标准品的液相色谱-串联质谱选择离子色谱图

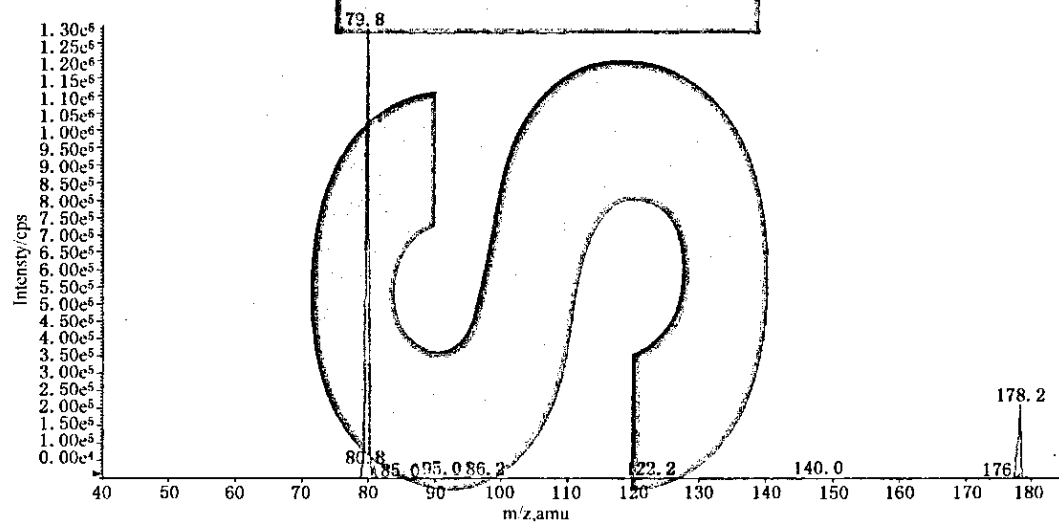


图 B.2 环己基氨基磺酸钠标准品的液相色谱-串联质谱图

Foreword

Annex A and Annex B of this standard are informative.

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

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

This standard was mainly drafted by Wang Mingtai, Mu Jun, Dai Hua, Ma Xiaogang, Zhang Daihui, Zhou Xiao, Li Aijun, and Chang Donghua.

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

Determination of sodium cyclamate in foods for import and export—LC-MS/MS method

1 Scope

The standard specifies the method for sample preparation and determination of sodium cyclamate in foods for import and export by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

This standard is applicable to determination and confirmation of sodium cyclamate content in foods including canned fruit, thick grape juice, wine, cake, candy, sweet sauce, and pickled vegetables for import and export.

2 Principle

The test sample is extracted with water by one ultrasonic processor. After centrifugation, the supernatant is determined and quantified by LC-MS/MS using external standard method.

3 Reagents and materials

All the reagents used should be of analytical grade unless specified. "Water" is redistilled water.

3.1 Acetic acid.

3.2 Acetonitrile, GC grade.

3.3 0.1% acetic acid-water solution (V/V); take 4 mL of acetic acid and dilute with water to 4 000 mL.

3.4 Sodium cyclamate standard ($C_6H_{12}NNaO_3S$, CAS No. 139-05-9); purity $\geq 99\%$.

3.5 Standard stock solution; accurately weigh appropriate amount of sodium cyclamate standard and dissolve with a little volume of water. Dilute with water to 500 $\mu\text{g}/\text{mL}$ as standard stock solution. The solution is stored in a refrigerator at range of $0^\circ\text{C} \sim 4^\circ\text{C}$.

3.6 Intermediate standard solution; transfer a certain volume of the standard stock solution. Dilute with water to make 1 $\mu\text{g}/\text{mL}$ of intermediate standard solution. The solution is stored in a refrigerator at range of $0^\circ\text{C} \sim 4^\circ\text{C}$.

3.7 Standard working solution; transfer a certain volume of the intermediate standard solution. Dilute with water to make required standard solution. The solution is stored in refrigerator at

0℃ ~4℃.

