SN

中华人民共和国出入境检验检疫行业标准

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进出口保健食品中伐地那非、西地那非、他达那非的检测方法 液相色谱-质谱/质谱法

Determination of vardenafil, sildenafil and tadalafil in health foods for import and export—LC-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。

本标准由中华人民共和国湖南出入境检验检疫局起草。

本标准主要起草人：王美玲、万向阳、戴华、李拥军、王正良、王象贤、黄萍。

本标准系首次发布的出入境检验检疫行业标准。

进出口保健食品中伐地那非、西地那非、他达那非的检测方法 液相色谱-质谱/质谱法

1 范围

本标准规定了保健食品中伐地那非、西地那非、他达那非的液相色谱-质谱/质谱检测方法。

本标准适用于片剂、胶囊剂和口服液剂类保健食品中伐地那非、西地那非、他达那非的检测。

2 测定方法

2.1 方法提要

片剂、胶囊剂试样中的伐地那非、西地那非、他达那非用甲醇超声提取，提取液经稀释后过滤。口服溶液剂经直接稀释后过滤，用配有电喷雾离子源的液相色谱-质谱/质谱仪进行测定，外标法定量。

2.2 试剂和材料

所有试剂除特殊注明外，均为分析纯，水为二次蒸馏水。

2.2.1 乙腈：高效液相色谱级。

2.2.2 甲醇：高效液相色谱级。

2.2.3 冰乙酸：优级纯。

2.2.4 盐酸：优级纯。

2.2.5 0.1 mol/L 盐酸＋甲醇混合液(9＋1，体积比)。

2.2.6 伐地那非(vardenafil，CAS 号：224785-90-4，分子式：$C_{23}H_{32}N_6O_4S \cdot HCl \cdot 3H_2O$)标准品：纯度大于等于 99.5%。

2.2.7 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 99.5%。

2.2.8 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 99.5%。

2.2.9 标准溶液：分别准确称取适量伐地那非、西地那非、他达那非标准品，用甲醇配制成浓度为 10 μg/mL的混合标准储备液，于4℃下保存。根据需要用0.1 mol/L 盐酸＋甲醇混合液(2.2.5)将标准储备液稀释成 1 ng/mL、2 ng/mL、4 ng/mL、10 ng/mL、50 ng/mL、100 ng/mL 的混合标准工作溶液。

2.3 仪器和设备

2.3.1 高效液相色谱-质谱/质谱仪：三重四极杆质谱检测器，配电喷雾离子源(ESI)。

2.3.2 超声波清洗器。

2.3.3 旋涡混匀器。

2.3.4 电子天平：感量 1 mg。

2.3.5 研钵。

2.4 测定步骤

2.4.1 试样的制备

2.4.1.1 片剂

随机取同一批号的供试品 20 片，研细为试样。

2.4.1.2 硬胶囊

随机取同一批号的供试品 20 粒,倾出所有内容物,研细为试样。

2.4.1.3 软胶囊

随机取同一批号的供试品 20 粒,将内容物全部挤到一离心管中,充分混匀后,作为试样。

2.4.1.4 口服溶液剂

随机抽取同一批号的供试品 10 支(瓶),取等量体积样品到同一容器中混匀,作为试样。

2.4.2 提取

2.4.2.1 片剂、硬胶囊、软胶囊试样

称取 1 g 试样(精确到 0.01 g)于 100 mL 容量瓶中,加入 90 mL 甲醇,盖上盖混匀,置于超声波清洗器中超声 1 h,冷却至室温后用甲醇定容至刻度,混匀。取 1.0 mL 于 10 mL 容量瓶中,用 0.1 mol/L 盐酸+甲醇混合液(2.2.5)定容后混匀,溶液过 0.45 μm 微孔滤膜后,供液相色谱-质谱/质谱仪测定。

2.4.2.2 口服溶液剂

准确移取 1.0 mL 试样于 10 mL 容量瓶中,用 0.1 mol/L 盐酸+甲醇混合液(2.2.5)定容,混匀,溶液过 0.45 μm 微孔滤膜后,供液相色谱-质谱/质谱仪测定。

