ICS 67.050
CCS X 04

SN

中华人民共和国出入境检验检疫行业标准

SN/T 5520—2023

动物源食品中苯乙醇胺A的测定 液相色谱-质谱/质谱法

Determination of phenylethanol amine A residues in foodstuffs for animal origin—Liquid chromatography tandem mass spectrometry method

2023-11-01 发布　　2024-05-01 实施

中华人民共和国海关总署　　发布

前　言

本文件按照GB/T 1.1—2020《标准化工作导则　第1部分：标准化文件的结构和起草规则》的规定起草。

请注意本文件的某些内容可能涉及专利。本标准的发布机构不承担识别专利的责任。

本文件由中华人民共和国海关总署提出并归口。

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动物源食品中苯乙醇胺 A 的测定 液相色谱-质谱/质谱法

1 范围

本文件规定了动物源食品中苯乙醇胺 A 残留量的液相色谱-质谱/质谱检测方法。

本文件适用于畜肉及内脏、猪肠衣、羊肠衣、鸡肉、牛奶等动物源食品中苯乙醇胺 A 的测定。

2 规范性引用文件

下列文件中的内容通过文中的规范性引用而构成本文件必不可少的条款。其中，注日期的引用文件，仅该日期对应的版本适用于本文件；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 术语和定义

本文件没有需要界定的术语和定义。

4 方法提要

试样中苯乙醇胺 A 残留用 β-葡萄糖醛酸甙酶/芳香硫酸酯酶解后乙酸铵溶液提取，正己烷脱脂，经混合型阳离子交换固相萃取柱净化后，液相色谱-质谱/质谱仪测定，同位素内标法定量。

5 试剂和材料

除另有规定外，所有实验用试剂均为分析纯，水为 GB/T 6682 中规定的一级水。

5.1 甲醇：色谱纯。

5.2 乙腈：色谱纯。

5.3 乙酸乙酯：色谱纯。

5.4 正己烷：色谱纯。

5.5 乙酸铵：色谱纯。

5.6 甲酸：色谱纯。

5.7 冰乙酸。

5.8 氨水：25%～28%(ω/%)。

5.9 盐酸：36%～38%(ω/%)。

5.10 β-葡萄糖醛酸甙酶/芳香硫酸酯酶：含 β-葡萄糖醛酸甙酶 111 000 U/mL，芳香硫酸酯酶 1 079 U/mL。

5.11 0.1 mol/L 盐酸溶液：吸取 0.85 mL 盐酸，用水定容至 100 mL，混匀。

5.12 0.1%甲酸溶液：准确量取 1.0 mL 甲酸，用水定容至 1 000 mL，混匀。

5.13　乙腈-0.1%甲酸溶液(1∶9,体积比):移取50 mL水,加入0.1 mL甲酸和10 mL乙腈,用水定容至100 mL混匀。

5.14　0.02 mol/L乙酸铵溶液:称取1.54 g乙酸铵加入600 mL～700 mL水溶解,用冰乙酸调节pH值为5.2±0.2,用水定容至1 000 mL。

5.15　洗脱溶液,氨水-甲醇-乙酸乙酯(5∶45∶50,体积比):准确量取5 mL氨水、45 mL甲醇、50 mL乙酸乙酯混合均匀。

5.16　标准品:苯乙醇胺A($C_{19}H_{24}N_2O_4$,CAS号:1346746-81-3),纯度≥99%。

5.17　内标:苯乙醇胺A-D_3($C_{19}H_{21}D_3N_2O_4$),纯度≥99%。

5.18　标准储备溶液:准确称取苯乙醇胺A标准品(5.16)、苯乙醇胺A-D3标准品(5.17)各10 mg(精确到0.1 mg)于100 mL容量瓶中,用甲醇溶解并定容至刻度,配制成质量浓度为100 μg/mL的标准储备液,于－18 ℃冰箱中保存,有效期9个月。

5.19　标准中间工作溶液:分别吸取1 mL标准储备溶液(5.18)用甲醇稀释至100 mL,该标准中间工作溶液1.0 μg/mL的标准工作液,于－18 ℃冰箱中保存,有效期2个月。

5.20　标准工作溶液:分别吸取适量标准中间工作液(5.19)用0.1%甲酸溶液-乙腈(5.13)稀释,得到0.1 μg/mL的标准工作液,该溶液临用前配置。

5.21　系列标准工作溶液:分别取不同体积的标准工作溶液(5.18)和50 μL的内标标准工作溶液(5.18)配置标准系列浓度为0.3 ng/mL、0.5 ng/mL、1.0 ng/mL、2.0 ng/mL、5.0 ng/mL、10 ng/mL(内标浓度为5.0 ng/mL),该溶液临用前配置。

