



中华人民共和国出入境检验检疫行业标准

SN/T 1867—2007

进出口贝类中软骨藻酸的检测方法 液相色谱-串联质谱法

Determination of domoic acid in shellfish for import and export—
LC-MS/MS method

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中 华 人 民 共 和 国 发 布
国家质量监督检验检疫总局

前 言

本标准的附录 A 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：中华人民共和国上海出入境检验检疫局、中华人民共和国辽宁出入境检验检疫局。

本标准主要起草人：方晓明、唐毅锋、樊祥、卫锋。

本标准系首次发布的出入境检验检疫行业标准。

进出口贝类中软骨藻酸的检测方法

液相色谱-串联质谱法

1 范围

本标准规定了进出口贝类样品中软骨藻酸的液相色谱-串联质谱检测方法。

本标准适用于进出口贝类样品中的软骨藻酸检测。

2 测定方法

2.1 方法提要

试样中的软骨藻酸用甲醇-水提取,固相萃取柱净化后,液相色谱分离,串联质谱检测,外标法定量。

2.2 试剂和材料

除另有规定外,所用试剂均为分析纯,水为二次蒸馏水或相适应的去离子水。

2.2.1 甲醇:液相色谱级。

2.2.2 乙腈:液相色谱级。

2.2.3 甲酸:纯度大于等于 98.0%。

2.2.4 软骨藻酸标准品(domoic acid,分子式 $C_{15}H_{21}NO_6$,CAS No. 14277-97-5):纯度大于等于 97.0%。

2.2.5 甲醇:水(50:50,体积比)。

2.2.6 乙腈:水(10:90,体积比)。

2.2.7 0.1 mol/L 甲酸水溶液。

2.2.8 0.05%甲酸的乙腈溶液:取 0.50 mL 甲酸加入至 1 L 乙腈中。

2.2.9 0.05%甲酸水溶液。

2.2.10 软骨藻酸标准储备液(100 $\mu\text{g/mL}$):准确称取 0.010 g 软骨藻酸于 100 mL 容量瓶中,用甲醇溶解并定容至刻度。此储备液于 -18°C 中可保存半年。

2.2.11 软骨藻酸中间溶液(10 $\mu\text{g/mL}$):移取 1.00 mL 软骨藻酸标准储备液(2.2.10)于 10 mL 容量瓶中,用甲醇定容至刻度。此溶液于 -18°C 中可保存 3 个月。

2.2.12 空白样品提取液:空白样品提取液的制备同 2.4.2。

2.2.13 软骨藻酸基质标准工作溶液:根据需要吸取适量软骨藻酸标准储备液(2.2.10)或软骨藻酸中间溶液(2.2.11),用空白样品提取液配制成适当浓度的基质标准工作溶液。基质标准工作溶液在 -18°C 保存,可使用 1 周。

2.2.14 SAX 固相萃取柱:3 mL(500 mg),使用前小柱预先用 6 mL 甲醇、3 mL 水和 3 mL 甲醇水溶液(2.2.5)淋洗,流速为 2 mL/min。

2.3 仪器和设备

2.3.1 液相色谱-串联质谱仪:配有电喷雾离子源。

2.3.2 固相萃取装置。

2.3.3 恒温氮吹装置。

2.3.4 组织捣碎机

2.3.5 均质机。

2.3.6 旋涡混合器。

2.3.7 离心机:最大转速 4 000 r/min。

2.3.8 离心管:50 mL。

2.4 测定步骤

2.4.1 样品的制备

从抽取的原始样品中取可食部分,放入组织捣碎机中搅碎至匀浆状,均分成两份,装入清洁的容器内,作为试样。密封,并标明标记。将试样于-18℃以下冷冻保存。在制样操作过程中,应防止样品污染或发生残留物含量的变化。