3.8 Film:0.45 μm (water phase).

4 Apparatus and equipments

4.1 LC-MS/MS: Equipped with electrospray ionization (ESI).

4.2 Vortex mixer.

4.3 Ultrasonic processor.

4.4 Centrifuge.

5 Preparation and storage of test sample

5.1 Preparation of test sample

5.1.1 Canned fruit

Open original sample at the room temperature. Weigh the liquid and the solid separately. Take approximate 1 kg of representative sample. Smash thoroughly by a vortex mixer. Put in clean containers. Seal and label them.

5.1.2 Wine and juice

Take approximately 1 kg of original sample. Smash thoroughly in a ceramic barrel. Put into clean containers. Seal and label them.

5.1.3 Cake and candy

Take approximate 1 kg of representative sample. Quarter the sample to 200 g according to diagonal method. Grind into mince in a glass motor. Mix thoroughly. Put in clean containers. Seal and label them.

5.1.4 Sweet sauce and pickled vegetable

Take approximate 1 kg of representative sample. Quarter the sample to 500 g according to diagonal method. Crush by a crusher. Put in clean containers. Seal and label them.

5.2 Storage of test sample

The samples should be stored at 0℃ ~4℃. In the course of sampling and sample preparation, it

should be taken to avoid contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction

6.1.1 Canned fruit, cakes, candy, sweet sauce, pickled vegetables and concentrated juice

Weigh approximately 1.0 g (accurate to 0.01 g) of the test sample into a 50 mL centrifuge tube. Add 8.0 mL water and mix thoroughly. Then extract for 20 min in the ultrasonic processor. Centrifuge for 10 min at 4 000 r/min. Filter the extract into a calibrated flask and dilute with water to final volume of 10 mL. The supernatant layer is passed through a 0.45 μm film. The filtrate is for the LC-MS/MS determination and confirmation.

6.1.2 Wine

Accurately transfer 2 mL of wine. Pass through 0.45 μm film. The filtrate is for the LC-MS/MS determination.

6.2 Determination

6.2.1 LC operating condition

- Chromatographic column: Extend-C 18, 250 mm \times 4.6 mm (i. d.), size 5 μm , or equivalent;
- 0.1% acetic acid aqueous solution-methanol; (4 + 6, V/V);
- Flow rate: 0.6 mL/min;
- Injection volume: 5 μL ;
- Column temperature: room temperature.

6.2.2 MS/MS condition

- Ionization source: ESI;
- Scan mode: negative ion mode;
- Monitor mode: multiple reaction monitoring (MRM);
- Nebulizer gas, curtain gas, auxiliary gas, and collision gas are all ultra pure nitrogen. Before usage

all gas flow must be optimized to guarantee the required sensitivity of the MS detector. The detailed parameters are shown in Annex A. 1.

e) IS,GS1,CUR,GS2,DP, and CE should be optimized to the most sensitive level. The referred conditions and the ions for confirmation and quantification are showed in Annex A. 1.

6.2.3 LC-MS/MS determination and confirmation

6.2.3.1 According to the above LC-MS/MS operating condition,determine the sample solution and the working standard solution. The responses should be within the linear range of the instrument detection. Quantification is calculated by chromatographic peak area using external standard method. At the above-mentioned chromatographic operating condition,the retention time of sodium cyclamate is about 5.0 min. Selected ion current chromatogram of sodium cyclamate is figure B. 1 in Annex B. The mass spectrum of the MS2 fragments is figure B. 2 in Annex B.

6.2.3.2 If a respected peak of sample solution appears at the same retention time as that of the peak of the standard in the selected ion current chromatograph,both positive ion mode and negative ion mode can be scanned (choose 202. 2/122. 0 at positive ion mode; 178. 2/79. 8 at negative ion mode),and confirmation is performed according to the abundance ratio of these two groups of ions. The parameters of LC-MS/MS at negative ion mode and positive ion mode are listed in Annex A.

