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中华人民共和国出入境检验检疫行业标准

SN/T 4263—2015

出口食品中多种食欲抑制剂的测定 液相色谱-质谱/质谱法

Determination of multi-appetite suppressants in food 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：中华人民共和国重庆出入境检验检疫局、中国检验检疫科学研究院，中华人民共和国黑龙江省出入境检验检疫局。

本标准主要起草人：马微、李晓娟、王俊苏、刘磊、代汉慧、马强、朱明达、王海波、何浩。

出口食品中多种食欲抑制剂的测定

液相色谱-质谱/质谱法

1 范围

本标准规定了出口食品中芬氟拉明、苯丙醇胺、西布曲明、舍曲林、安非他酮、西酞普兰、氟西汀、苯氟雷司的液相色谱-质谱/质谱检测和确证。

本标准适用于片剂、胶囊、散剂、饼干、口服溶液、咖啡和茶中 8 种食欲抑制剂的检测和确证。

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 方法提要

将试样中的 8 种食欲抑制剂用甲醇溶液超声提取，用 0.1% 甲酸-甲醇混合液稀释；采用液相色谱-质谱/质谱检测，外标法定量。

4 试剂和材料

除非另有规定外，所有试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1 甲醇：HPLC 级。

4.2 甲酸：纯度 99%，HPLC 级。

4.3 乙酸铵：优级纯。

4.4 10 mmol/L 乙酸铵溶液：精确称取 0.771 g 乙酸铵(4.3)，超纯水溶解，稀释至 1 000 mL。

4.5 0.1% 甲酸溶液：移取 1.0 mL 甲酸用水定容至 1 000 mL。

4.6 0.1% 甲酸-甲醇混合液(3+7, 体积比)：移取 30 mL 0.1% 甲酸水溶液和 70 mL 甲醇，混匀。

4.7 标准物质：芬氟拉明、苯丙醇胺、西布曲明、舍曲林、安非他酮、西酞普兰、氟西汀、苯氟雷司标准品；纯度大于等于 95%，见附录 A。

4.8 标准储备溶液：分别称取 0.01 g(精确至 0.000 1 g)的标准品，除舍曲林用少量水溶解，用甲醇定容，其他标准品均用甲醇溶解定容至 100 mL，配成浓度为 100.0 mg/L 的标准储备溶液，置于 -18 °C 保存，有效期 3 个月。

4.9 混合标准中间溶液：分别吸取适量的 8 种食欲抑制剂标准储备液于 100 mL 容量瓶中，用甲醇定容至刻度，配成混合标准中间液。使得混合标准工作溶液中芬氟拉明 250 ng/mL、苯丙醇胺 500 ng/mL、西布曲明 100 ng/mL、舍曲林 500 ng/mL、安非他酮 500 ng/mL、西酞普兰 1 000 ng/mL、氟西汀 1 000 ng/mL、苯氟雷司 500 ng/mL。置于 -18 °C 保存，有效期 1 个月。

4.10 混合标准工作溶液：根据需要用甲醇将混合标准中间溶液(4.9)逐级稀释成适当浓度的混合标准工作溶液，需用现配。

4.11 塑料离心管：50 mL，具塞。

4.12 0.22 μm 滤膜:双性滤膜。

5 仪器和设备

5.1 超高效液相色谱-质谱/质谱仪:配有电喷雾离子源。

5.2 分析天平:感度为 0.001 g 和 0.1 mg。

5.3 涡旋混合器。

5.4 超声波清洗器。

5.5 离心机:10 000 r/min。

6 试样制备

6.1 片剂

随机取同一批号的供试品 20 片,研细混匀,均分成两份,分别装入洁净容器中,密封并标明标记。

6.2 胶囊

随机取同一批号的供试品 20 粒,倾出所有内容物混匀,均分成两份,分别装入洁净容器中,密封并标明标记。

6.3 饼干

随机取同一批号的供试品 10 片,研细混匀,均分成两份,分别装入洁净容器中,密封并标明标记。

6.4 散剂

随机取同一批号的供试品 10 袋,混匀,均分成两份,分别装入洁净容器中,密封并标明标记。

6.5 咖啡

随机取同一批号的供试品 10 袋,混匀,均分成两份,分别装入洁净容器中,密封并标明标记。

6.6 茶剂

随机取同一批号的供试品 10 袋,混匀,均分成两份,分别装入洁净容器中,密封并标明标记。

6.7 口服溶液剂

随机抽取同一批号的供试品 10 支(瓶),取等量体积溶液到同一洁净容器中混匀,均分成两份,密封并标明标记。

6.8 注意事项

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 分析步骤

7.1 提取

7.1.1 片剂、胶囊、散剂、饼干、咖啡和茶叶样品

称取 1.0 g 试样(精确至 0.01 g)于具塞锥形瓶中,加入 20 mL 甲醇溶液(4.1),盖上盖混匀,置于超

声波清洗器中超声 15 min, 萃取完毕后转置离心管中, 以 6 000 r/min 离心 5 min。取 1.0 mL 于 5 mL 容量瓶中, 用 0.1% 甲酸-甲醇混合液(4.6)定容后并混匀, 溶液过 0.22 μm 滤膜, 供液相色谱-质谱/质谱进行测定。