2.4.2 提取及净化

称取试样 4 g(精确到 0.01 g),置于 50 mL 离心管中,加入 2×8 mL 甲醇:水(50:50,体积比),于 12 000 r/min 均质 3 min,于 3 800 r/min 离心 10 min,合并上清液,再用甲醇:水(50:50,体积比)加补到 20.0 mL。取 5.0 mL 上清液(相当于含 1.0 g 样品)过 SAX 固相萃取柱,加 5 mL 乙腈:水(10:90,体积比)和 0.5 mL 0.1 mol/L 甲酸溶液淋洗,流速 2 mL/min,最后用 3.0 mL 0.1 mol/L 甲酸溶液洗脱,并收集,柱中液体流完后,保持抽气 2 min,最后用 0.1 mol/L 甲酸溶液定容到 3.0 mL,供测试。

2.4.3 测定

2.4.3.1 液相色谱条件

- a) 色谱柱:150 mm×2.1 mm,粒径 3.5 μm,Zorbax XDB-C₁₈,或相当者;预柱 20 mm×3.9 mm,粒径 5 μm,Symmetry C₁₈柱,或相当者;
- b) 流动相:溶剂 A,0.05%甲酸的乙腈溶液;溶剂 B,0.05%甲酸水溶液;
- c) 梯度淋洗:梯度淋洗见表 1;
- d) 流速:0.2 mL/min;
- e) 柱温:22℃;
- f) 进样量:10 μL。

表 1 梯度淋洗表

时间/min	溶剂 A/(%)	溶剂 B/(%)	梯度曲线
0	5	95	6
20	20	80	6
30	5	95	6

2.4.3.2 质谱条件

- a) 离子化模式:电喷雾正离子模式(ESI⁺);
- b) 锥电压:50 V;
- c) 源温度:105℃;
- d) 喷雾温度:250℃;
- e) 毛细管电压:3.0 kV;
- f) 碰撞电压:10 V;
- g) 碰撞气:氦气;
- h) 锥气流速:60 L/h;
- i) 喷雾气流速:480 L/h;
- j) MS1(m/z):312;TOF MS(m/z):100~500。

2.4.3.3 液相色谱-串联质谱测定

按浓度由小到大的顺序,依次分析基质标准工作溶液(2.2.13),得到浓度与峰面积的工作曲线。样品溶液中被测物的响应值应在工作曲线范围内。在上述液相色谱-串联质谱条件下,软骨藻酸的保留时

间约 17.5 min, 色谱图和质谱图参见附录 A 中图 A.1 和图 A.2。

样品中被测物保留时间与标准品一致, 选择的两个特征子离子 $m/z312 \rightarrow m/z266$, $m/z312 \rightarrow m/z294$ 同时存在, 且两者丰度比与标准品丰度比的偏差小于 10%, 以此来确证样品中存在软骨藻酸。

选择丰度最大的特征子离子的峰面积, 或所有子离子加和的峰面积进行外标法定量。

2.4.4 空白试验

除不加试样外, 均按上述操作步骤进行。

2.4.5 结果计算和表述

试样中被测物含量利用数据处理系统计算或按式(1)计算, 计算结果应扣除空白值。

$$X = c \times \frac{V}{m} \quad \dots\dots\dots (1)$$

式中:

X ——试样中被测组分残留量, 单位为微克每克($\mu\text{g/g}$);

c ——从标准工作曲线得到的被测组分溶液浓度, 单位为微克每毫升($\mu\text{g/mL}$);

V ——试样溶液定容体积, 单位为毫升(mL);

m ——试样溶液所代表试样的质量, 单位为克(g)。

3 测定低限、回收率

3.1 测定低限

本方法的测定低限为 $0.1 \mu\text{g/g}$ 。

3.2 回收率

软骨藻酸在贻贝和蛤蜊中添加浓度及其回收率数据:

——添加浓度在 $0.1 \mu\text{g/g}$ 时, 回收率为 78%~104%;

——添加浓度在 $10 \mu\text{g/g}$ 时, 回收率为 81%~110%;