2.4.3 测定

2.4.3.1 液相色谱-质谱/质谱条件

a) 色谱柱:柱填料为十八烷基硅烷键合相的色谱柱,2.1×150 mm,5 μm 或相当者;

b) 流动相:梯度洗脱程序见表 1;

c) 流速:0.20 mL/min;

d) 柱温:40℃;

e) 进样量:10 μL;

f) 离子源:电喷雾离子源;

g) 扫描方式:正离子;

h) 检测方式:多反应监测(MRM);

i) 雾化气、窗帘气、辅助加热气、碰撞气等气体使用前应调节各气体流量以使质谱灵敏度达到检测要求,参考条件参见附录 A;

j) 喷雾电压、去集簇电压、碰撞能等电压值应优化至最优灵敏度,参考条件参见附录 A;

k) 监测离子对(m/z):他达那非 390.4/268.3(定量离子对)、390.4/135.1、390.4/169.1;西地那非 475.3/100.1(定量离子对)、475.3/58.0、475.3/311.4;伐地那非 489.5/151.1(定量离子对)498.5/72.1、489.5/312.1。

表 1 流动相梯度洗脱程序

时间/min	0.1%乙酸水溶液/%	乙腈/%
0.00	85.0	15.0
3.00	85.0	15.0
8.00	5.0	95.0
13.00	5.0	95.0
13.01	85.0	15.0
20.00	85.0	15.0

2.4.3.2 液相色谱-质谱/质谱测定

按照 2.4.3.1 液相色谱-质谱/质谱条件测定样液和标准工作溶液,标准曲线法测定样液中的伐地那非、西地那非、他达那非含量。样液中被测物的响应值应在仪器线性范围之内,如果超出仪器线性范

围，应用0.1 mol/L盐酸＋甲醇混合液（2.2.5）进行适当稀释。在上述色谱条件下，伐地那非、西地那非、他达那非的质量色谱峰保留时间约为10.5 min、10.7 min、12.4 min。标准溶液的提取离子流色谱图参见附录B中的图B.1。

在相同的实验条件下，样液中被测物的质量色谱峰保留时间与标准工作溶液一致，并且在扣除背景后的样品色谱图中，所选择的离子对均出现，各定性离子的相对丰度与浓度相当的标准溶液的离子相对丰度一致，误差不超过表2规定的范围，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误差

相对离子丰度/%	允许的相对误差/%
＞50	±20
＞20～50	±25
＞10～20	±30
≤10	±50

2.4.3.3　空白试验

除不加试样外，均按上述测定步骤进行。

2.5　结果计算和表述

用液相色谱-质谱/质谱数据处理机或按照式(1)计算样品中伐地那非、西地那非、他达那非含量，计算结果需扣除空白。

$$X=\frac{(A-A_0)\cdot c_S\cdot V}{A_S\cdot m} \qquad \cdots\cdots\cdots\cdots(1)$$

式中：

X——试样中伐地那非、西地那非、他达那非的含量，单位为毫克每千克(mg/kg)或者毫克每升(mg/L)；

A——样液中伐地那非、西地那非、他达那非的峰面积或峰高；

c_S——标准工作液中伐地那非、西地那非、他达那非的浓度，单位为微克每毫升(μg/mL)；

A_S——标准工作液中伐地那非、西地那非、他达那非的峰面积或峰高；

V——样液最终定容体积，单位为毫升(mL)；

A_0——空白实验液中伐地那非、西地那非、他达那非的峰面积或峰高；

m——最终样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)。

3　测定低限、回收率

3.1　测定低限

本方法片剂、胶囊剂样品中伐地那非、西地那非、他达那非的测定低限均为1.0 mg/kg；测定口服溶液剂样品中伐地那非、西地那非、他达那非的测定低限均为0.010 mg/L。

3.2　回收率

3.2.1　保健食品中伐地那非添加浓度及其回收率数据

片剂、胶囊剂添加浓度在1.0 mg/kg～10 mg/kg时，回收率在89.3%～104%之间。

口服溶液添加浓度在0.010 mg/L～0.10 mg/L时，回收率在94.5%～114%之间。

3.2.2　保健食品中西地那非添加浓度及其回收率数据

片剂、胶囊剂添加浓度在1.0 mg/kg～10 mg/kg时，回收率在83.8%～101%之间。

口服溶液添加浓度在0.010 mg/L～0.10 mg/L时，回收率在85.4%～108%之间。

3.2.3　保健食品中他达那非添加浓度及其回收率数据

片剂、胶囊剂添加浓度在1.0 mg/kg～10 mg/kg时，回收率在81.5%～108%之间。

口服溶液添加浓度在0.010 mg/L～0.10 mg/L时，回收率在85.7%～100%之间。

附　录　A

（资料性附录）

API 4 000 LC-MS/MS 系统检测伐地那非、西地那非、他达那非参考条件[1)]