5.22　固相萃取(SPE)柱:混合型阳离子交换固相萃取柱,填料为苯磺酸化的聚苯乙烯-二乙烯基苯高聚物,60 mg/3 mL或相当者,使用前依次用3 mL甲醇、3 mL水、3 mL0.1 mol/L盐酸活化,保持柱体湿润。

5.23　有机相滤膜:0.22 μm。

5.24　定性滤纸。

6　主要仪器和设备

6.1　液相色谱-质谱/质谱仪:配备电喷雾离子源(ESI)。

6.2　天平:感量为0.1 mg和0.01 g。

6.3　恒温振荡水浴锅。

6.4　氮吹仪。

6.5　涡旋振荡混合器。

6.6　离心机:转速≥10 000 r/min。

6.7　固相萃取仪。

6.8　具塞离心管:10 mL。

6.9　均质器:转速大于10 000 r/min。

6.10　组织绞碎机。

7　样品制备与保存

7.1　动物肌肉、内脏

取出代表性样品约500 g,组织绞碎机绞碎,均分成两份,分别装入洁净容器中,密封,并标明标记,于－18 ℃以下冷冻存放。

7.2 肠衣

盐渍肠衣样品先用剪刀剪成小于 5 mm 宽的段，用清水洗去盐分，再用蒸馏水冲洗，在丝网器皿上摊开沥去水分 5 min，混合均匀，均分成两份，分别装入洁净容器内密封并做好标识，于－18 ℃以下冷冻保存。

7.3 牛奶

取 500 mL 牛奶于洁净容器中，混合均匀，于－18 ℃以下冷冻存放。

在样品制备和保存过程中，应防止样品受到污染或者发生残留物含量的变化。

8 测定步骤

8.1 提取

8.1.1 动物肌肉、内脏、肠衣

称取 2.0 g 试样（精确至 0.01 g），置于 50 mL 离心管内。加入 0.1mL 内标液（5.20）、10 mL 0.02 mol/L 乙酸铵溶液（5.14）和 0.05 mLβ-葡萄糖醛酸甙酶/芳香硫酸酯酶溶液（5.10）均质（6.9）提取 1 min，涡旋混合 3 min，置于 37 ℃±2 ℃水浴锅（6.3）振荡酶解 16 h，4 ℃条件下 10 000 r/min 下离心 5 min，转移全部上清液与一新离心管内，加入 15 mL 正己烷（5.4）涡旋混合 3 min，4 ℃条件下 10 000 r/min 下离心 5 min，弃去上层正己烷，制得样品提取液待净化。

8.1.2 牛奶

称取 2.0 g 试样（精确至 0.01 g），置于 50 mL 离心管内。加入 0.1mL 内标液（5.20）、10 mL 0.02 mol/L 乙酸铵溶液（5.14）和 0.05 mLβ-葡萄糖醛酸甙酶/芳香硫酸酯酶溶液（5.10）涡旋混合 3 min，置于 37±2℃水浴锅（6.3）振荡酶解 16 h，－18 ℃下冷冻 10 min，4 ℃条件下 10 000 r/min 下离心 3 min，转移全部上清液，加入 15 mL 正己烷（5.4）涡旋混合 3 min，4 ℃条件下 10 000 r/min 下离心 5 min，弃去上层正己烷，下层液体经滤纸（5.23）过滤，并用 2 mL 乙酸铵溶液（5.14）洗涤滤纸，合并滤液制得样品提取液待净化。

8.2 净化

转移全部样品提取液进行固相萃取柱（5.22）净化，使液体流速小于 1.0 mL/min，待样品提取液全部通过后，依次用 3 mL 水、3 mL 甲醇淋洗固相萃取柱，保持抽干 1 min，用 4 mL 洗脱溶液（5.15）洗脱，收集流出液，并于 40 ℃氮气流下吹干。准确加入 2 mL 乙腈-0.1%甲酸溶液（1∶9，体积比）（5.13）溶解残渣，经 0.22 μm 滤膜（5.23）过滤，滤液供液相色谱-质谱/质谱测定。

8.3 测定

8.3.1 液相色谱仪器条件

液相色谱条件如下：

a) 色谱柱：C_{18} 柱，100 mm×2.1 mm（内径），粒径 1.8 μm，或性能相当者；

b) 柱温箱：25 ℃；

c) 进样量：5 μL；

d) 流速：0.3 mL/min；

e) 流动相梯度洗脱程序见表 1；

表 1　流动相洗脱梯度表

时间/min	乙腈/%	0.1%甲酸溶液/%
0	10	90
1.5	10	90
2.5	50	50
4	90	10
6	90	10
7	10	90
9	10	90

