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中华人民共和国出入境检验检疫行业标准

SN/T 4051—2014

出口保健食品中奥利斯他的测定 液相色谱-质谱/质谱法

Determination of orlistat in health foods for export—HPLC-MS/MS method

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前　言

本标准按照 GB/T 1.1—2009 给出的规定起草。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。

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：中华人民共和国湖南出入境检验检疫局、湖南省检验检疫科学技术研究院、湖南师范大学。

本标准主要起草人：王美玲、颜鸿飞、付善良、张莹、张帆、李拥军、陈波。

出口保健食品中奥利斯他的测定
液相色谱-质谱/质谱法

1 范围

本标准规定了保健食品中奥利斯他的液相色谱-质谱/质谱测定方法。

本标准适用于减肥胶囊、减肥咀嚼片、减肥咖啡、减肥口服溶液、减肥饼干、减肥茶中奥利斯他含量的测定和确证。

2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本文件。凡是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 原理

试样中的奥利斯他用甲醇超声提取，提取液经稀释、过滤后，用液相色谱-质谱/质谱仪测定，外标法定量。

4 试剂和材料

除特殊注明外，所有试剂均为分析纯，水为符合 GB/T 6682 规定的一级水。

4.1 甲醇：液相色谱级。

4.2 乙腈：液相色谱级。

4.3 乙酸铵。

4.4 5 mmol/L 乙酸铵溶液：称取 0.385 g 乙酸铵，用水溶解并稀释定容至 1 000 mL。

4.5 甲醇-水混合溶液（1+1，体积比）：量取 100 mL 甲醇，加入 100 mL 水，混匀备用。

4.6 奥利斯他标准品（CAS 号：96829-58-2，分子式：$C_{29}H_{53}NO_5$）：纯度≥99％。

4.7 奥利斯他标准储备液：准确称取适量的奥利斯他标准品（4.6），用甲醇溶解配制成浓度为 1.0 mg/mL 的标准储备溶液，4 ℃下避光保存。

4.8 奥利斯他标准工作溶液：准确移取一定体积的奥利斯他标准储备溶液（4.7），用甲醇-水混合溶液（4.5）稀释成 0.2 ng/mL、0.5 ng/mL、1.0 ng/mL、5.0 ng/mL、10 ng/mL、50 ng/mL、100 ng/mL 不同浓度系列的标准工作溶液，使用前配制。

5 仪器和设备

5.1 高效液相色谱-质谱/质谱仪：配电喷雾离子源（ESI）。

5.2 超声波清洗器。

5.3 捣碎机。

5.4 分析天平：感量为 0.000 1 g 和 0.01 g。

5.5 离心机：大于等于 6 000 r/min。

6 试样制备与保存

6.1 试样制备

6.1.1 固体试样

片剂、咖啡、茶和饼干研细，混匀，均分成两份，分别装入洁净容器内；胶囊连同胶囊壳一起捣碎，混匀，均分成两份，分别装入洁净容器内。

6.1.2 液体试样

口服液样品直接混匀，均分成两份，分别装入洁净容器内。密封作为试样，标明标记。

6.2 试样的保存

将试样于 0 ℃～4 ℃下保存。在取样、制样过程中，应防止样品受到污染或发生目标物含量的变化。

7 测定步骤

7.1 提取

7.1.1 片剂、胶囊、饼干、咖啡、茶叶样品

称取 1 g(精确至 0.01 g)试样于 50 mL 具塞锥形瓶中，加入 30 mL 甲醇超声提取 30 min。取出冷却至室温，将样品全部转移至 50 mL 容量瓶中，用 15 mL 甲醇分数次清洗锥形瓶，合并清洗液至容量瓶中并用甲醇定容至刻度，摇匀后，静置 2 min。移取 5 mL 提取液至 15 m 离心管中，在 6 000 r/min 离心 5 min 后，取 0.5 mL 上清液，加入 0.5 mL 甲醇-水混合溶液(4.5)，混匀后过 0.45 μm 有机微孔滤膜，供液相色谱-质谱/质谱仪检测。

7.1.2 口服液

准确移取 1.0 mL 试样于 10 mL 容量瓶中，用甲醇-水混合溶液(4.5)稀释并定容至刻度，混匀后，过 0.45 μm 有机微孔滤膜，供液相色谱-质谱/质谱仪检测。