——添加浓度在 $20 \mu\text{g/g}$ 时, 回收率为 81%~107%。

附录 A
(资料性附录)
液相色谱图和质谱图

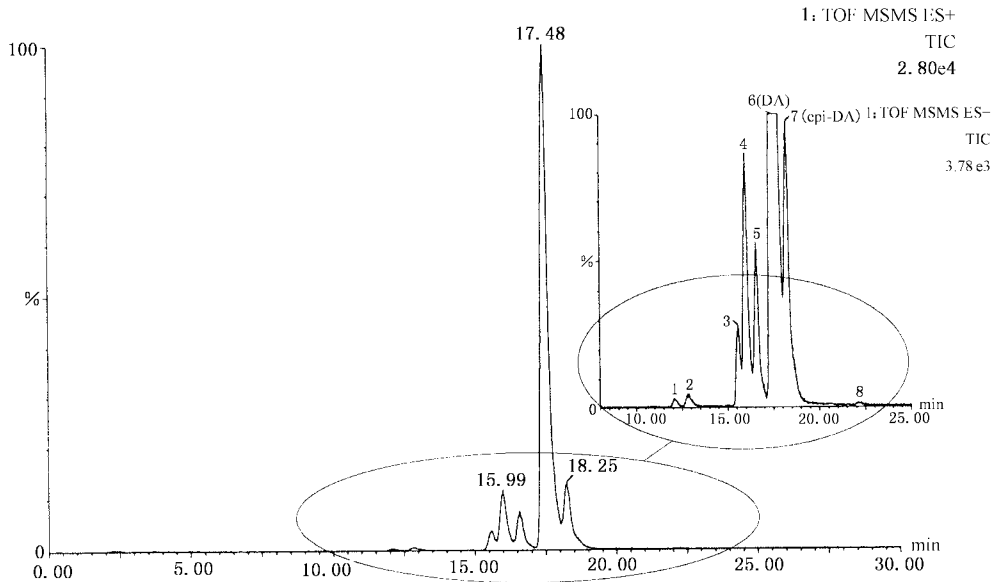


图 A.1 软骨藻酸(DA)及其异构体组织标样的总离子色谱图

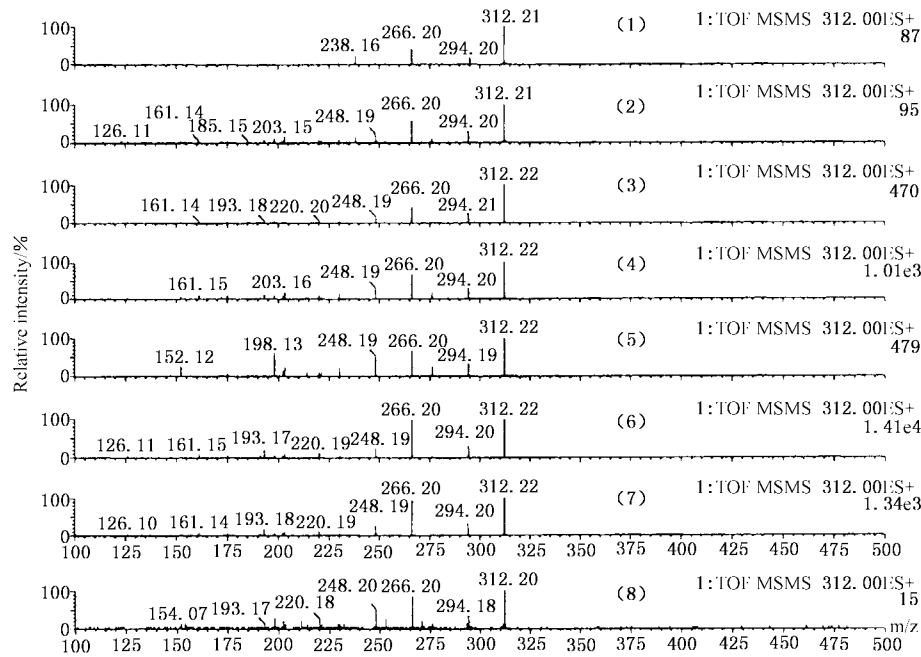


图 A.2 软骨藻酸及其异构体的质谱图

Foreword

Annex A of this standard is an informative annex.