8.3.2　质谱条件

质谱条件如下：

a)　电离方式：电喷雾(ESI+)；

b)　扫描方式：正离子扫描；

c)　检测模式：多反应监测(MRM)；

d)　雾化气、气帘气、辅助气、碰撞气均为高纯氮气；使用前应调节各参数使质谱灵敏度达到检测要求，参考条件及监测离子对(m/z)参见表 A.1。

8.3.3　定性测定

按照液相色谱-质谱/质谱条件测定试样和内标混合标准工作液，试样中目标化合物的保留时间与内标混合标准工作液中对应化合物的保留时间偏差在±2.5%之内；定性离子对的相对丰度与浓度相当的基质混合标准工作液的相对丰度一致，相对丰度偏差不超过表 2 的规定，且每个离子的信噪比均≥3，则可判断试样中存在相应的被测物。

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

8.3.4　定量测定

本方法苯乙醇胺 A 采用同位素内标法定量测定，根据样液中被测物的含量情况，选定浓度相近的标准工作溶液。标准溶液和样液中苯乙醇胺 A 的响应值均应在仪器检测线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述仪器条件下，苯乙醇胺 A 的参考保留时间为 6.14 min。苯乙醇胺 A 的 MRM 图参见附录 B。

8.3.5　空白试验

除不称取试样外，均按上述测定条件和步骤进行。

9 结果计算与表述

用 LC-MS/MS 数据处理系统或按公式(1)计算试样中苯乙醇胺 A 的残留量：

$$X=\frac{c\times c_i\times A\times A_{si}\times V}{c_{si}\times A_i\times A_s\times m}\times\frac{1\ 000}{1\ 000} \quad \cdots\cdots(1)$$

式中：

X ——试样中苯乙醇胺 A 的残留量，单位为微克每千克(μg/kg)；

c ——标准工作溶液中苯乙醇胺 A 的浓度，单位为纳克每毫升(ng/mL)；

c_i ——样品中内标物苯乙醇胺 A-D_3 的浓度，单位为纳克每毫升(ng/mL)；

A ——样品中苯乙醇胺 A 的峰面积；

A_{si} ——标准工作溶液中内标物苯乙醇胺 A-D_3 的峰面积；

V ——样品定容液体积，单位为毫升(mL)；

c_{si} ——标准工作溶液中内标物苯乙醇胺 A-D_3 的浓度，单位为纳克每毫升(ng/mL)；

A_i ——样品溶液中内标物苯乙醇胺 A-D_3 的峰面积；

A_s ——标准工作溶液中苯乙醇胺 A 的的峰面积；

m ——最终样液所代表的试样质量，单位为克(g)。

计算结果需将空白值扣除结果，结果保留 3 位有效数字。

10 定量限与回收率

10.1 定量限

本方法的定量限为 0.5 μg/kg。

10.2 回收率

样品的添加浓度及回收率的试验数据见表 C.1。

附 录 A
（资料性）
质谱系统参考条件

定量离子对、定性离子对、去簇电压、碰撞气能量见表 A.1。

a） 碰撞气压力(CAD)：68.9 kPa；
b） 雾化气压力(GS1)：275.6 kPa；
c） 辅助器压力(GS2)：275.6 kPa；
d） 气帘气压力(CUR)：172.3 kPa；
e） 喷雾电压(IS)：4 500 V；
f） 辅助气温度(TEM)：550 ℃；
g） 每个离子对驻留时间：100 ms；
h） 射入电压(EP)：8 V；
I） 碰撞室出口电压(CXP)：10V。

表 A.1 定量离子对、监测离子对、去簇电压、碰撞气能量[1)]

化合物	定量离子对 m/z	监测离子对 m/z	去簇电压(DP) V	碰撞气能量(CE) eV
苯乙醇胺 A	345.2/150.0	345.2/150.0	60	31
		345.2/326.8	70	17
苯乙醇胺 A-D3	348.2/153.1	348.3/153.1	60	30

1）非商业性声明：表 A.1 所列参数是在 API 5500Q 质谱仪完成的，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不同厂家和型号的仪器 。