7.1.2 口服溶液剂

准确移取 1.0 mL 试样于 10 mL 容量瓶中, 用 0.1% 甲酸-甲醇混合液(4.6)定容至刻度, 过 0.22 μm 滤膜, 供液相色谱-质谱/质谱进行测定。

7.2 液相色谱-质谱/质谱测定

7.2.1 色谱参考条件

色谱参考条件如下:

- 色谱柱: ACQUITY UPLC HSS T3 柱, 2.1 mm \times 100 mm, 粒度 1.8 μm , 或相当者;
- 流动相: 甲醇-10 mmol/L 乙酸铵水溶液, 梯度洗脱程序见表 1。

表 1 梯度洗脱程序

步骤	时间 min	10 mmol/L 乙酸铵水溶液 %	甲醇%	流速 mL/min	曲线 Curve
1	0	95	5	0.45	1
2	4	5	95	0.45	6
3	6	5	95	0.45	6
4	7	95	5	0.45	6
5	10	95	5	0.45	6

- 柱温: 30 $^{\circ}\text{C}$;
- 进样量: 5 μL ;
- 样品室温度: 20 $^{\circ}\text{C}$ 。

7.2.2 质谱参考条件

质谱参考条件如下:

- 离子源: 电喷雾离子源;
- 扫描方式: 正离子扫描;
- 检测方式: 多反应监测(MRM);
- 使用前应调节各参数使质谱灵敏度达到检测要求, 参考条件参见附录 B。

7.2.3 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一起进行色谱分析。混合标准工作液和待测液中 8 种食欲抑制剂的响应值均应在仪器线性响应范围内。对混合标准工作液和样液等体积进行测定。在上述色谱条件下, 8 种食欲抑制剂的参考保留时间分别约为: 苯丙醇胺 1.92 min、芬氟拉明 3.22 min、西酞普兰 3.47 min、氟西汀 3.82 min、安非他酮 4.0 min、舍曲林 4.22 min、苯氟雷司 4.40 min、西布曲明 5.51 min。标准溶液的 MRM 图参见附录 C。

7.2.4 定性测定

按照液相色谱-质谱/质谱条件测定样品和标准工作溶液,如果检测的质量色谱峰保留时间与标准工作溶液一致,允许偏差小于±2.5%。定性离子对的相对丰度应当与浓度相当标准工作溶液的相对丰度一致,相对丰度允许偏差不超过表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

7.2.5 空白试验

除不加试样外,均按上述操作步骤进行。

8 结果计算和表述

用液相色谱-质谱/质谱数据处理机或按式(1)计算试样中食欲抑制剂含量,计算结果扣除空白值:

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

式中:

X —— 试样中食欲抑制剂的含量,单位为毫克每千克(mg/kg)或者微克每升(μg/L);

c —— 由标准工作曲线计算得到的试样中食欲抑制剂的浓度,单位为微克每升(μg/L);

V —— 样液最终定容体积,单位为毫升(mL);

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)。

计算结果需扣除空白值。

9 测定低限和回收率

9.1 测定低限

测定低限见表3。

表3 不同样品中8种食欲抑制剂的测定低限

化合物	片剂 mg/kg	饼干 mg/kg	散剂 mg/kg	咖啡 mg/kg	胶囊 mg/kg	茶叶 mg/kg	口服溶液 μg/L
苯丙醇胺	0.5	0.5	0.5	5.0	5.0	5.0	5.0
芬氟拉明	0.2	0.2	0.2	2.0	2.0	2.0	2.0
西酞普兰	0.8	0.8	0.8	8.0	8.0	8.0	8.0
氟西汀	0.8	0.8	0.8	8.0	8.0	8.0	8.0
安非他酮	0.5	0.5	0.5	5.0	5.0	5.0	5.0
舍曲林	0.5	0.5	0.5	5.0	5.0	5.0	5.0
苯氟雷司	0.5	0.5	0.5	5.0	5.0	5.0	5.0
西布曲明	0.1	0.1	0.1	1.0	1.0	1.0	1.0