7.2 测定

7.2.1 液相色谱条件

液相色谱条件如下：

a) 色谱柱：C_{18}柱，150 mm×2.1 mm(内径)，粒径 5 μm 或相当者；

b) 流动相：乙腈-5 mmol/L 乙酸铵溶液，梯度洗脱程序见表 1；

c) 流速：0.3 mL/min；

d) 柱温：40 ℃；

e) 进样量：10 μL。

表 1　流动相梯度洗脱程序

时间 min	5 mmol/L 乙酸铵溶液 %	乙腈 %
0.0	40	60
8.0	20	80
12.0	10	90
15.0	10	90
16.0	40	60
20.0	40	60

7.2.2　质谱条件

质谱参考条件参见附录 A。

7.2.3　液相色谱-质谱检测及确证

7.2.3.1　定量测定

按照 7.2.1 色谱条件和 7.2.2 质谱条件测定样液和标准工作溶液，外标法定量。待测样液中奥利斯他的量应在标准曲线范围之内，如果超出标准曲线范围，应稀释到合适浓度后分析。在上述仪器条件下，奥利斯他保留时间约为 16.06 min。标准溶液的选择离子色谱图参见附录 B 中图 B.1。

7.2.3.2　定性测定

按照上述条件测定样液和标准溶液，如果样液中待测物的色谱峰保留时间与标准溶液一致，并且在扣除背景后的样液谱图中，所选择的离子对均出现，各定性离子的相对丰度与标准品离子的相对丰度相比，相对丰度偏差不超过表 2 的规定，则可判断样品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 %	允许的相对偏差 %
＞50	±20
＞20～50	±25
＞10～20	±30
≤10	±50

7.3　空白实验

除不加试样外，均按上述测定步骤进行。

7.4　结果计算和表述

用色谱数据处理机或按照式(1)计算样品中待测物的残留量，计算结果应扣除空白：

$$X = \frac{c \times V}{m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

式中

X —— 试样中奥利斯他含量，单位为毫克每千克(mg/kg)或者毫克每升(mg/L)；

c ——从标准工作曲线得到的奥利斯他浓度，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 —— 最终样液代表的试样质量，单位为克(g)；或者所代表的试样体积，单位为毫升(mL)。

8 定量限、回收率

8.1 定量限

本方法片剂、胶囊、饼干、咖啡和茶中奥利斯他的定量限为 0.05 mg/kg；口服液中奥利斯他的定量限为 0.005 mg/L。

8.2 回收率

减肥胶囊、减肥咀嚼片、减肥咖啡、减肥茶、减肥饼干和减肥口服液中奥利斯他的添加水平及回收率数据见表 3。

表 3 减肥胶囊、减肥咀嚼片、减肥咖啡、减肥茶、减肥饼干和减肥口服液中奥利斯他的添加水平及回收率数据

样品基质	添加水平 mg/kg	回收率范围 %
减肥胶囊	0.05	83.8～93.8
	0.10	87.4～98.3
	0.25	92.4～100.5
减肥咀嚼片	0.05	78.6～95.0
	0.10	85.5～98.1
	0.25	90.6～97.8
减肥茶	0.05	81.6～98.2
	0.10	83.6～98.8
	0.25	95.4～100.2
减肥咖啡	0.05	84.8～97.6
	0.10	86.7～97.8
	0.25	95.3～97.6
减肥饼干	0.05	89.8～99.8
	0.10	93.2～99.5
	0.25	91.6～101.2
减肥口服液	0.005	88.0～106.0
	0.01	92.0～105.1
	0.05	97.6～101.4

附　录　A
（资料性附录）
质谱测定参考条件[1]

参考质谱条件：

a）扫描方式：正离子扫描；

b）检测方式：多反应监测（MRM）；

c）气帘气（CUR）：137.9 kPa（20 psi）；

d）雾化气（GS1）：344.7 kPa（50 psi）；

e）辅助加热气（GS2）：379.2 kPa（55 psi）；

f）碰撞气（CAD）：48.3 kPa（7 psi）；

g）电喷雾电压（IS）：5 000 V；

h）离子源温度（TEM）：450 ℃；

i）定性离子对、定量离子对、碰撞能量、去簇电压、碰撞室出入口电压和碰撞室出口电压见表 A.1。

表 A.1　多反应监测条件

化合物名称	母离子 （*m/z*）	子离子 （*m/z*）	驻留时间 ms	去簇电压 （DP） V	碰撞能量 （CE） V	碰撞室入口 电压（EP） V	碰撞室出口 电压（CXP） V
奥利斯他	496.6	319.6*	100	85	18	10	15
		160.3	100	85	19	10	15
注：带“*”的离子为定量离子。							