附 录 B
（资料性）
苯乙醇胺 A MRM 谱图

苯乙醇胺 A MRM 谱图见图 B.1。

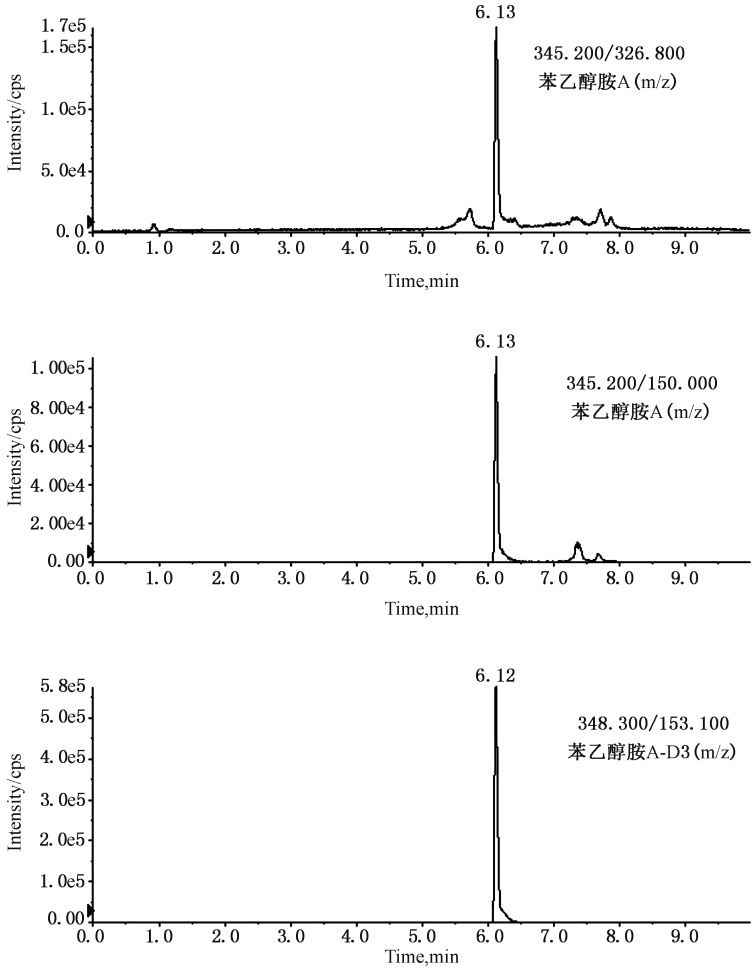

图 B.1 苯乙醇胺 A 标准溶液（0.5 ng/mL）液相色谱-质谱/质谱的多反应监测（MRM）色谱图

附 录 C
（资料性）
样品添加浓度及回收率范围

样品添加浓度及回收率范围见表 C.1。

表 C.1 样品添加浓度及回收率范围（n=6）

基质	添加水平/(μg/kg)	回收率/%	基质	添加水平/(μg/kg)	回收率/%
猪肉	0.5	97.0～103	羊肾脏	0.5	90.2～102
	1.0	88.9～105		1.0	88.8～106
	5.0	94.6～103		5.0	94.4～102
牛肉	0.5	97.2～100	猪肺脏	0.5	88.6～107
	1.0	94.5～109		1.0	87.0～95.7
	5.0	94.6～103		5.0	88.8～95.8
羊肉	0.5	93.6～97.8	牛肺脏	0.5	90.0～108
	1.0	93.0～103		1.0	79.9～105
	5.0	93.8～102		5.0	93.2～97.6
猪肝脏	0.5	90.4～100	羊肺脏	0.5	96.8～83.2
	1.0	90.2～98.0		1.0	86.4～98.7
	5.0	89.6～96.0		5.0	96.0～103
牛肝脏	0.5	92.8～99.6	猪肠衣	0.5	94.6～99.4
	1.0	82.5～106		1.0	97.5～105
	5.0	95.4～101		5.0	94.4～102
羊肝脏	0.5	78.2～93.0	羊肠衣	0.5	83.2～96.8
	1.0	87.7～99.7		1.0	86.4～98.7
	5.0	96.6～99.4		5.0	96.0～103
猪肾脏	0.5	75.6～94.8	牛奶	0.5	110～114
	1.0	84.0～94.5		1.0	99.9～106
	5.0	71.2～81.8		5.0	93.2～103
牛肾脏	0.5	87.0～96.0	鸡肉	0.5	91.0～110
	1.0	85.8～95.8		1.0	90.0～110
	5.0	88.6～98.6		5.0	88.6～103

Preface

The standard written in accordance with the rules given in GB/T 1.1—2020

"Directives for standardization—Part 1：Rules for the structure and draftng of standardizing documents".

Please note that some contents of this document may involve patents ，and the issuing agency of this document does not bear the responsibility of identifying patents.

This standard was proposed by and under the charge of General Administration of Customs，P. R. China.

This standard was drafted by Zhengzhou Customs Technology Center，Xiamen Customs Technology Center，Wuhan Customs Technology Center.

This Standard was mainly drafted by the following people：Zhang Shoujie，Zhu weixia，Wang xiangjun，Sun zhuanlian，Yang jizhou，Xu dunming，Zhang shuxia，Wang sufang，Zhao xiaoya，Shi fang，Liu zhaohui，Qiao qing，Liu junhong.