9.2 回收率范围

回收率见表4。

表4 不同样品中8种食欲抑制剂的添加回收率

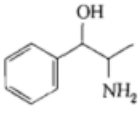
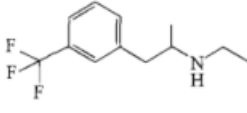
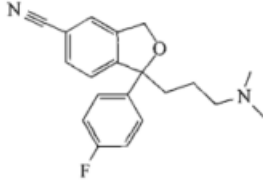
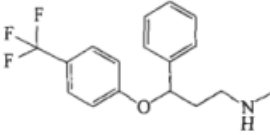
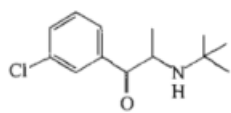
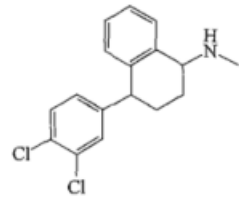
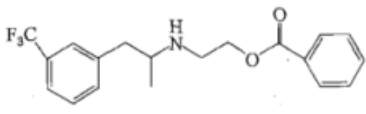
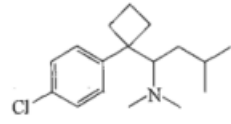
化合物	添加浓度 mg/kg	回收率范围 %			添加浓度 μg/L	回收率范围 %	添加浓度 mg/kg	回收率范围 %		
		片剂	散剂	饼干				口服液	茶叶	咖啡
苯丙醇胺	0.5	80.3~87.2	80.8~88.6	80.6~90.4	5.0	82.7~93.6	5.0	80.1~88.3	80.5~88.4	80.7~89.4
	1.0	81.3~88.6	81.6~89.7	81.3~92.2	10.0	84.1~94.8	10.0	81.3~89.4	81.5~90.5	81.9~91.2
	2.0	81.1~88.2	81.1~91.5	80.9~91.6	20.0	84.8~94.2	20.0	80.5~91.6	80.7~92.3	82.1~90.6
芬氟拉明	0.2	80.5~90.1	82.4~92.3	81.5~93.4	2.0	84.5~95.4	2.0	80.4~92.6	81.3~90.5	81.2~92.4
	0.4	81.3~91.7	82.5~91.4	82.3~95.1	4.0	86.2~96.7	4.0	82.3~94.3	83.5~92.9	84.5~93.6
	0.8	81.7~93.2	83.1~93.3	83.7~96.6	8.0	85.8~97.4	8.0	82.7~91.2	84.3~93.6	85.3~94.8
西酞普兰	0.8	80.9~94.1	80.9~91.3	80.4~89.3	8.0	85.2~96.7	8.0	82.5~93.4	81.5~89.5	83.5~93.4
	1.6	82.1~94.5	82.1~94.7	84.6~92.8	16.0	87.3~98.6	16.0	84.1~91.2	84.3~92.3	84.8~94.7
	3.2	81.6~95.1	81.6~95.3	87.2~93.9	32.0	88.2~99.1	32.0	83.9~93.1	84.5~93.3	85.1~95.2
氟西汀	0.8	83.5~ 101.2	82.3~96.3	87.1~96.3	8.0	85.7~98.6	8.0	85.7~99.4	89.4~ 100.6	90.3~ 100.3
	1.6	85.1~ 104.3	90.4~ 103.1	90.1~ 101.7	16.0	90.7~ 103.2	16.0	86.3~ 101.5	93.5~ 102.2	92.1~ 101.7
	3.2	86.3~ 103.8	91.1~ 104.3	89.8~ 103.9	32.0	91.4~ 102.1	32.0	88.6~ 102.4	94.1~ 101.9	91.7~ 103.9
安非他酮	0.5	81.3~94.3	81.1~89.6	82.1~93.3	5.0	82.6~95.5	5.0	80.5~89.1	80.5~87.6	83.5~95.4
	1.0	82.6~95.6	81.7~91.5	83.1~96.5	10.0	83.6~98.7	10.0	82.1~92.5	82.3~90.3	86.3~98.7
	2.0	83.2~96.2	80.6~92.2	82.4~97.2	20.0	83.1~98.2	20.0	81.2~93.6	82.5~91.1	84.5~97.9
舍曲林	0.5	84.3~97.5	89.4~96.1	87.3~96.8	5.0	87.3~98.6	5.0	82.4~97.5	85.3~98.7	85.2~98.7
	1.0	87.8~ 104.3	92.1~ 103.1	89.5~ 101.5	10.0	90.6~ 101.2	10.0	83.6~ 101.3	89.7~ 101.5	85.0~ 99.3
	2.0	89.4~ 106.2	91.7~ 102.3	90.3~ 106.3	20.0	92.3~ 105.1	20.0	83.3~ 105.2	90.4~99.3	93.3~ 101.5
苯氟雷司	0.5	82.3~96.5	83.7~96.6	83.1~97.8	5.0	88.8~ 104.6	5.0	85.3~ 102.7	91.2~98.4	90.6~ 102.4
	1.0	86.4~ 102.3	89.5~ 104.1	86.5~ 104.7	10.0	92.3~ 103.5	10.0	90.3~ 101.1	93.1~ 100.2	91.4~ 101.2
	2.0	85.5~ 104.8	88.9~ 103.3	87.4~ 103.9	20.0	90.7~ 106.2	20.0	88.7~ 102.3	92.7~ 102.3	92.5~ 105.1
西布曲明	0.1	85.7~96.5	86.1~97.4	86.6~98.4	1.0	85.4~98.1	1.0	85.1~96.3	86.4~96.6	89.5~97.3
	0.2	85.2~ 103.3	87.4~ 101.5	89.4~ 103.8	2.0	89.7~ 99.4	2.0	87.3~ 101.1	89.3~ 97.1	90.1~ 101.8
	0.4	86.5~ 102.5	89.2~ 102.4	90.7~ 102.9	4.0	90.1~ 98.3	4.0	88.1~ 99.2	90.4~ 101.4	89.4~ 103.9

附录 A

(规范性附录)