API 4 000 LC-MS/MS 系统电喷雾离子源参考条件：

a）窗帘气(CUR)：20.00 Psi；

b）雾化气(GS1)：40.00 Psi；

c）辅助加热气(GS2)：45.00 Psi；

d）碰撞气(CAD)：7.00 Psi；

e）离子源喷雾器电压(IS)：5 000.00 V；

f）喷雾针温度(TEM)：550.00℃；

g）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　3 种待测物定性离子对、定量离子对、去簇电压、碰撞能量和碰撞室出口电压

待测物	Q1 m/z	Q3 m/z	去簇电压/V	碰撞能量/V	碰撞室出口电压/V
伐地那非	489.5[a]	151.1[a]	120.00	60.00	13.00
	489.5	72.1	120.00	85.00	13.00
	489.5	312.1	120.00	55.00	19.00
西地那非	475.3[a]	100.1[a]	110.00	42.85	17.00
	475.3	58.0	110.00	85.00	10.00
	475.3	311.4	110.00	41.70	23.00
他达那非	390.4[a]	268.3[a]	70.00	18.50	17.00
	390.4	135.1	70.00	31.40	23.00
	390.4	169.1	70.00	50.00	13.00

[a] 为定量离子对。

1）非商业性声明：本标准所采用仪器设备及型号不涉及商业目的，鼓励标准使用者尝试不同厂家或型号的仪器。

附 录 B

（资料性附录）

伐地那非、西地那非、他达那非混合标准溶液提取离子流色谱图

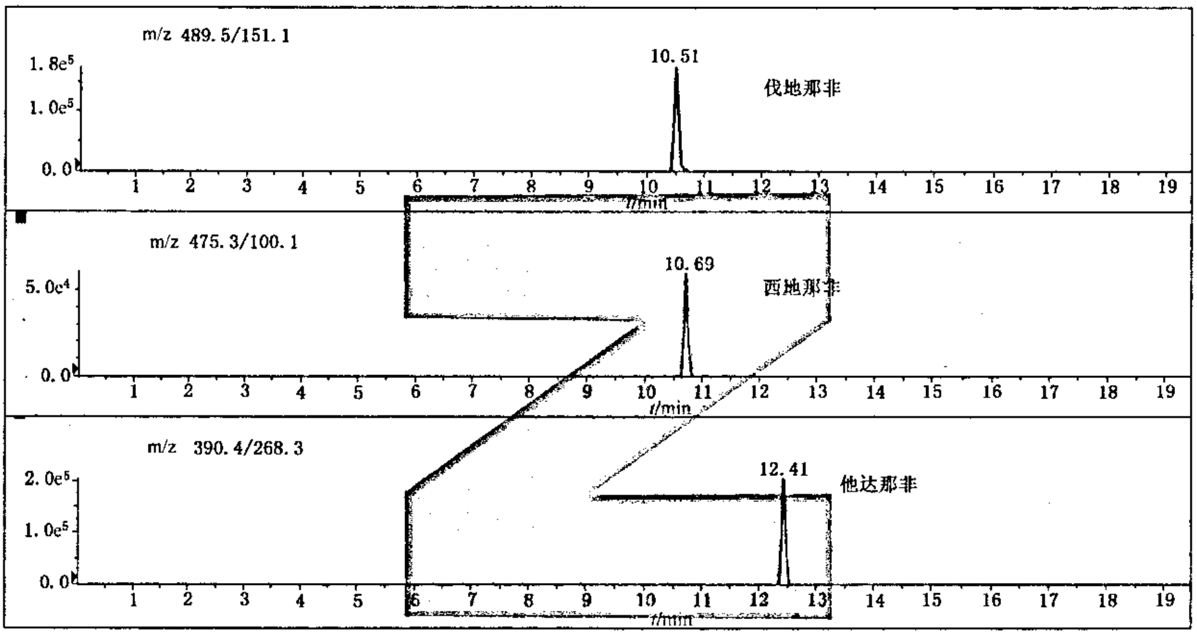

图 B.1 伐地那非、西地那非、他达那非混合标准溶液提取离子流色谱图

Foreword

Annex A and annex B of this standard are informative annexes.

