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中华人民共和国出入境检验检疫行业标准

SN/T 4054—2014

出口保健食品中育亨宾、伐地那非、西地那非、他达那非的测定 液相色谱-质谱/质谱法

Determination of yohimbin, tadalafil, sildenafil and vardenafil in health foods for export—LC-MS/MS method

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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

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。

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出口保健食品中育亨宾、伐地那非、西地那非、他达那非的测定 液相色谱-质谱/质谱法

1 范围

本标准规定了保健食品中育亨宾、伐地那非、西地那非、他达那非的液相色谱-质谱/质谱测定方法。

本标准适用于片剂、胶囊和口服液类保健食品中育亨宾、伐地那非、西地那非、他达那非的定量测定和确证。

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

片剂、胶囊试样中的育亨宾、伐地那非、西地那非、他达那非用甲醇超声提取，提取液经稀释后过滤；口服液经直接稀释后过滤，用液相色谱-质谱/质谱仪进行测定，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：优级纯。

4.4 0.1%乙酸溶液：取 1 mL 乙酸，用水稀释并定容至 1 000 mL。

4.5 0.1%乙酸-甲醇混合溶液(1+1，体积比)：量取 100 mL 0.1%乙酸溶液，加入 100 mL 甲醇，混匀备用。

4.6 育亨宾(yohimbin，CAS 号：146-48-5，分子式：$C_{21}H_{26}N_2O_3$)标准品：纯度大于等于 99.0%。

4.7 他达那非(tadalafil，CAS 号：171596-29-5，分子式：$C_{22}H_{19}N_3O_4$)标准品：纯度大于等于 99.0%。

4.8 西地那非(sildenafil，CAS 号：139755-83-2，分子式：$C_{22}H_{30}N_6O_4S$)标准品：纯度大于等于 99.0%。

4.9 伐地那非(vardenafil，CAS 号：224785-91-5，分子式：$C_{23}H_{32}N_6O_4S \cdot HCl$)标准品：纯度大于等于 99.0%。

4.10 标准储备液：准确称取适量的上述标准品，用甲醇分别配制成浓度为 1.0 mg/mL 的标准储备溶液，4 ℃下避光保存。

4.11 混合标准中间溶液：取上述标准贮备液适量，用甲醇配制成浓度为 10 μg/mL 的混合标准中间溶液，4 ℃下避光保存。

4.12 标准工作溶液的配制：根据需要用0.1%乙酸-甲醇混合溶液(4.5)将混合标准中间溶液(4.11)稀释成1 ng/mL、5 ng/mL、10 ng/mL、50 ng/mL、100 ng/mL的混合标准工作溶液，使用前配制。

5 仪器

5.1 高效液相色谱-质谱/质谱仪：配有电喷雾离子源(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。

6 试样制备与保存

6.1 试样制备

6.1.1 固体试样

片剂研细，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、胶囊试样

称取1 g(精确至0.01 g)试样于100 mL具塞锥形瓶中，加入80 mL甲醇超声提取30 min。取出冷却至室温，将样品全部转移至100 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5 min。移取1.0 mL提取液至10 mL容量瓶中，用0.1%乙酸-甲醇混合溶液(4.5)定容后混匀，溶液过0.45 μm微孔有机滤膜后，供液相色谱-质谱/质谱仪测定。

7.1.2 口服液

准确移取1.0 mL试样于10 mL容量瓶中，用0.1%乙酸-甲醇混合溶液(4.5)定容至刻度，摇匀，溶液过0.45 μm有机滤膜后，供液相色谱-质谱/质谱仪测定。

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×4.6 mm(内径)，粒径5 μm或相当者；

b) 流动相：梯度洗脱程序见表 1；

c) 流速：0.5 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	0.1%乙酸溶液 %	乙腈 %
0.00	80.0	20.0
3.00	80.0	20.0
8.00	5.0	95.0
13.00	5.0	95.0
13.01	80.0	20.0
20.00	80.0	20.0