8 种食欲抑制剂标准物质的化学信息

表 A.1 8 种食欲抑制剂的基本信息

中文名称	英文名称	CAS号	结构式	分子式	相对分子质量
苯丙醇胺	Phenylpropanolamine	14838-15-4		$C_9H_{13}NO$	151.10
芬氟拉明	Fenfluramine	458-24-2		$C_{12}H_{16}F_3N$	231.12
西酞普兰	Citalopram	59729-33-8		$C_{20}H_{21}FN_2O$	324.16
氟西汀	Fluoxetine	54910-89-3		$C_{17}H_{18}F_3NO$	309.13
安非他酮	Bupropion	34911-55-2		$C_{13}H_{16}ClNO$	239.11
舍曲林	Sertraline	79617-96-2		$C_{17}H_{17}Cl_2N$	305.07
苯氟雷司	Benfluorex	23602-78-0		$C_{19}H_{20}F_3NO_2$	351.14
西布曲明	Sibutramine	106650-56-0		$C_{17}H_{26}ClN$	279.18

附录 B
(资料性附录)
质谱参考参数

质谱参考参数如下:

- a) 毛细管电压:3.5 kV;
- b) 射频透镜电压:0.5 V;
- c) 离子源温度:150 ℃;
- d) 去溶剂气温度:500 ℃;
- e) 去溶剂气流量:800 L/h;
- f) 锥孔气流量:50 L/h;
- g) 光电倍增器电压:650 V;
- h) 碰撞气体为氩气,碰撞气压 2.6×10^{-4} Pa;
- i) 监测离子对、锥孔电压、碰撞能量见表 B.1。

表 B.1 监测离子对、锥孔电压、碰撞能量

化合物	电离模式	监测离子对 <i>m/z</i>	锥孔电压 V	碰撞能量 eV
苯丙醇胺	ESI ⁺	152.1/134.0 ^a	12	8
		152.1/116.9		15
芬氟拉明	ESI ⁺	232.1/159.0	25	20
		232.1/108.9 ^a		40
西酞普兰	ESI ⁺	325.1/262.0	30	15
		325.1/108.9 ^a		20
氟西汀	ESI ⁺	310.1/148.1	13	6
		310.1/43.9 ^a		10
安非他酮	ESI ⁺	240.0/184.1 ^a	18	10
		240.0/131.0		25
舍曲林	ESI ⁺	305.9/274.9 ^a	12	10
		305.9/158.9		25
苯氟雷司	ESI ⁺	352.1/230.1 ^a	25	15
		352.1/149.0		15
西布曲明	ESI ⁺	280.1/139.0	18	15
		280.1/124.9 ^a		20

^a 定量离子(the ion for quantification)

附录 C

(资料性附录)