6.2.3.3 At the same experimental status,if the deviation between the retention time of the test sample and the time of the standard working solution is within ± 2. 5%,and the tolerances between the relative abundance of the qualification ions of the test sample and that of the standard working solution is not over the range described in Table 1,the targeted compound exists in the sample is confirmed.

Table 1—Maximum permitted tolerances for relative ion intensities while confirmation

Relative ionic abundance/%	>50	>20~50	>10~20	≤10
Allowed relative deviation/%	± 20	± 25	± 30	± 50

6.4 Blank test

Blank test will be conducted according to the procedures above without sample addition.

7 Calculation and expression of result

Calculate the content of sodium cyclamate in the test sample by data processor of the work station of this spectrometer or according to the formula (1) The result of calculation should be deducted with blank value.

$$X = \frac{A \cdot c \cdot V}{A_s \cdot m} \dots\dots\dots(1)$$

where

X —the residue content of sodium cyclamate in the test sample, mg/kg.

A —the peak height of sodium cyclamate in the sample solution.

A_s —the peak height of sodium cyclamate in the standard working solution.

c —the concentration of sodium cyclamate in the standard working solution, $\mu\text{g/mL}$.

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

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

8 Detection limit and recovery

8.1 Limit of quantification

The limit of quantification of the method is 0.10 mg/kg.

8.2 Range of fortification and recovery

The ranges of fortification and recovery of this method are shown in table 2.

Table 2—The range of fortification and recovery of this method

Sample	Fortification range/(mg/kg)	Recovery range/%
concentrate grape juice	0.10~1.00	81.2~101.0
cake	0.10~1.00	80.2~99.4
sweet sauce	0.10~1.00	81.5~99.9
pickled vegetable	0.10~1.00	82.6~101.3
candy	0.10~1.00	80.1~100.0
wine	0.10~1.00	84.9~100.0
can	0.10~1.00	82.1~102.0

Annex A
(informative annex)

Referred LC-MS/MS parameters under negative ion mode and positive ion mode¹⁾

A. 1 Referred LC-MS/MS parameters under negative ion mode

Monitoring ions and voltages:

- a) Electrospray voltage (IS): -4 500 V;
- b) Ion source gas 1 (GS1): 10 Psi;
- c) Curtain gas pressure (CUR): 10 Psi;
- d) Ion source gas 2 (GS2): 30 Psi;
- e) Temperature (TEM): 500°C;
- f) Collisionally activated dissociation gas (CAD): 10 mL/min;
- g) Qualification ion pairs, quantitation ion pair, declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), entrance potential (EP) is shown in Table A. 1

Table A. 1—Qualification ion pairs, quantitation ion pair, declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), entrance potential (EP)

Compound	Q1/Q3(m/z)	Dwell time/ms	DP/V	EP/V	CE/V	CXP/V
sodium cyclamate	178. 2/79. 8	200	-60	-9	-37	-8

A. 2 Referred LC-MS/MS parameters under positive ion mode

Monitoring ions and voltages:

- a) Electrospray voltage (IS): 5 000 V;
- b) Ion source gas 1 (GS1): 40 Psi;
- c) Curtain gas pressure (CUR): 20 Psi;
- d) Ion source gas 2 (GS2): 45 Psi;

1) Non-commercial statement: the reference mass parameters in Annex A are accomplished by API 4 000 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.

- e) Temperature (TEM): 500°C ;
- f) Collisionally activated dissociation gas (CAD): 7 mL/min ;
- g) Qualification ion pairs, quantification ion pair, declustering potential (DP) , collision energy (CE), collision cell exit potential (CXP), entrance potential (EP) is shown in Table A. 2

Table A. 2—Qualification ion pairs, quantification ion pair, declustering potential (DP) ,
collision energy (CE), collision cell exit potential (CXP), entrance potential (EP)

Compound	Q1/Q3(m/z)	Dwell time/ms	DP/V	EP/V	CE/V	CXP/V
sodium cyclamate	202. 2/122. 0	500	48. 08	9. 14	12. 50	6. 30

Annex B
(informative annex)

LC-MS/MS selected ion chromatogram and LC-MS/MS spectrum of sodium cyclamate standard