7.2.2 质谱条件

试验所用质谱条件参见附录 A。

7.2.3 液相色谱-质谱/质谱检测及确证

7.2.3.1 定量测定

根据试样中被测物的含量情况，选取响应值相近的标准工作液进行分析。标准工作液和待测样液中被测物的响应值均应在仪器线性响应范围内。如果超出仪器线性范围，应稀释到合适浓度后分析。在上述色谱条件下育亨宾、伐地那非、西地那非、他达那非的参考保留时间为 9.4 min、10.0 min、10.2 min、11.9 min。外标法定量。标准溶液的多反应监测色谱图参见附录 B 中的图 B.1。

7.2.3.2 定性测定

在相同的实验条件下，样液中被测物的色谱峰保留时间与标准工作液相同，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，偏差不超过表 2 规定的范围内，则可判断样品中存在对应的被测物。

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 空白试验

除不加试样外，均按上述测定步骤进行样品空白试验。

7.4 结果计算和表述

用色谱数据处理软件或按式(1)计算试样中被测组分的含量，计算结果需扣除空白值：

$$X_i = \frac{c_i \times V \times 1\,000}{m \times 1\,000} \quad \cdots\cdots(1)$$

式中：

X_i ——试样中被测组分的含量，单位为毫克每千克(mg/kg)或者毫克每升(mg/L)；

c_i ——标准工作溶液中被测组分的浓度，单位为微克每毫升(μg/mL)；

V ——试样溶液定容体积，单位为毫升(mL)；

m ——最终试样溶液所代表的试样质量，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、胶囊样品中育亨宾、伐地那非、西地那非、他达那非的定量限均为 1.0 mg/kg；口服液样品中育亨宾、伐地那非、西地那非、他达那非的定量限均为 0.01 mg/L。

8.2 回收率

片剂、胶囊和口服液样品中育亨宾、伐地那非、西地那非、他达那非的不同添加水平及回收率数据见表 3。

表 3 片剂、胶囊和口服液样品中育亨宾、伐地那非、西地那非、他达那非的不同添加水平及回收率

化合物名称	样品类型					
	片剂		胶囊剂		口服液	
	添加水平 mg/kg	回收率范围 %	添加水平 mg/kg	回收率范围 %	添加水平 mg/L	回收率范围 %
育亨宾	1.0	81.0～109.0	1.0	78.0～101.0	0.01	93.6～112
	2.0	80.0～94.5	2.0	77.5～98.0	0.05	96.6～104.0
	4.0	88.3～97.3	4.0	80.7～99.3	0.10	94.4～104.0
伐地那非	1.0	82.0～107.0	1.0	82.0～108.0	0.01	81.1～97.9
	2.0	80.5～95.5	2.0	80.5～94.5	0.05	91.2～103.0
	4.0	85.0～98.5	4.0	80.0～98.7	0.10	95.8～105.0
西地那非	1.0	80.0～106.0	1.0	79.0～108.0	0.01	91.1～107.0
	2.0	90.5～102.0	2.0	80.5～103.0	0.05	91.2～103.0
	4.0	88.8～101.0	4.0	83.3～102.0	0.10	95.8～105.0
他达那非	1.0	81.0～106.0	1.0	81.0～106.0	0.01	82.1～97.7
	2.0	85.0～97.0	2.0	81.0～99.5	0.05	89.8～101.0
	4.0	82.3～97.8	4.0	80.5～103.0	0.10	92.8～103.0

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）：138 kPa（20 psi）；

d） 雾化气（GS1）：276 kPa（40 psi）；

e） 辅助气（GS2）：310 kPa（45 psi）；

f） 碰撞气（CAD）：55 kPa（8 psi）；

g） 电喷雾电压（IS）：5 000 V；

h） 离子源温度（TEM）：550.0 ℃。

定性离子对、定量离子对、去簇电压、碰撞能量、碰撞室出口电压见表 A.1。

表 A.1 目标化合物定性离子对、定量离子对、去簇电压、碰撞能量和碰撞室出口电压

目标化合物	母离子（m/z）	子离子（m/z）	去簇电压（DP）V	碰撞能量（CE）V	碰撞室出口电压（CXP）V
育亨宾	355.2	144.1*	108	50	15
		212.4	108	30	15
伐地那非	489.5	151.1*	120	60	14
		312.1	120	56	18
西地那非	475.3	100.1*	110	40	16
		58.0	110	82	12
他达那非	390.4	268.3*	70	18	17
		135.1	70	30	22
注：“*”为定量离子对。					