食欲抑制剂标准溶液多反应监测色谱图

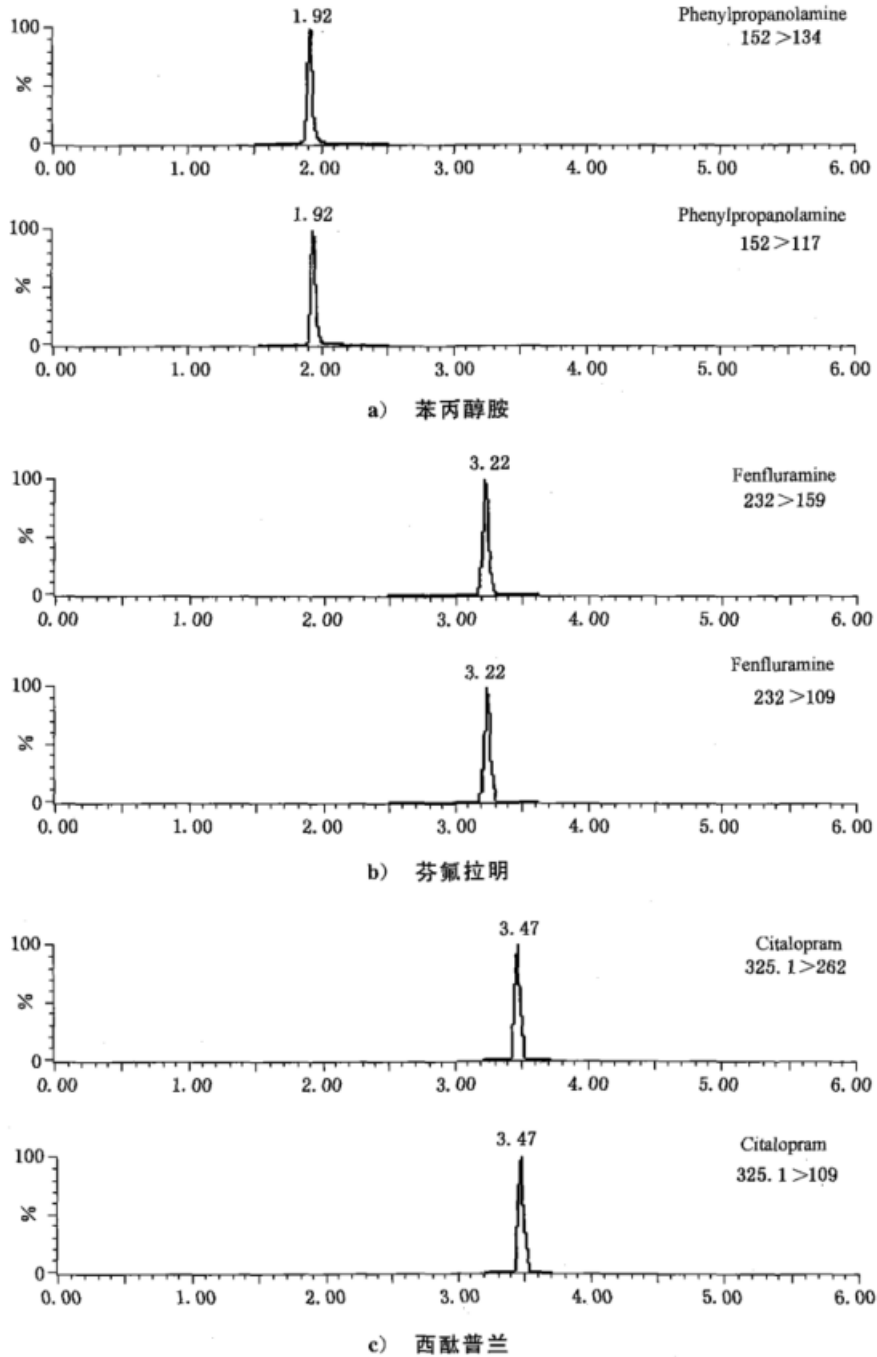
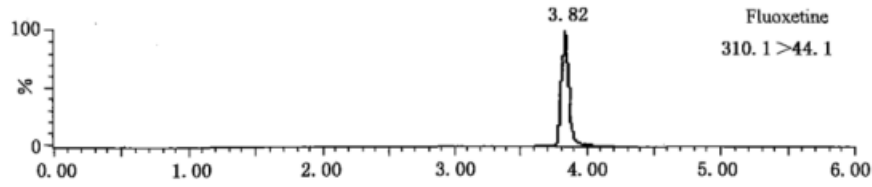
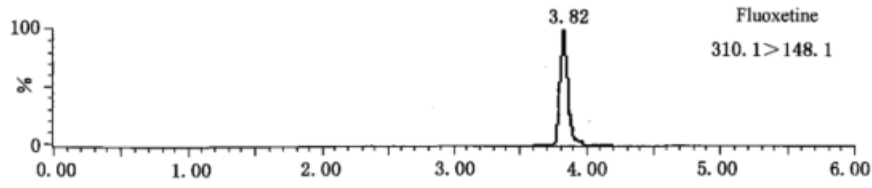
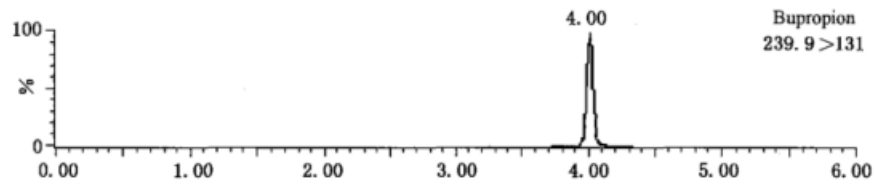
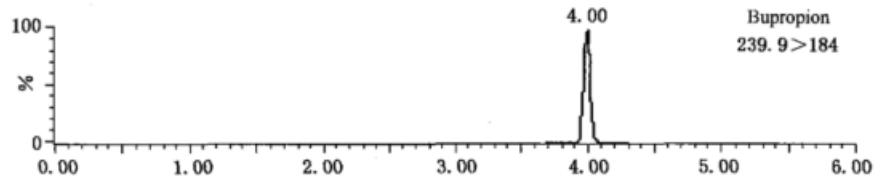


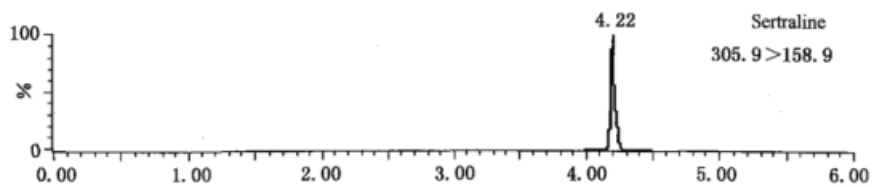
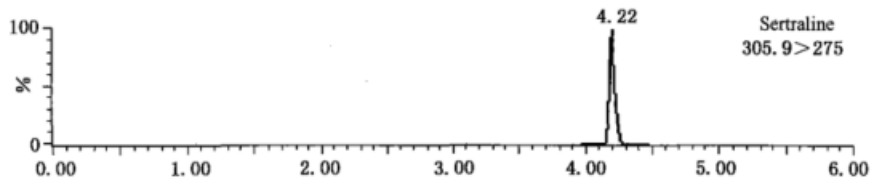
图 C.1 8 种食欲抑制剂标准品多反应监测色谱图