Determination of phenylethanol amine A residues in foodstuffs for animal origin—Liquid chromatography tandem mass spectrometry method

1 Scope

This standard specifies the method of Determination of Phenylethanol amine A residues in foodstuffs of animal origin-LC-MS/MS.

This standard is applicable to the determination of Phenylethanol amine A in livestock meats and entrails , pig casing,sheep casing,chicken,milk etc. Other foods may be also tested by consulting this standard.

2 Normative references

The contents of the following documents constitute essential provisions of this document through normative references in the text. Among them, the date of the reference document, only the date of the corresponding version applicable to this document; no date of the reference document, its latest version (including all amendments) applicable to this document.

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

3 Terms and Definitions

There are no terms and definitions to be defined in this document.

4 Principle

The samples were extracted with ammonium acetate solution after enzymolysised, degreased by n-hexane, cleaned up by Mixed cation exchange solid phase extraction column, detected by liquid chromatography-mass spectrometry / mass spectrometry, and quantified by isotope internal standard method.

5 Reagents and materials

Unless otherwise specified, all reagents used should be of analytically pure, water is the first grade water prescribed by GB / T 6682.

5.1 Methanol: HPLC grade.

5.2 Acetonitrile: HPLC grade.

5.3 Ethyl acetate: HPLC grade.

5.4 N-hexane: HPLC grade.

5.5 Ammonium acetate: HPLC grade.

5.6 Formic acid: HPLC grade.

5.7 Glacial acetic acid.

5.8 Ammonia: 25%～28%(ω/%).

5.9 Hydrochloric acid: 36%～38%(ω/%).

5.10 β-Glucuronidase / aromatic sulfatase solution: contents β-Glucuronidase111 000 U/mL; aromatic sulfatase 1 079 U/mL.

5.11 0.1mol/L Hydrochloric acid: Transfer 0.85 mL hydrochloric acid to 100 mL water and mix well.

5.12 0.1% Formic acid: Transfer1mLFormic acid , make the volume to 1000 mL with water and mix well.

5.13 Acetonitrile-0.1% Formic acid(1 + 9, Volume ratio): Transfer 0.1 mL Formic acid to 50 mL water, add 10 mLAcetonitrile, make the volume to 100 mL with water and mix well.

5.14 0.02mol/L Ammonium acetate solution: Transfer1.54 gAmmonium acetateDissolve in water and bring to Volume 1 000 mL, charge PH = 5.2 ± 0.2 with Glacial acetic acid.

5.15 Eluant: Ammonia + Methanol + Ethyl acetate = 5 + 45 + 50, Volume ratio.

5.16 Phenylethanol amine A, $C_{19}H_{24}N_2O_4$, CAS NO: 1346746-81-3: Purity≥99%.

5.17 Phenylethanol amine A-D_3, $C_{19}H_{21}D_3N_2O_4$: Purity≥99%.

5.18 Standard stock solution: Accurately weigh 10 mg (accurate to 0.1 mg) of standard phenylethanol amine A(5.16), Phenylethanol amine A-D3(5.17) into a 10 ml volumetric flask, Dissolve in methanol and bring to volume, the oncentration is 1 mg / mL, Keep in refrigerator below −18 ℃, term of validity is 9 months.

5.19 Intermediate working solution: Transfer 1 mL Phenylethanol amine A(5.18), Phenylethanol amine A-D_3(5.18) Standard stock solution, Dilute to 100 mL with methanol, The standard working solution of 0.01 mg / ml was obtained, Keep in refrigerator below −18 ℃, term of validity is 2 months.

5.20 Standard working solution: Draw some Phenylethanol amine A (5.19), Phenylethanol amine A-D3(5.19) Standard Intermediate working solutiondiluted to 0.1 μg/mL Standard working solution with0.1% Formic acid-Acetonitrile(5.13), The solution is prepared before use.

5.21 Take different volumes of standard working solution (5.20) and 50 μL The standard concentration of the internal standard working solution is 0.3, 0.5, 1.0, 2.0, 5.0, 10 ng / mL (the internal standard concentration is 5.0 ng / mL).

5.22 SPE Column: Mixed cation exchange solid phase extraction column, the filler is benzene sulfonated polystyrene divinylbenzene polymer, 60 mg/3 mLOr equivalent, Before used, activated the column with 3 mL methanol, 3 mL water, 3 mL0.1 mol/LHydrochloric acid, Keep the column moist.

5.23 Organic phase membrane: 0.22 μm.

5.24 Qualitative filter paper.

6 Apparatus and equipment

6.1 High performance liquid chromatography-mass spectrometry / mass spectrometry: equipped with electrospray ionization source(ESI).