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 Brureau of the People's Republic of China.

The standard was mainly drafted by Wang Meiling, Wan Xiangyang, Dai Hua, Li Yongjun, Wang Zhengliang, Wang Xiangxian and Huang Ping.

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

Note: This English version, a translation from the Chinese text, is solely for guidance.

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

1 Scope

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

This standard is applicable to the determination of vardenafil, sildenafil and tadalafil in health foods for troche, capsule, soft capsule and oral solution.

2 Method of determination

2.1 Abstract of method

vardenafil, sildenafil and tadalafil in the troche, capsule and soft capsule samples are extracted with methanol in a 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.

2.2 Reagents and materials

Unless otherwise specified, all the reagents should be of analytical grade and double-distilled water is used.

2.2.1 Acetonitrile: HPLC grade.

2.2.2 Methanol: HPLC grade.

2.2.3 Acetic acid: Guaranteed grade.

2.2.4 Hydrochloric acid: Guaranteed grade.

2.2.5 0.1 mol/L hydrochloric acid solution-methanol (9+1, *V*/*V*).

2.2.6 Vardenafil (CAS No: 224785-90-4, molecular formula: $C_{23}H_{32}N_6O_4S \cdot HCl \cdot 3H_2O$) standard: Purity≥99.5%.

2.2.7 Sildenafil (CAS No: 139755-83-2, molecular formula: $C_{22}H_{30}N_6O_4S$) standard: Purity≥99.5%.

2.2.8 Tadalafil (CAS No: 171596-29-5, molecular formula: $C_{22}H_{19}N_3O_4$) standard: Purity≥99.5%.

2.2.9 Standard solution: accurately weight an adequate amount of vardenafil, sildenafil and tadalafil standard in an volumetric flask and dissolve with methanol to form a mixed standard stock solution of 10 μg/mL in concentration, and store at 4℃. Dilute the mixed standard stock solution with 0.1 mol/L hydrochloric acid solution-methanol (2.2.5) to obtain a series of the mixed standard working solutions with concentrations of 1, 2, 4, 10, 50 and 100 ng/mL.

2.3 Apparatus and equipment

2.3.1 High performance liquid chromatography triple-quadrupole tandem mass spectrometer equipped with electrospray ionization source (ESI).

2.3.2 Ultrasonic washer.

2.3.3 Vortex mixer.

2.3.4 Electronic balance: readability 1 milligram.

2.3.5 Mortar.

2.4 Procedure

2.4.1 Preparation of test sample

2.4.1.1 Troche

Twenty troches are sampled at random and ground into powder which is used as test sample.

2.4.1.2 Capsule

Twenty pills are sampled at random and the materials in every capsule are spilled out and ground into power which is used as test sample.

2.4.1.3 Soft capsule

Twenty pills are sampled at random and the materials in every capsule are spilled into the same centrifuge tube and sufficiently mixed. The mixed sample is used as test sample.

2.4.1.4 Oral solution

Ten bottles of oral solution are sampled at random. Transfer the same volume of solution from every bottle into the same container and sufficiently mixed. The mixed sample is used as test sample.

2.4.2 Extraction

2.4.2.1 Troche, capsule and soft capsule

Weigh 1 g (accurate to 0.01 g) of the test sample into a 100 mL volumetric flask, add 90 mL methanol, put the stopper and extract in a ultrasonic washer for 1 h. Cool down to room temperature, dilute to mark with methanol and mix the contents. Transfer 1.0 mL of the extract into a 10 mL volumetric flask and dilute to mark with 0.1 mol/L hydrochloric acid aqueous solution-methanol (2.2.5). The diluted solution is filtered through a 0.45 μm membrane and determined by LC-MS/MS.

2.4.2.2 Oral solution

Accurately transfer 1.0 mL of test sample into a 10 mL volumetric flask and dilute to mark with 0.1 mol/L hydrochloric acid aqueous solution-methanol (2.2.5). The diluted solution is filtered through a 0.45 μm membrane and determined by LC-MS/MS.