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）
标准溶液多反应监测（MRM）色谱图

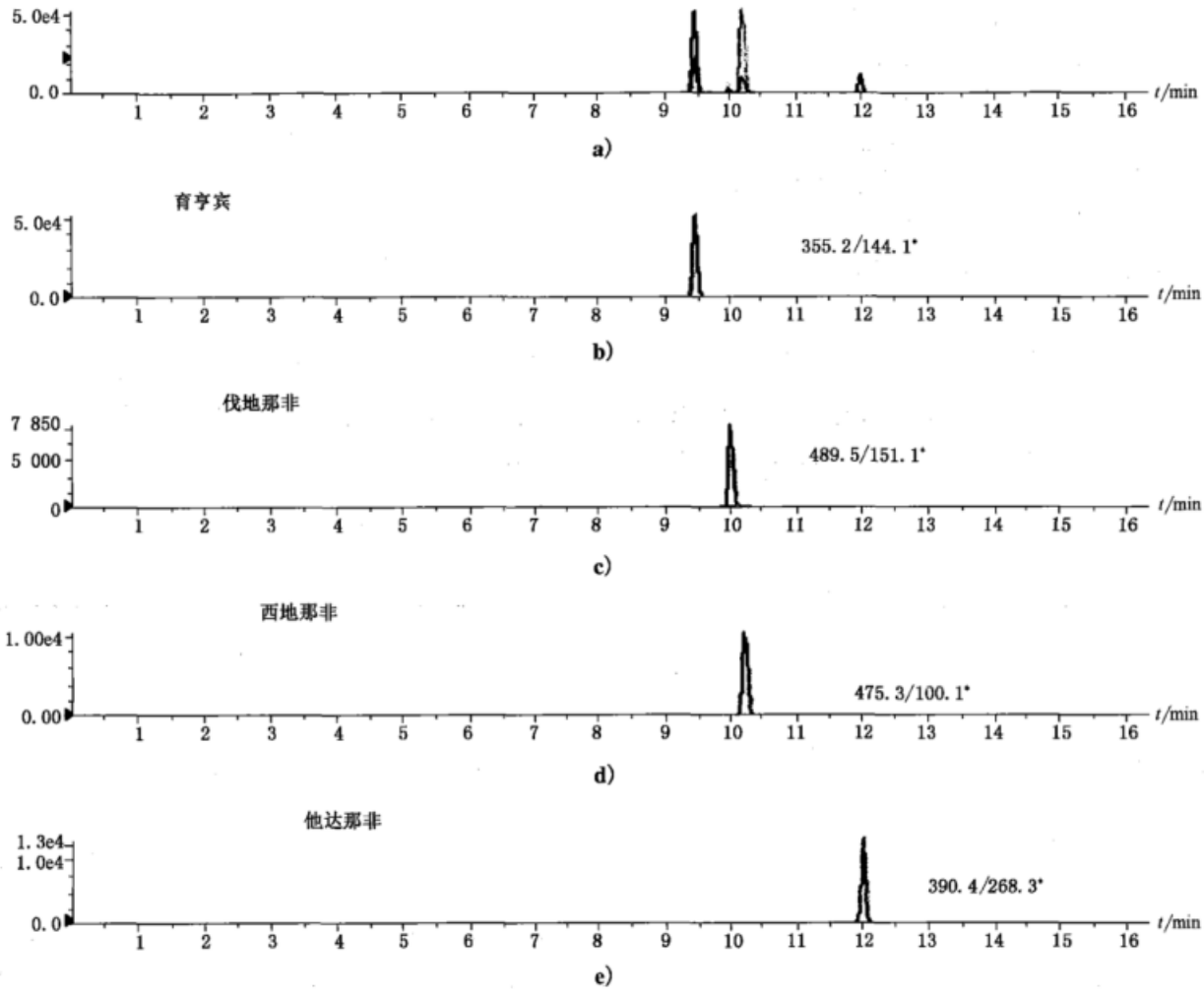

图 B.1 标准溶液多反应监测（MRM）色谱图

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 and Quarantine and Hunan Normal University.