6.2 Balance: sensitivity is 0.1 mg and 0.01 g.

6.3 Constant temperature oscillation water bath.

6.4 Nitrogen evaporator.

6.5 Vortex mixer.

6.6 Centrifuge: ≥10 000 r/min.

6.7 Solid phase extractor.

6.8 Graduated centrifuge tube:10 mL.

6.9 Homogenizer:≥10 000 r/min.

6.10 Tissue grinder.

7 Sample preparation and storage

7.1 Animal muscle, viscera

About 500 g of representative samples were taken from all the samples, thawed at room temperature and broken, and divided into two parts, respectively put into clean containers, sealed and marked. One was used as the test sample, and the other was frozen below −18 ℃.

7.2 Casings

The salted casings were first cut into sections less than 5 mm wide with scissors, washed with clean water to remove the salt, then washed with distilled water, spread on the screen utensils to drain the water for 5 min, mixed evenly, and divided into two parts, sealed and marked in clean containers, and frozen below −18 ℃.

7.3 Milk

Warm the milk sample to room temperature, mix thoroughly.

In the course of sampling and sample preparation, precautions must be taken to avoid the contamination or any factors which may cause the change of residues content.

8 Procedure

8.1 Extraction

8.1.1 Animal muscle, viscera, casings

Weigh 2.0 g(accurate to 0.01 g)of the test sample into a 50 mL centrifuge tube, add 0.1 mL

Phenylethanol amine A-D_3 Internal Standard working solution(5.20),10 mL 0.02 mol/L Ammonium acetate solution(5.14)and 0.05 mL β-Glucuronidase / aromatic sulfatase solution(4.23)homogenized (6.9) 1 min,Vortex mixed 3 min,Put in 37±2 ℃ Constant temperature oscillation water bath(6.3)

enzymolysised 16 h, 4 ℃ Centrifugated 5 min at 10 000 r/min, Transfer all of the supernatant to a new centrifuge tube, add 15mL N-hexane (5. 4), Vortex mixed 3 min, 4 ℃ Centrifugated 5 min at 10 000 r/min, Discard the supernatant, Lower liquid to be cleanup.

8. 1. 2 Milk

Weigh 2. 0 g(accurate to 0. 01g)of the test sample into a 50 mL centrifuge tube, add 0. 1 mL Phenylethanol amine A-D3 Internal Standard working solution(5. 20), 10 mL0. 02mol/L Ammonium acetate solution(5. 14)and 0. 05 mLβ-Glucuronidase/aromatic sulfatase solution(5. 10)Vortex mixed 3 min, Put in 37±2℃ Constant temperature oscillation water bath(6. 3)enzymolysised 16 h, Put in Refrigerator at -18 ℃ for 10 min, 4 ℃ Centrifugated 3 min at 10 000 r/min, Transfer all the supernatant, add 15mL N-hexane(5. 4), Vortex mixed 3 min, 4 ℃ Centrifugated 3 min at 10 000 r/min, Discard the supernatant, Lower liquid to be filtered(5. 24), with 2 mL0. 02mol/L Ammonium acetate solution(5. 14) washed the filter paper, Combined filtrate to be cleanup.

8. 2 Cleanup

Take all the extract or filtrate and pass through the SPE column(5. 22), make the liquid flow rate less than 1. 0 ml / min, Drip washing the SPE successively with 3 mLwater, 3 mLmethanol, after Drain, use 4 mL eluant(5. 15)to eluent, the eluant is evaporated to near dryness at 40 ℃ under a stream of nitrogen, then 2. 0 mL Acetonitrile-0. 1% Formic acid(1+9, Volume ratio) (5. 13)is added to reconstitute the residue. After being filtrated with a 0. 22 μm organic membrane(5. 23), the final solution is ready for analysis by LC-MS / MS.

8. 3 Determination

8. 3. 1 LC operating condition

LC operating condition:

a) LC column: C_{18}, 100 mm × 2. 1 mm(i. d.), 1. 8 μm, equivalent;

b) Column temperature: 25 ℃;

c) Injection volume: 5 μL;

d) Flow rate: 0. 3 mL / min;

e) The grade of mobile phase is listed in Table 1.

Table 1 —The grade of mobile phase list

Time/min	Acetonitrile/%	0.1% Formic acid/%
0	10	90
1.5	10	90
2.5	50	50
4	90	10
6	90	10
7	10	90
9	10	90

8.3.2 Mass spectrometry conditions

Mass spectrometry conditions:

a) Ion source: electrospray ionization source(ESI+);

b) Scanning model: positive ion.;

c) Monitoring model: multiple reaction monitor(MRM);

d) Nebulizer gas, curtain gas and auxiliary gas are high purity nitrogen. They are optimized by adjusting the gas flow parameters. Ion spray voltage, deflector voltage, collision energy and so on should be optimized to reach the highest sensitive of mass spectrometer, referenced conditions are showed in Table A.1.