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[1] 非商业性声明：附录 A 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）

标准溶液的选择反应监测(SRM)色谱图

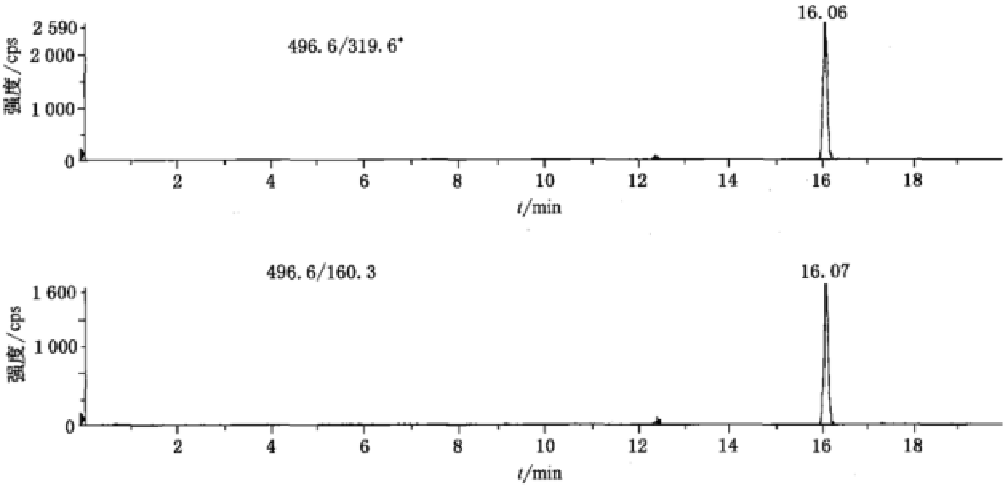

图 B.1　标准溶液的选择反应监测(SRM)色谱图

Foreword

This standard was drafted according with GB/T 1.1—2009.

Please note that some of the content of this document may involve patents, the publisher of this document does not assume the responsibility to identify these patents.

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

This standard was drafted by the Hunan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Hunan Academy of Inspection & Quarantine and Hunan Normal University.

The standard was mainly drafted by Meiling Wang, Hongfei Yan, Shanliang Fu, Ying Zhang, Fan Zhang, Yongjun Li and Bo Chen.

Determination of orlistat in health foods for export—HPLC-MS/MS method

1 Scope

This standard specifies the sample preparation and determination of orlistat in heath foods by HPLC-MS/MS method.

This standard is applicable to the determination and the confirmation of orlistat in diet capsule, diet chewable tablet, diet coffee, diet biscuit, oral solution and diet tea.

2 Cited normative documents

The following normative documents are indispensable to the application of this standard. For dated references, only the edition bearing such date applies to th is standard. For undated references, the latest edition of the normative document referred to(including all the amendments) applies.

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

3 Abstract of method

Orlistat in sample is extracted with methanol in an ultrasonic washer. Then the extract is diluted and filtrated before injection. The filtrate is determined by HPLC-MS/MS. External standards method is used for quantitative measurement.

4 Reagents and materials

Unless otherwise specified, all the reagents should be of analytical grade, "Water" is the first grade water prescribed by GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Acetonitril: HPLC grade.

4.3 Ammonium acetate.

4.4 5 mmol/L ammonium acetate solution: Accurately weight 0.385 g ammonium acetate, dissolve in water to make a solution of 1 000 mL in concentration.

4.5 Methanol-water mixed solution (1+1, *V*/*V*): Transfer 100 mL methanol into 100 mL water, mix adequately.

4.6 Orlistat (CAS No.: 96829-58-2, molecular formula: $C_{29}H_{53}NO_5$) standard: Purity≥99.0%.

4.7 Standard stock solution: Accurately weight an adequate amount of orlistat (4.6), dissolve in methanol to make a standard stock solution of 1.0 mg/mL in concentration. The standard solution stored below 4 ℃ avoiding sunlight.

4.8 Standard working solution: dilute the standard stock solution with methanol-water mixed solution (4.5) to prepare a series of standard working solutions with concentrations of 0.2 ng/mL, 0.5 ng/mL, 1.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 50 ng/mL, 100 ng/mL, just before use.