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

Determination of yohimbin, tadalafil, sildenafil and vardenafil in health foods for export—LC-MS/MS method

1 Scope

This standard specifies the determination of yohimbin, tadalafil, sildenafil and vardenafil in heath foods by LC-MS/MS.

This standard is applicable to the determination and the confirmation of yohimbin, tadalafil, sildenafil and vardenafil in troche, capsule and oral solution.

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 this 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

Yohimbin, tadalafil, sildenafil and vardenafil in troche, capsule and oral solution are extracted with methanol in an ultrasonic washer and then the extract is diluted and filtered off. The oral solution sample is only diluted and filtered off. The diluted solution is determined by LC/MS/MS, using external standard method.

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 Acetonitrile: HPLC grade.

4.3 Acetic acid.

4.4 0.1% acetic acid solution: Accurately transfer 1 mL acetic acid into 1 000 mL volumetric flask, then make up to graduation with water.

4.5 0.1% acetic acid-methanol mixed solution (1+1, *V*/*V*): Transfer 100 mL methanol into 100 mL 0.1% acetic acid solution, mix adequately.

4.6 Yohimbin (CAS NO.: 146-48-5, molecular formula: $C_{21}H_{26}N_2O_3$) standard: Purity≥99.0%.

4.7 Tadalafil (CAS NO.: 171596-29-5, molecular formula: $C_{22}H_{19}N_3O_4$) standard: Purity≥99.0%.

4.8 Sildenafil (CAS NO.: 139755-83-2, molecular formula: $C_{22}H_{30}N_6O_4S$) standard: Purity≥99.0%.

4.9 Vardenafil (CAS NO.: 224785-91-5, molecular formula: $C_{23}H_{32}N_6O_4S \cdot HCl$) standard: Purity ≥99.0%.

4.10 Standard stock solution: Accurately weight an adequate amount of yohimbin, tadalafil, sildenafil and vardenafil, dissolve in methanol to make a mixed standard stock solution of 1.0 mg/mL in concentration. The standard solution should be stored below 4 ℃ avoiding sunlight.

4.11 Mixed standard solution: Dilute the standard stock solution with methanol to obtain a solution of 10 μg/mL as mixed standard solution, stored below 4 ℃ avoiding sunlight.

4.12 Standard working solution: Dilute the mixed standard solution (4.11) with 0.1% acetic acid-methanol mixed solution (4.5) to prepare a series of standard working solutions with concentrations of 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL and 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.

6 Sample preparation and storage

6.1 Preparation of test sample

6.1.1 Solid sample

Tablet, 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 Troche and capsule

Weigh ca 1 g (accurate to 0.01 g) of the test sample into a 100 mL conical flask with stopper. Add 80 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 100 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 5 min. Transfer 1.0 mL the extract upper layer into a 10 mL volumetric flask and dilute to mark with 0.1% acetic acid-methanol mixed solution (4.5). The diluted solution is filtered throught a 0.45 μm membrane and determined by LC-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 0.1% acetic acid-methanol mixed solution (4.5). The diluted solution is filtered throught a 0.45 μm membrane and determined by LC-MS/MS.

7.2 Determination

7.2.1 LC operating condition

LC operating condition is as following:

a) Chromatographic column: Octadecylsilica (ODs) or equivalent column [150 mm × 4.6 mm (i.d.), 5 μm];

b) Mobile phase and gradient program: See Table 1;

c) Flow rate:0.5 mL/min;

d) Column temperature:40 ℃;

e) Injection volume:10 μL.

Table 1 Gradient program of mobile phase

Time min	0.1% Acetic acid %	Acetonitrile %
0.00	80.0	20.0
3.00	80.0	20.0
8.00	5.0	95.0
13.00	5.0	95.0
13.01	80.0	20.0
20.00	80.0	20.0

7.2.2 MS operating condition

MS operating referenced conditions are shown in Annex A.