d) 氟西汀

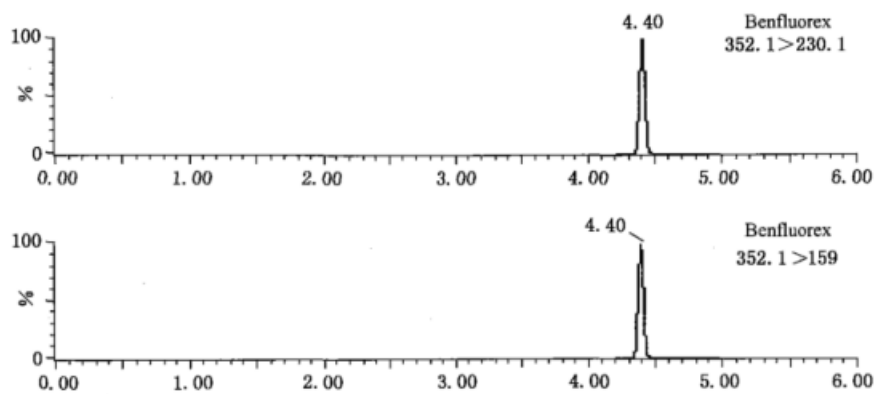


e) 安非他酮

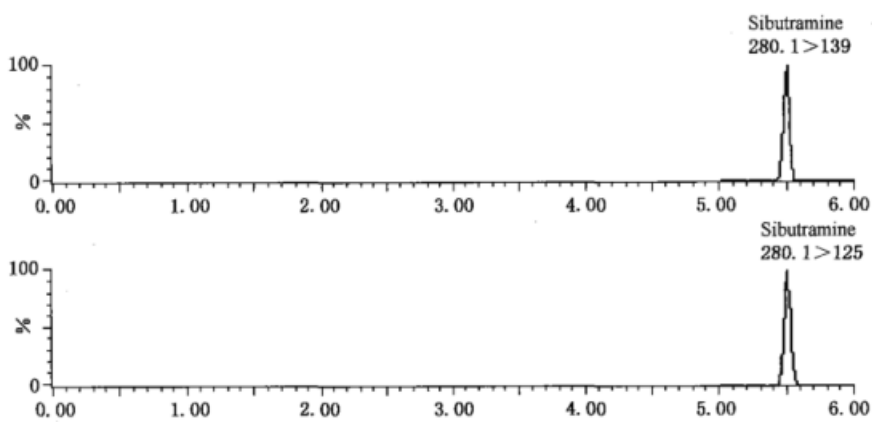


f) 舍曲林

图 C.1 (续)



g) 苯氟雷司



h) 西布曲明

图 C.1 (续)

Foreword

This standard is drafted according to the rules given by the 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 is proposed by and is under the jurisdiction of National Regulatory Commission for Certification and Accreditation.

This standard was drafted by Chongqing Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, China Inspection and Quarantine Institute of Science and Technology, Heilongjiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

This standard was mainly drafted by Ma wei, Li xiaojuan, Wang junsu, Liu lei, Dai hanhui, Ma qiang, Zhu mingda, Wang haibo, He hao.

Determination of multi-appetite suppressants in food for export—LC-MS/MS method

1 Scope

This standard specifies the determination and confirmation of Phenylpropanolamine, Fenfluramine, Citalopram, Fluoxetine, Bupropion, Sertraline, Benfluorex, Sibutramine in food for export by LC-MS/MS.

This standard is applicable to the determination and confirmation of phenylpropanolamine, fenfluramine, citalopram, fluoxetine, bupropion, sertraline, benfluorex, sibutramine in diet tablet, slimming capsule, slimming powder, diet cookies, slimming oral liquid, slimming coffee, slimming tea in food.

2 Normative references

The following documents is necessary for this standard. For dated references, Only dated editions shall apply to this standard. For undated references, the latest edition of the normative document referred to applies.

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

3 Principle

The 8 kinds of appetite suppressants in the test sample are extracted with methanol by the ultrasonic extraction method. After diluted by 0.1% methanoic-methanol mixture, the appetite suppressants are determined by LC-MS/MS, quantified by external standard method.

4 Reagents and materials

Unless specifically mentioned, all reagents used should be of analytically grade; Water is the first grade water prescribed by GB/T 6682.

4.1 Methanol; HPLC grade.

4.2 Methane acid; Purity is 99%, HPLC grade.

- 4.3 Ammonium acetate:GR.
- 4.4 10 mmol/L ammonium acetate:Accurately measure 0.771 g ammonium acetate (4.3), dissolve with ultrapure water and dilute to 1 000 mL.
- 4.5 0.1% methane acid:Transfer 1.0 mL methane acid into 1 000 mL volumetric flask,dissolve with water and dilute to the scale reading.
- 4.6 0.1% methanoic-methanol mixture (3 + 7, V/V): Add 30 mL 0.1% methane acid into 70 mL methanol,mix adequately.
- 4.7 Standards:Phenylpropanolamine, fenfluramine, citalopram, fluoxetine, bupropion, sertraline, benfluorex, sibutramine, purities are all $\geq 95\%$.Standards see Annex B.
- 4.8 Standard stock solution:Accurately weight 0.01 g (accurate to 0.000 1 g) of appetite suppressant standard (except for Fluoxetine) into 100 mL volumetric flask, respectively, then make up to the scale reading with methanol,prepare a solution of 100.0 mg/L.(Fluoxetine should be dissolved with little water first then processed with methanol same as other appetite suppressants.) Stored below $-18\text{ }^{\circ}\text{C}$, valid for 3 months.
- 4.9 Middle standard solution:Accurately transfer an adequate amount of standard stock solution into 100 mL volumetric flask, respectively, then make up to the scale reading with methanol,prepare a solution as the middle standard solution.Prepare the solution concentration as 500 ng/mL phenylpropanolamine, 250 ng/mL fenfluramine, 100 ng/mL citalopram, 500 ng/mL fluoxetine, 500 ng/mL bupropion, 1 000 ng/mL sertraline, 1 000 ng/mL benfluorex, 500 ng/mL sibutramine. Stored below $-18\text{ }^{\circ}\text{C}$, valid for 1 month.
- 4.10 Mix standard working solution:According to the requirement, dilute middle standard solution (4.9) to appropriate concentration with methanol, just before use.
- 4.11 Plastic centrifuge tube with cap:50 mL.
- 4.12 Membrane filter:0.22 μm .