5 Apparatus and equipment

5.1 Liquid chromatography-tandem mass spectrometry, equipped with electrospray ion source (ESI).

5.2 Ultrasonic washer.

5.3 Triturator.

5.4 Electronic balance: Readability 0.000 1 g and 0.01 g.

5.5 Centrifuge: Max 6 000 r/min.

6 Sample preparation and storage

6.1 Preparation of test sample

6.1.1 Solid sample

Chewable tablet, coffee, biscuit, tea, capsule contents and capsule shell should be grinded and blended to produce homogeneous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled.

6.1.2 Liquid sample

The oral solution sample should be blended to produce homogeneous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled.

6.2 Storage of test sample

The test sample should be stored at 0 ℃ ～4 ℃. While sampling and sample preparation, precaution must be taken to avoid contamination or any factors that may cause the change of residue content.

7 procedure

7.1 Extraction

7.1.1 Capsule, chewable tablet, tea, coffee and biscuit

Weigh ca 1 g (accurate to 0.01 g) of the test sample into a 50 mL conical flask with stopper. Add 30 mL methanol and extracted in a ultrasonic washer for 30 min. Take it out and cool down to room temperature. Transfer all the sample into 50 mL volumetric flask and wash the beaker with 15 mL methanol in several times. Combine the washing fluids into the volumetric flask and dilute to mark with methanol and mix the contents. Let it stand for 2 min, transfer 5 mL the extract upper layer into a 15 mL centrifuge tube. Centrifuge at 6 000 r/min for 5 min. Transfer the upper layer 0.5 mL of the extract and add 0.5 mL methanol-water mixed solution(4.5). The diluted solution is filtered throught a 0.45 μm membrane and determined by HPLC-MS/MS.

7.1.2 Oral solution

Accurately transfer 1.0 mL of test sample into a 10 mL volumetric flask and dilute to mark with methanol-water mixed solution(4.5). The diluted solution is filtered throught a 0.45 μm membrane and determined by HPLC-MS/MS.

7.2 Determination

7.2.1 HPLC operating condition

LC operating condition is as following:

a) Chromatographic column: C_{18} chromatographic column or equivalent column (2.1 mm × 150 mm, 5 μm);

b) Mobile phase: Acetonitrile-5 mmol/L-ammonium acetate solution, gradient program see Table 1;

c) Flow rate: 0.3 mL/min;

d) Column temperature: 40 ℃;

e) Injection volume: 10 μL.

Table 1—gradient program of mobile phase

Time min	5 mmol/L ammonium acetate solution %	Acetonitrile %
0.0	40	60
8.0	20	80
12.0	10	90
15.0	10	90
16.0	40	60
20.0	40	60

7.2.2 MS operating condition

MS operating referenced conditions are shown in Annex A.

7.2.3 HPLC-MS determination and confirmation

7.2.3.1 Qualitative determination

According to HPLC operating condition assigned 7.2.1 and MS operating condition assigned 7.2.2, analyze the standard solution and the sample solution. The quality of the results obtained using external standards. The responses of orlistat in the sample solution should be in the linear range of standard solution. If the response is above the linear range, dilute the sample solution. Under the above HPLC-MS operating conditions, the retention time of orlistat is ca 16.06 min. The selected reaction monitoring (SRM) chromatograms are shown in Figure B.1 in Annex B.

7.2.3.2 Confirmation

Under above determination conditions, if the retention time of sample chromatogram peaks are consistent with the standards, and subtracted from background compensation, selected ions are all present and the relative ion abundance of the selected ions according with that of the calibration standard at comparable concentrations, within the tolerances (see Table 2). The corresponding analyte could be confirmed.

Table 2—Maximum permitted tolerances for relative ion intensities while confirmation

Relative ion intensities %	Permitted relative tolerances %
>50	±20
>20~50	±25
>10~20	±30
⩽10	±50

7.3 Blank test

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

7.4 Calculation and expression of result

Calculate the content of orlistat in the test sample by HPLC-MS data processor or using the followed formula (1), the blank value should be subtracted from the result of calculation:

$$X = \frac{c \times V}{m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots\cdots\cdots (1)$$

Where:

X —the content of orlistat in the test samples, mg/kg or mg/L;

c —the concentration of orlistat in the standard working solution, μg/mL;

V —the final volume of sample solution, mL;

m —the corresponding mass of test sample in the final sample solution, g; or the corresponding volume of test sample, mL.

8 Limit of quantitation and recovery

8.1 Limit of quantitation

The limit of quantitation of orlistat is 0.05 mg/kg for capsule, chewable tablet, tea, coffee and biscuit; and 0.005 mg/L for oral solution.