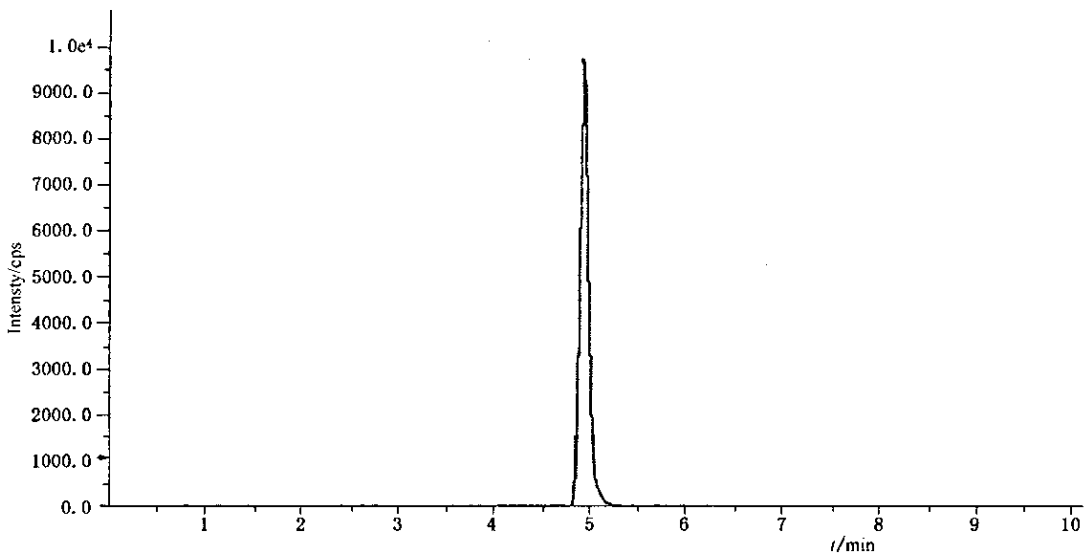


Figure B. 1—LC-MS/MS selected ion chromatogram of sodium cyclamate standard

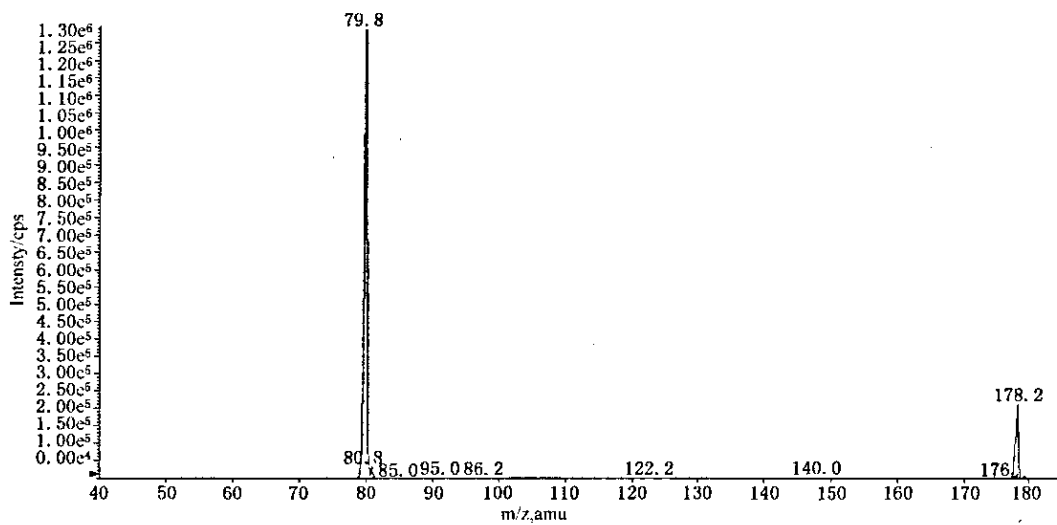


Figure B. 2—LC-MS/MS spectrum of sodium cyclamate standard

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