2.4.3 Determination

2.4.3.1 LC-MS/MS operating conditions

a) Chromatographic column: octadecylsilica (ODS) or equivalent column (2.1 × 150 mm, 5 μm);

b) Mobile phase and gradient program see table 1;

c) Flow rate: 0.20 mL/min;

d) Column temperature: 40℃;

e) Injection volume: 10 μL;

f) Ion source: electrospray ionization source (ESI);

g) Scan mode: positive ion;

h) Detection mode: multiple reaction monitoring (MRM);

i) Nebulizer Gas, curtain gas, collision gas and auxiliary gas should be optimized by adjusting the gas flow parameters. Reference conditions are shown in Annex A;

j) Ion spray voltage, deflector voltage, collision energy and so on should be optimized to reach the highest sensitive of mass spectrometer. Referenced conditions are shown in Annex A;

k) Monitoring ions pairs (m/z): tadalafil 390.4/268.3 (quantitation ion pair), 390.4/135.1, 390.4/169.1; sildenafil 475.3/100.1 (quantitation ion pair), 475.3/58.0, 475.3/311.4; vardenafil 489.5/151.1 (quantitation ion pair), 498.5/72.1, 489.5/312.1.

Table 1—Mobile phase and gradient program

Time/min	0.1% Acetic acid aqueous solution/%	Acetonitrile/%
0.00	85.0	15.0
3.00	85.0	15.0
8.00	5.0	95.0
13.00	5.0	95.0
13.01	85.0	15.0
20.00	85.0	15.0

2.4.3.2 LC-MS/MS determination

According to the LC-MS/MS operating conditions (2.4.3.1), the standard working solution and the sample solution are determined. Quantitative analysis of the vardenafil, sildenafil and tadalafil in the sample solution is done using standard curve method. The responses of vardenafil, sildenafil and tadalafil in the sample solution should be within the linear range of the instrumental detection. If the response is above the linear range, dilute the sample solution with 0.1 mol/L hydrochloric acid aqueous solution-methanol (2.2.5). Under the above chromatographic condition, the retention time of vardenafil, sildenafil and tadalafil are ca 10.5 min, 10.7 min and 12.4 min, respectively. For LC-MS/MS chromatogram of the standard, see Figure B.1 in annex B.

According to the LC-MS/MS operating conditions (2.4.3.1), 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 ratio of the selected ions according with that of the calibration standard, at comparable concentrations, within the tolerances (seen table 2). The corresponding analyte could be confirmed.

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

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

2.4.3.3 Blank test

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

2.5 Calculation and expression of result

Calculate the content of vardenafil, sildenafil and tadalafil in the test sample by LC/MS/MS data processor or according to the formula (1), the blank value should be subtracted from the result of calculation:

$$X=\frac{(A-A_0)\cdot c_S\cdot V}{A_S\cdot m} \quad\cdots\cdots (1)$$

where

X—the content of tadalafil, sildenafil, vardenafil in the test sample, mg/kg or mg/L;

A—the peak area or peak height of tadalafil, sildenafil, vardenafil in the sample solution;

c_S—the concentration of tadalafil, sildenafil, vardenafil in the standard working solution, μg/mL;

A_S—the peak area or peak height of tadalafil, sildenafil, vardenafil in the standard working solution;

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

A_0—the peak area or peak height of tadalafil, sildenafil, vardenafil in the blank solution;

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

3 Limit of determination and recovery

3.1 Limit of determination

The limit of quantification is 1.0 mg/kg for troche, capsule and soft capsule and 0.010 mg/L for oral solution in this method.

3.2 Recovery

3.2.1 According to the experiment data, the fortified concentrations of vardenafil in health foods and its corresponding recoveries are:

Spike 1.0 mg/kg～10 mg/kg in capsule, troche and soft capsule, the recovery is 89.3%～104%.

Spike 0.010 mg/L～0.10 mg/L in oral solution, the recovery is 94.5%～114%.

3.2.2 According to the experiment data, the fortified concentrations of sildenafil in health foods and its corresponding recoveries are:

Spike 1.0 mg/kg～10 mg/kg in capsule, troche and soft capsule, the recovery is 83.8%～101%.

Spike 0. 010 mg/L～0. 10 mg/L in oral solution, the recovery is 85. 4%～108%.

3.2.3 According to the experiment data, the fortified concentrations of tadalafil in health foods and its corresponding recoveries are:

Spike 1. 0 mg/kg～10 mg/kg in capsule, troche and soft capsule, the recovery is 81. 5%～108%.