8.2 Recovery

According to the experimental data for diet capsule, diet chewable tablet, diet coffee and diet tea, the fortifying concentration of orlistat for each sample and the range of recovery are shown in Table 3.

Table 3—The fortifying concentration and the range of recovery of orlistat in diet capsule, diet chewable tablet, diet coffee, diet tea, diet biscuit and oral solution

Sample	Fortified level mg/kg	Range of recovery %
Diet capsule	0.05	83.8～93.8
	0.10	87.4～98.3
	0.25	92.4～100.5

Table 3 (continued)

Sample	Fortified level mg/kg	Range of recovery %
Diet chewable tablet	0.05	78.6～95.0
	0.10	85.5～98.1
	0.25	90.6～97.8
Diet tea	0.05	81.6～98.2
	0.10	83.6～98.8
	0.25	95.4～100.2
Diet coffee	0.05	84.8～97.6
	0.10	86.7～97.8
	0.25	95.3～97.6
Diet biscuit	0.05	89.8～99.8
	0.10	93.2～99.5
	0.25	91.6～101.2
Diet oral solution	0.005	88.0～106.0
	0.01	92.0～105.1
	0.05	97.6～101.4

Annex A
(Informative)
HPLC-MS reference condition[1]

HPLC-MS/MS reference condition:

a) Scan mode: Positive ion;

b) Detection mode: Multiple reaction monitoring(MRM);

c) Curtain gas(CUR): 137.9 kPa(20 psi);

d) Nebulizer gas(GS1): 344.7 kPa(50 psi);

e) Auxiliary gas(GS2): 379.2 kPa(55 psi);

f) Collision gas(CAD): 48.3 kPa(7 psi);

g) Ion spray voltage(IS): 5 000 V;

h) Ion source temperature(TEM): 450 ℃;

i) Confirmation ion, quantification ion, declustering Potential (DP), collision Energy (CE), and collision cell Exit Potential(CXP) see Table A.1.

Table A.1—Multiple reaction monitoring condition

Compound	Parent ion (m/z)	Daughter ion (m/z)	Dwell time ms	Declustering Potential(DP) V	Collision Energy (CE) V	Collision cell Entrance Potential(EP) V	Collision cell Exit Potential(CXP) V
Orlistat	496.6	319.6*	100	85	18	10	15
		160.3	100	85	19	10	15
Annotation: the symbol " * " represents the quantitative ion.							

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[1] Non-commercial statement: The reference mass parameters in Annex A are accomplished by API 4000 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 B

(informative)

Selected reaction monitoring(SRM) chromatograms of orlistat standard solution

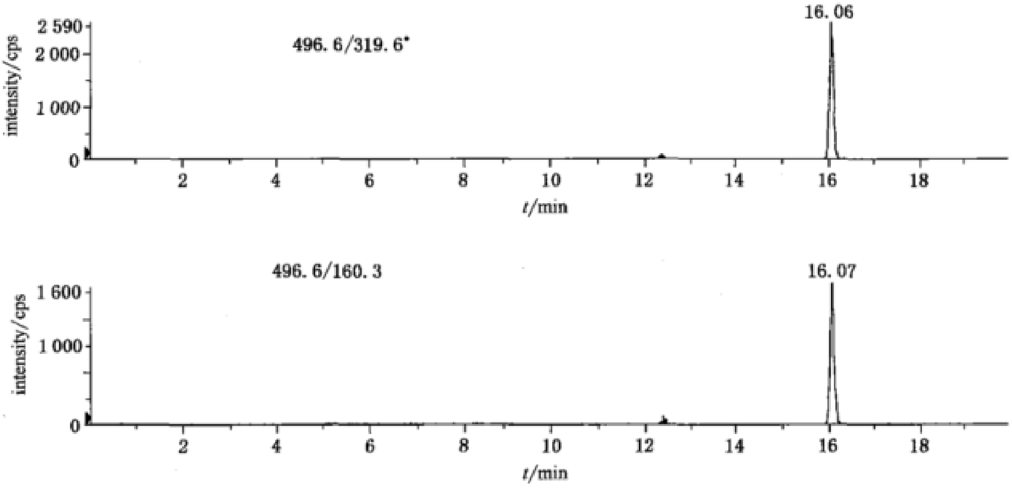

Figure B.1—Selected reaction monitoring(SRM) chromatograms of orlistat standard solution