7.2.3 LC-MS/MS determination

7.2.3.1 Qualitative determination

According to the estimated approximate concentration of yohimbin, tadalafil, sildenafil and vardenafil in the sample solution, select the standard working solution of suitable concentration to that of sample solution. The responses of yohimbin, tadalafil, sildenafil and vardenafil in the standard working solution and the sample solution should be in the linear range of the instrumental detection. If the response is above the linear range, dilute the sample solution. Under the above LC-MS/MS operating conditions, the retention time of yohimbin, tadalafil, sildenafil and vardenafil are ca 9.4 min, 10.0 min, 10.2 min, 11.9 min, respectively. Quantitative analysis was carried out by external standard method. LC-MS/MS multiple reactions monitoring chromatograms of standard solution 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 yohimbin, tadalafil, sildenafil and vardenafil in the test sample by LC-MS/MS data processor or using the followed formula (1), the blank value should be subtracted from the result of calculation:

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

Where:

X_i —the content of tested composition in the test samples, mg/kg or mg/L;

c_i —the concentration of tested composition 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 yohimbin, tadalafil, sildenafil and vardenafil is 1.0 mg/kg for troche and capsule and 0.01 mg/L for oral solution.

8.2 Recovery

According to the experimental data for troche, capsule and oral solution, the fortifying concentration of yohimbin, tadalafil, sildenafil and vardenafil for each sample and the range of recovery is shown in Table 3.

Table 3 The fortifying concentration and the range of recovery of yohimbin, tadalafil, sildenafil and vardenafil in troche, capsule and oral solution

Compound	Sample					
	Troche		Capsule		Oral solution	
	Fortified level mg/kg	Range of recovery %	Fortified level mg/kg	Range of recovery %	Fortified level mg/L	Range of recovery %
Yohimbin	1.0	81.0～109.0	1.0	78.0～101.0	0.01	93.6～112
	2.0	80.0～94.5	2.0	77.5～98.0	0.05	96.6～104.0
	4.0	88.3～97.3	4.0	80.7～99.3	0.10	94.4～104.0
Vardenafil	1.0	82.0～107.0	1.0	82.0～108.0	0.01	81.1～97.9
	2.0	80.5～95.5	2.0	80.5～94.5	0.05	91.2～103.0
	4.0	85.0～98.5	4.0	80.0～98.7	0.10	95.8～105.0
Sildenafil	1.0	80.0～106.0	1.0	79.0～108.0	0.01	91.1～107.0
	2.0	90.5～102.0	2.0	80.5～103.0	0.05	91.2～103.0
	4.0	88.8～101.0	4.0	83.3～102.0	0.10	95.8～105.0
Tadalafil	1.0	81.0～106.0	1.0	81.0～106.0	0.01	82.1～97.7
	2.0	85.0～97.0	2.0	81.0～99.5	0.05	89.8～101.0
	4.0	82.3～97.8	4.0	80.5～103.0	0.10	92.8～103.0

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

LC-MS/MS reference condition:

a) Scan mode:Positive ion;

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

c) Curtain gas(CUR):138 kPa(20 psi);

d) Nebulizer gas(GS1):276 kPa(40 psi);

e) Auxiliary gas(GS2):310 kPa(45 psi);

f) Collision gas(CAD):55 kPa(8 psi);

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

h) Ion source temperature(TEM):550.0 ℃.

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

Table A.1—Confirmation ion,quantification ion,declustering potential,collision energy and collision cell exit potential for compound

Compound	Parant ion (*m/z*)	Daughter ion (*m/z*)	Declustering Potential(DP) V	Collision Energy(CE) V	Collision cell Exit Potential (CXP) V
Yohimbin	355.2	144.1*	108	50	15
		212.4	108	30	15
Vardenafil	489.5	151.1*	120	60	14
		312.1	120	56	18
Sildenafil	475.3	100.1*	110	40	16
		58.0	110	82	12
Tadalafil	390.4	268.3*	70	18	17
		135.1	70	30	22
Annotation: the symbol" * "represents the quantitative ion.					