Spike 0. 010 mg/L～0. 10 mg/L in oral solution, the recovery is 85. 7%～100%.

Annex A
(informative)
Referenced conditions for analysing vardenafil, sildenafil and tadalafil by API 4 000 LC-MS/MS system[1)]

Referenced conditions for electrospray ionization source of API 4 000 LC-MS/MS system:

a) Curtain gas (CUR): 20.00 Psi;

b) Nebulizer gas (GS1): 40.00 Psi;

c) Auxiliary gas (GS2): 45.00 Psi;

d) Collision gas (CAD): 7.00 Psi;

e) Ion spray voltage (IS): 5 000.00 V;

f) Probe temperature(TEM): 550.00℃;

g) Transitions for confirmation and quantification, DP, CE, CXP see table A.1.

Table A.1—Transitions for confirmation and quantification, DP, CE, CXP

Target analyte	Q1 m/z	Q3 m/z	DP/V	CE/V	CXP/V
vardenafil	489.5[a]	151.1[a]	120.00	60.00	13.00
	489.5	72.1	120.00	85.00	13.00
	489.5	312.1	120.00	55.00	19.00
sildenafil	475.3[a]	100.1[a]	110.00	42.85	17.00
	475.3	58.0	110.00	85.00	10.00
	475.3	311.4	110.00	41.70	23.00
tadalafil	390.4[a]	268.3[a]	70.00	18.50	17.00
	390.4	135.1	70.00	31.40	23.00
	390.4	169.1	70.00	50.00	13.00

[a] Quantitation ion pair.

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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.

Annex B
(informative)
Extracted ion current chromatograms for a mixture of vardenafil, sildenafil and tadalafil standard

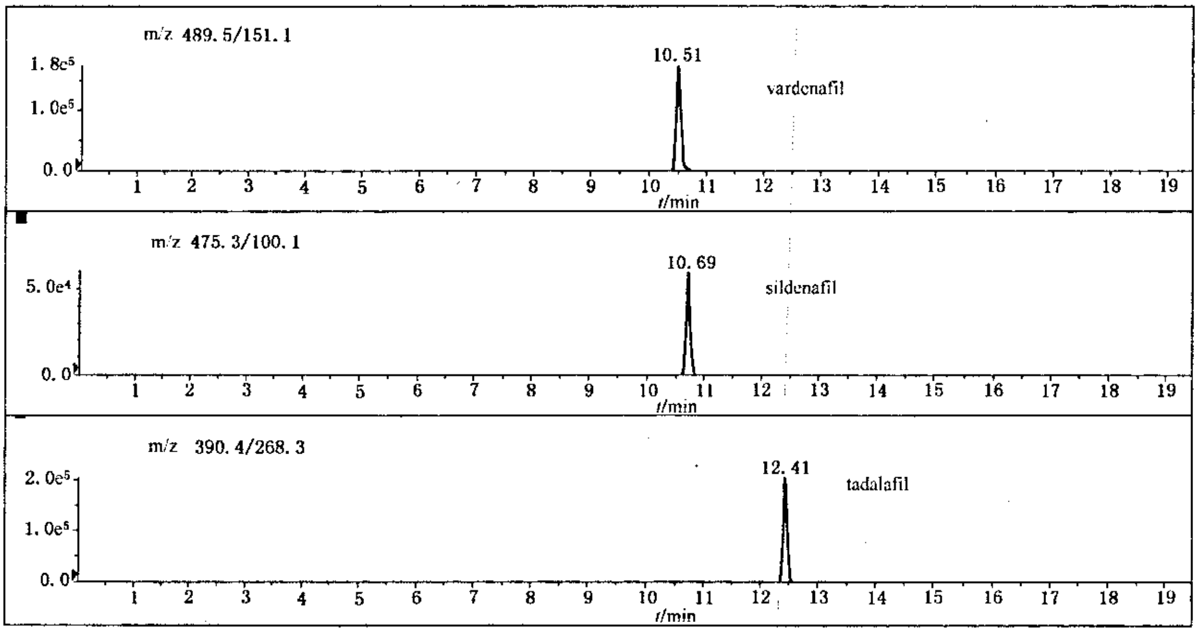

Figure B.1—Extracted ion current chromatograms for a mixture of vardenafil, sildenafil and tadalafil standard

SN/T 1951-2007

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