5 Apparatus and equipment

- 5.1 Liquid chromatography-mass spectrometer equipment, triple quadrupole mass spectrometry detector, equipped with electro-spray ionization source (ESI).
- 5.2 Balance, Sensitivity:0.001 g and 0.1 mg.
- 5.3 Vortex mixer.
- 5.4 Ultrasonic cleaner.

5.5 Centrifuge: 10 000 r/min.

6 Sample preparation and storage

6.1 Tablet

Random sample 20 pieces of same lot identification mark tablets, grind and mix adequately, then divide into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.2 Capsule

Random sample 20 same lot identification mark capsules and pour the contents, grind and mix adequately, then divide into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.3 Cookies

Random sample 10 pieces of same lot identification mark cookies, grind and mix adequately, then divide into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.4 Powder

Random sample 10 pouches of same lot identification mark powder, grind and mix adequately, then divide into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.5 Coffee

Random sample 10 pouches of same lot identification mark powder, grind and mix adequately, then divide into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.6 Tea

Random sample 10 pouches of powder, grind and mix adequately, then divide into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.7 Oral liquid

Random sample 10 vials of same lot identification mark oral liquid, mix with same volume liquid, then divide in two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled.

6.8 Note

Prevent contamination of the samples during the sample preparation.

7 procedure

7.1 Extract

7.1.1 Tablet, capsule, powder, cookies, oral liquid, coffee, tea

Accurately weigh 1.0 g of the test sample (accurate to 0.01 g) into a Erlenmeyer flask with cap, add 20 mL methanol (4.1), mix adequately, clean 15 min in ultrasonic cleaner, then centrifuge at 6 000 r/min for 5 min. Transfer 1.0 mL supernatant into a 5 mL volumetric flask, dissolve with 0.1% methanoic-methanol mixture(4.6) and dilute to the scale reading. Then mix adequately and filter solution through a 0.22 μm membrane. The filtrate is ready for LC-MS/MS determination.

7.1.2 Oral liquid

Accurately transfer 1.0 mL of the test sample into 10 mL volumetric flask, dissolve with 0.1% methanoic-methanol mixture(4.6) and dilute to the scale reading. Then mix adequately and filter solution through a 0.22 μm membrane. The filtrate is ready for LC-MS/MS determination.

7.2 LC-MS/MS determination

7.2.1 LC operation reference conditions

LC operation reference conditions are as follows:

- a) LC column: ACQUITY UPLC HSS T3, 2.1 (i.d.) mm \times 100 mm, 1.8 μm , (or other conformable column).
- b) Mobile phase: methanol – 10 mmol/L ammonium acetate, elute condition: See Table 1;

Table 1—Elute condition

Step	Time min	10 mmol/L ammonium acetate %	Methanol %	Flow rate mL/min	Curve
1	0	95	5	0.45	1
2	4	5	95	0.45	6
3	6	5	95	0.45	6
4	7	95	5	0.45	6
5	10	95	5	0.45	6

- c) Column temperature: 30 °C ;
- d) Injector volume: 5 μL ;
- e) Sample cell temperature: 20 °C .

7.2.2 MS/MS operating reference conditions

MS/MS operating reference conditions are as follows:

- a) Ion source: ESI ;
- b) Scanning model: positive ion ;
- c) Monitoring model: multiple reaction monitoring (MRM) ;
- d) MS/MS reference conditions listed in Annex B.

7.2.3 Quantification test

According to the estimated approximate concentration of analytes in the sample solution, select the mix standard working solution of similar responses to that of sample solution. The responses of analytes in the sample solution should be in the linear range of the instrumental detection. The mix standard working solution should be injected randomly in-between the injections of the sample solution of equal volume. Under the above instrumental condition, the retention time of 8 kinds of appetite suppressants are phenylpropanolamine 1.92 min, fenfluramine 3.22 min, citalopram 3.47 min, fluoxetine 3.82 min, bupropion 4.0 min, sertraline 4.22 min, benfluorex 4.40 min, and sibutramine 5.51 min, respectively. For the chromatogram of standards, see Annex C.