This standard was proposed by and is under the charge of Nation Regulatory Commission for Certification and Accreditation.

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

This standard was mainly drafted by Fang Xiaoming, Tang Yifeng, Fan Xian and Wei Feng.

This standard is promulgated for the first time.

Determination of domoic acid in shellfish for import and export—LC-MS/MS method

1 Scope

This standard specifies the method of determination of domoic acid in shellfish by liquid chromatography-tandem mass spectrometry.

This standard applies to determination of domoic acid in shellfish.

2 Method of Determination

2.1 Principle

A shellfish tissue was extracted with methanol-water solution. Cleanup of the extract was used with a SAX solid-phase extraction column. Separation was carried out on a C18 column. Determination was made by means of a tandem mass spectrometry in a positive electrospray ionization, external standard method to quantify.

2.2 Reagents and materials

Reagents were of analytical-reagent grade except for the specific reagents. The water used was doubly distilled or deionized water.

2.2.1 Methanol: LC grade.

2.2.2 Acetonitrile: LC grade.

2.2.3 Formic acid: purity $\geq 98.0\%$.

2.2.4 Domoic acid standard: purity $\geq 97.0\%$, Formula $C_{15}H_{21}NO_6$, CAS No. 14277-97-5.

2.2.5 Methanol : water (50 : 50, V/V).

2.2.6 Acetonitrile : water (10 : 90, V/V).

2.2.7 0.1 mol/L formic acid.

2.2.8 0.05% formic acid in acetonitrile: 0.5 mL formic acid is added into 1 L acetonitrile.

2.2.9 0.05% formic acid water solution.

2.2.10 Domoic acid stock solution, 100 $\mu\text{g/mL}$: Accurately weigh 0.010 g domoic acid standard (2.2.3) into a 100 mL volumetric flask, dilute to volume with methanol. Stored at -18°C for half year.

2.2.11 Domoic acid intermediate solution, 10 $\mu\text{g/mL}$: Transfer 1.00 mL stock solution (2.2.10) into a 10 mL volumetric flask and dilute to volume with methanol, stored at -18°C for 3 months.

2.2.12 Extract of blank sample: Prepare extract of blank sample as Section 2.4.2.

2.2.13 Matrix-matched standards for domoic acid: to the extract of blank sample is added proper domoic acid stock solution (2.2.10) or intermediate solution (2.2.11). The matrix-matched standards are stored at -18°C for one week.

2.2.14 SAX SPE column: 3 mL (500 mg). Conditioned with 6 mL methanol, 3 mL water and 3 mL methanol-water solution (2.2.5) prior to use, the flow rate of 2 mL/min.

2.3 Apparatus and equipments

2.3.1 Liquid chromatography-electrospray tandem mass spectrometry.

2.3.2 Vacuum manifold for solid-phase extraction.

2.3.3 Nitrogen evaporator.

2.3.4 Pulper.

2.3.5 Homogenizer.

2.3.6 Vortex mixer.

2.3.7 Centrifuge: $<4\,000$ r/min.

2.3.8 Centrifuge tubes: 50 mL.

2.4 Procedure

2.4.1 Sample preparation

The edible part of original sample is taken and homogenized, then it is divided in two and sealed, labeled for lab test. The test sample should be stored at -18°C .