8.3.3 Confirmation

Under the LC-MS / MS operating conditions, the Internal matched mixed standard working solution and sample solution are injected. If the retention times of sample chromatogram peaks are consistent with that of standard solution with the difference less than ±2.5%. The relative intensities of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of standard solution should be the same with those of sample solution. The signal-to-noise ratio of monitoring ions of sample should not be lower than 3. The permitted tolerances lists in Table 2, then the corresponding analyte must be presented in sample.

Table 2 —Maximum permitted tolerances relative ion intensities while confirmation

Relative intensity/%	>50	>20~50(inclusive)	>10~20(inclusive)	≤10
Permitted tolerances/%	±20	±25	±30	±50

8.3.4 Quantitation determination

The internal standard method was used for quantitative determination of Phenylethanol amine A, According to the content of the tested substance in the sample solution, the standard working solution with similar concentration is selected. The responses of analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. Determination of standard working solution and sample solution by equal volume reference injection. Under the above instrument conditions, The reference retention time of Phenylethanol amine A is 6.14 min. See MRM chromatogram of Phenylethanolamine A in annex B.

8.3.5 Blank test

The operation of the blank test is the same as that described in the method of determination, but with the omission of sample addition.

9 Calculation and expression of the result

Calculate the content of Phenylethanol amine A residues in the test sample by LC-MS / MS data processor or according to the followed formula (1):

$$X = \frac{c \times c_i \times A \times A_{si} \times V}{c_{si} \times A_i \times A_s \times m} \times \frac{1\,000}{1\,000} \quad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

Where:

X —the residue content of Phenylethanol amine A in the test sample, μg/kg;

c —the concentration of Phenylethanol amine A obtained from the sample solution, ng/mL;

c_i —the concentration of Phenylethanol amine A-D_3 Internal Standard obtained from the sample solution, ng/mL;

A —the area of Phenylethanol amine A in sample;

A_{si} —the area of Phenylethanol amine A-D_3 in Standard working solution;

V —Constant volume of sample, mL;

c_{si} —the concentration of Phenylethanol amine A-D_3 Internal Standard obtained from the Standard working solution, ng/mL;

A_i —the area of Phenylethanol amine A-D_3 in sample solution;

A_s —the concentration of Phenylethanol amine A Standard obtained from the Standard working solu-

tion，ng/mL；

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

Note：The blank values should be deducted from the calculation result，Three significant figures should be retained.

10 Limit of Quantitation and Recovery rate

10.1 Limit of Quantitation

The limit of Quantitation of method is 0.5 μg/kg.

10.2 Recovery rate

The fortifying concentration of Phenylethanol amine A for each sample and the range of recovery are showed in Table C.1.

Annex A
(Informative)
MS / MS condition1

Quantitative ion pair(m/z),Quantitative ion pair(m/z),DP,CE in Table A.1.

a) CAD(N2):68.9 kPa;

b) GS1(N2):275.6 kPa;

c) GS2(N2):255.6 kPa;

d) CUR(N2):172.3 kPa;

e) IS:4 500 V;

f) TEM:550 ℃;

g) Residence time of each ion pair:100 ms;

h) EP:8 V;

I) CXP:10V.

Table A.1 Quantitative ion pair(m/z),Quantitative ion pair(m/z),DP,CE[1)]

Compound	Quantitative ion pair(m/z)	Monitoring ion pairs (m/z)	DP(V)	CE(eV)
Phenylethanol amine A	345.2/150.0	345.2/150.0	60	31
		345.2/326.8	70	17
Phenylethanol amine A-D3	348.2/153.1	348.3/153.1	60	30

1) Non-commercial statement:The parameters listed in this standard are completed in the ABSCIEX 5 500 mass spectometer; This information is given for the convenience of users of this standard,and does not mean approval of the product. If other equivalent products have the same effect,they can be used

Annex B
（Informative annex）
MRM chromatograms of Phenylethanol amine A

MRM chromatograms of Phenylethanol amine A see Figure B. 1.