1) Non-commercial statement:The reference mass parameters in Annex A are accomplished by API4000 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)
Multiple reaction monitor(MRM)chromatograms of yohimbin, tadalafil, sildenafil and vardenafil standard solution

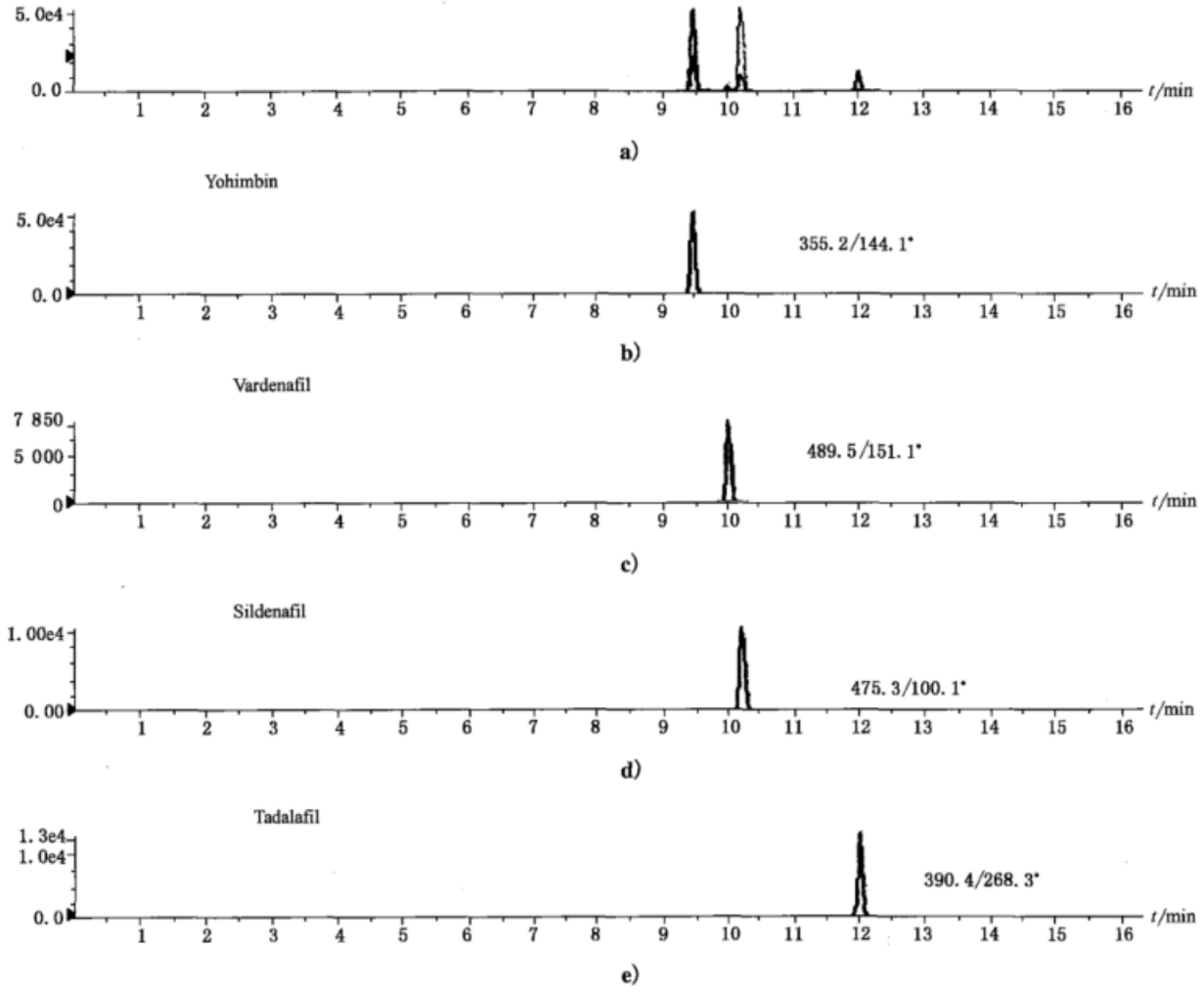

Figure B.1—Multiple reaction monitor(MRM)chromatograms of yohimbin, tadalafil, sildenafil and vardenafil standard solution