2.4.2 Extraction and Cleanup

A 4.00 g of homogenate was weighed into a 50 mL centrifuge tube, and extracted by adding 2 × 8 mL of methanol water solution (50 : 50, v/v) by stirring at 12 000 r/min for 3 min using a homogenizer. The mixture was centrifuged at approximately 3 800 r/min for 10 min. Combine the supernatant. A 5.0 mL of supernatant (equivalent to 1.0 g sample) was transferred onto a SAX SPE column. The column was then washed with 5 mL acetonitrile water (10 : 90, v/v) and 0.5 mL 0.1 mol/L formic acid solution. Finally, the toxins were eluted with another 3.0 mL 0.1 mol/L formic acid solution, and the eluate was diluted to 3.0 mL with 0.1 mol/L formic acid solution, collected for analysis.

2.4.3 Determination

2.4.3.1 Liquid Chromatographic Conditions

- Column: Zorbax XDB-C₁₈ (150 mm × 2.1 mm, 3.5 μm) or equivalent; safeguard column: Symmetry C₁₈ (20 mm × 3.9 mm, 5 μm) or equivalent;
- Mobile Phase: A, 0.05% formic acid in acetonitrile; B, 0.05% formic acid water solution;
- Gradient elution: See table 1;
- Flow rate: 0.20 mL/min;
- Column temperature: 22°C;
- Injection volume: 10 μL.

Table 1 —Gradient elution

Time/min	Solvent A/(%)	Solvent B/(%)	Gradient curve
0	5	95	6
20	20	80	6
30	5	95	6

2.4.3.2 MS/MS Conditions

- Ionization mode: ESI+;
- Cone voltage: 50 V;
- Source temperature: 105°C;
- Spray temperature: 250°C;
- Capillary voltage: 3.0 kV;
- Collision voltage: 10 V;
- Collision gas: argon;
- Cone gas flow: 60 L/h;
- Nebuliser gas flow: 48 L/h;
- MS1(m/z)312; TOF MS(m/z): 100~500.

2.4.3.3 LC-MS/MS determination

Analyze matrix-matched standards (2.2.13) in order of increasing concentration, obtaining the ma-

trix-matched calibration curve of peak area against concentration. The detector response of analyte should be in the range of calibration curve. Under above LC-MS/MS conditions, the retention time of domoic acid is ca. 17.5 min. The mass chromatograms refer to Figure A. 1 and A. 2 in annex A.

The peak has the same retention time on LC as the standards, the selected ions of m/z 312→266 and 312→294 are present as seen in the standard after subtraction of the background, and the deviation of the abundance ratio between sample and standard within 10%. The maximum abundance of daughter ion or the sum of all daughter ions is used for quantitation by external standard method.

2.4.4 Blank test

The operation of the blank test is the same as that described in the method of determination but without addition of the sample.

2.4.5 Calculations

The concentration of analyte in sample is calculated with chromatographic data processor or with the following equation (1):

$$X = c \times \frac{V}{m} \quad \dots\dots\dots (1)$$

Where:

X —the concentration of analyte of interest in sample, $\mu\text{g/g}$;

c —the concentration of analyte from calibration curve, $\mu\text{g/mL}$;

V —final volume of sample extract, mL;

m —weight of sample, g.

3 Limit of Quantitation and Recovery

3.1 Limit of quantitation

The limit of quantitation of this method is $0.1 \mu\text{g/g}$.

3.2 Recovery

The fortifying concentration of domoic acid in mussel and clam, and its corresponding recoveries are:

— $0.1 \mu\text{g/g}$, recovery were 78%~104%;

— $10 \mu\text{g/g}$, recovery were 81%~110%;