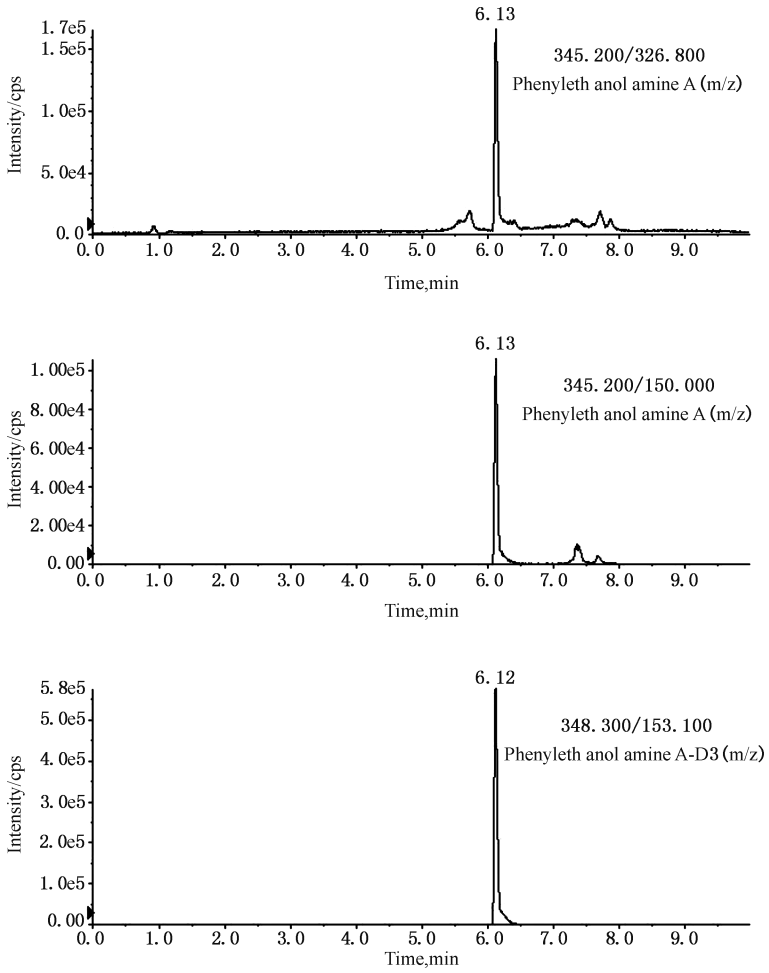

Figure B. 1 —MRM chromatograms of Phenylethanol amine A（0. 5 ng/mL）

Annex C
(Informative)
The fortifying concentration and the range of recovery($n=6$)

The fortifying concentration and the range of recovery($n=6$) see Table C. 1.

Table C. 1—The fortifying concentration and the range of recovery($n=6$)

Base material	Add level/(μg/kg)	Recovery rate/%	Base material	Add level/(μg/kg)	Recovery rate/%
Pork	0. 5	97. 0～103	Sheep kidney	0. 5	90. 2～102
	1. 0	88. 9～105		1. 0	88. 8～106
	5. 0	94. 6～103		5. 0	94. 4～102
Beef	0. 5	97. 2～100	Pig lung	0. 5	88. 6～107
	1. 0	94. 5～109		1. 0	87. 0～95. 7
	5. 0	94. 6～103		5. 0	88. 8～95. 8
Mutton	0. 5	93. 6～97. 8	Cow lung	0. 5	90. 0～108
	1. 0	93. 0～103		1. 0	79. 9～105
	5. 0	93. 8～102		5. 0	93. 2～97. 6
Pig liver	0. 5	90. 4～100	Sheep lung	0. 5	96. 8～83. 2
	1. 0	90. 2～98. 0		1. 0	86. 4～98. 7
	5. 0	89. 6～96. 0		5. 0	96. 0～103
Bovine liver	0. 5	92. 8～99. 6	Pig casing	0. 5	94. 6～99. 4
	1. 0	82. 5～106		1. 0	97. 5～105
	5. 0	95. 4～101		5. 0	94. 4～102
Sheep liver	0. 5	78. 2～93. 0	Sheep casing	0. 5	83. 2～96. 8
	1. 0	87. 7～99. 7		1. 0	86. 4～98. 7
	5. 0	96. 6～99. 4		5. 0	96. 0～103
Pig kidney	0. 5	75. 6～94. 8	Milk	0. 5	110～114
	1. 0	84. 0～94. 5		1. 0	99. 9～106
	5. 0	71. 2～81. 8		5. 0	93. 2～103
Cow kidney	0. 5	87. 0～96. 0	Chicken	0. 5	91. 0～110
	1. 0	85. 8～95. 8		1. 0	90. 0～110
	5. 0	88. 6～98. 6		5. 0	88. 6～103

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中国海关出版社有限公司出版发行
北京市朝阳区东四环南路甲 1 号(100023)
编辑部:(010)65194242-7530
中国标准出版社秦皇岛印刷厂印刷
*
开本 880×1230 1/16 印张 1.75 字数 45 千字
2024 年 4 月第一版 2024 年 4 月第一次印刷
印数 1—500
*
书号:155175·1042 定价 28.00 元