— $20 \mu\text{g/g}$, recovery were 81%~107%。

Annex A
(informative)
Mass chromatogram

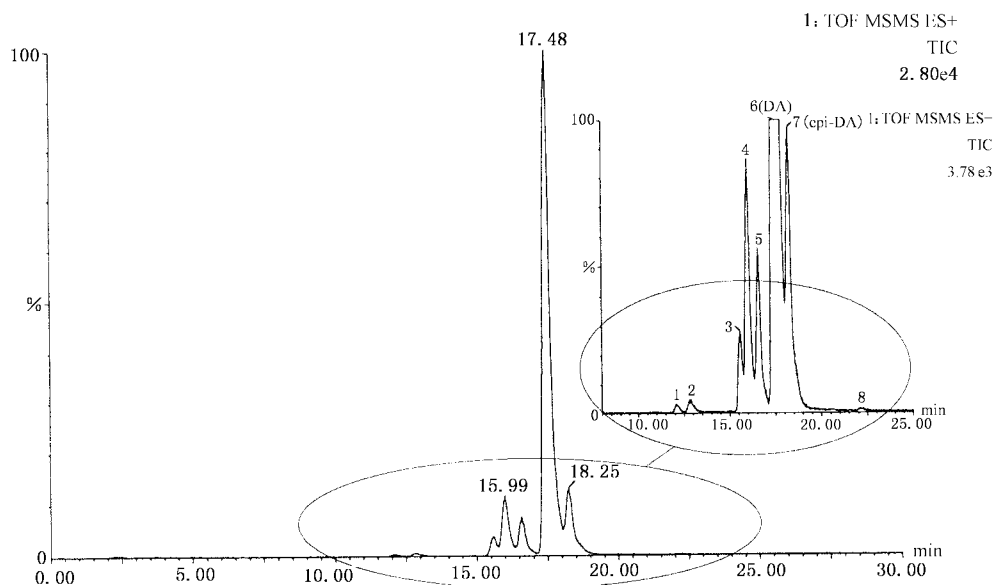


Figure A. 1—Chromatogram of certified reference material, a homogenized mussel tissue for domoic acid(DA)and its isomers

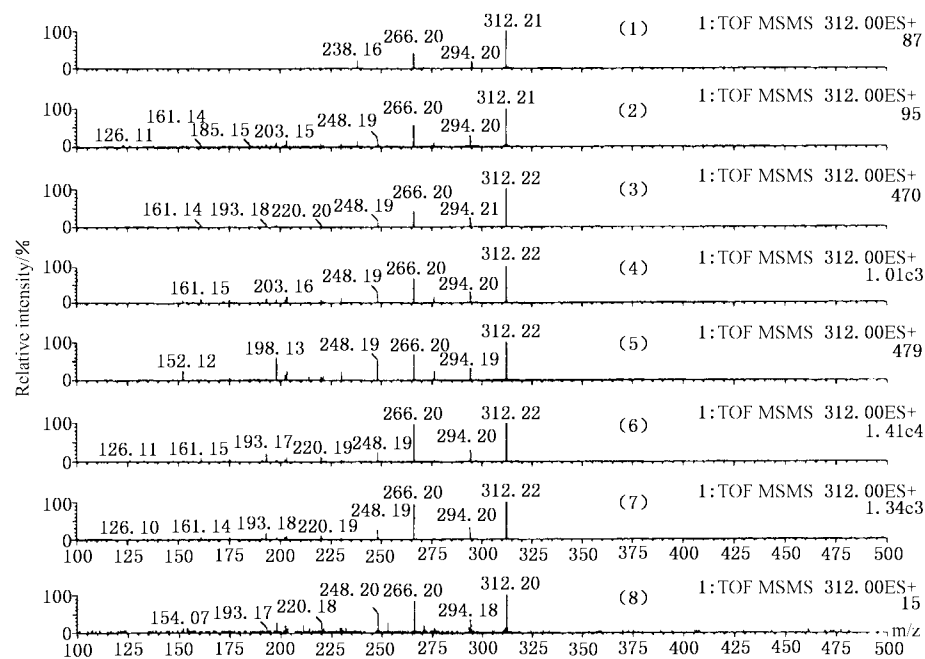


Figure A. 2 —Full-scan mass spectra of domoic acid and its isomers

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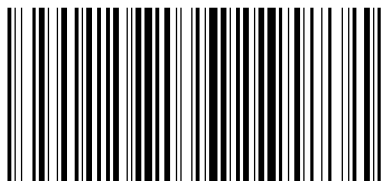
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