7.2.4 Qualification test

Under above determination condition, the variation range of the retention time for the peak of analyte in unknown sample and in the mix standard working solution can not be out of range of $\pm 2.5\%$. For the same analysis batch and the same compound, the variation range of the ion ratio between the two daughter ions for the unknown sample and the standard working solution at the mix standard working solution at the similar concentration cannot be out of range of Table 2, and then the corresponding analyte must be present in the sample.

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

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

7.2.5 Blank test

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

8 Calculation and expression of result

Calculate the appetite suppressants content of analyte in the test sample according to the data processing software of LC-MS/MS equipment or formula(1):

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

Where:

X —the appetite suppressants content of analyte in the test sample, mg/kg or $\mu\text{g/L}$;

c —the concentration of appetite suppressants in the mix standard working solution, calculated by calibration curve, $\mu\text{g/L}$;

V —the final volume of sample solution, mL;

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

The blank value should be subtracted from the result of calculation.

9 Limit of determination and recovery

9.1 Limit of determination

The limit of determination see Table 3.

Table 3—Limit of determination of appetite suppressants in different samples(LOQ)

Compound	Tablet LOQ mg/kg	Cookies LOQ mg/kg	Powder LOQ mg/kg	Coffee LOQ mg/kg	Capsule LOQ mg/kg	Tea LOQ mg/kg	Oral Liquid LOQ $\mu\text{g/L}$
Phenylpropanolamine	0.5	0.5	0.5	5.0	5.0	5.0	5.0
Fenfluramine	0.2	0.2	0.2	2.0	2.0	2.0	2.0
Citalopram	0.8	0.8	0.8	8.0	8.0	8.0	8.0

Table 3 (continued)

Compound	Tablet LOQ mg/kg	Cookies LOQ mg/kg	Powder LOQ mg/kg	Coffee LOQ mg/kg	Capsule LOQ mg/kg	Tea LOQ mg/kg	Oral Liquid LOQ µg/L
Fluoxetine	0.8	0.8	0.8	8.0	8.0	8.0	8.0
Bupropion	0.5	0.5	0.5	5.0	5.0	5.0	5.0
Sertraline	0.5	0.5	0.5	5.0	5.0	5.0	5.0
Benfluorex	0.5	0.5	0.5	5.0	5.0	5.0	5.0
Sibutramine	0.1	0.1	0.1	1.0	1.0	1.0	1.0

9.2 Recovery and precision

Recovery and precision see Table 4.

Table 4—Recovery of appetite suppressants in different samples

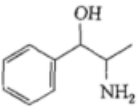
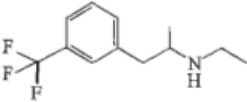
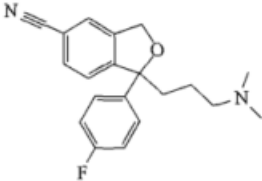
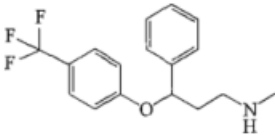
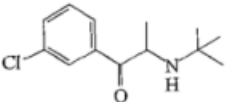
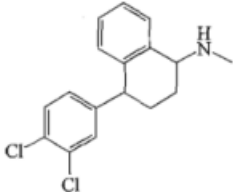
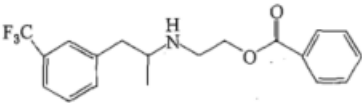
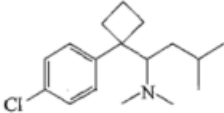
Compound	add concentration mg/kg	Recovery %			add concentration µg/L	Recovery %	add concentration mg/kg	Recovery %		
		Tablet	Powder	Cookies				Tea	Coffee	Capsule
Phenylpropanolamine	0.5	80.3~87.2	80.8~88.6	80.6~90.4	5.0	82.7~93.6	5.0	80.1~88.3	80.5~88.4	80.7~89.4
	1.0	81.3~88.6	81.6~89.7	81.3~92.2	10.0	84.1~94.8	10.0	81.3~89.4	81.5~90.5	81.9~91.2
	2.0	81.1~88.2	81.1~91.5	80.9~91.6	20.0	84.8~94.2	20.0	80.5~91.6	80.7~92.3	82.1~90.6
Fenfluramine	0.2	80.5~90.1	82.4~92.3	81.5~93.4	2.0	84.5~95.4	2.0	80.4~92.6	81.3~90.5	81.2~92.4
	0.4	81.3~91.7	82.5~91.4	82.3~95.1	4.0	86.2~96.7	4.0	82.3~94.3	83.5~92.9	84.5~93.6
	0.8	81.7~93.2	83.1~93.3	83.7~96.6	8.0	85.8~97.4	8.0	82.7~91.2	84.3~93.6	85.3~94.8
Citalopram	0.8	80.9~94.1	80.9~91.3	80.4~89.3	8.0	85.2~96.7	8.0	82.5~93.4	81.5~89.5	83.5~93.4
	1.6	82.1~94.5	82.1~94.7	84.6~92.8	16.0	87.3~98.6	16.0	84.1~91.2	84.3~92.3	84.8~94.7
	3.2	81.6~95.1	81.6~95.3	87.2~93.9	32.0	88.2~99.1	32.0	83.9~93.1	84.5~93.3	85.1~95.2
Fluoxetine	0.8	83.5~101.2	82.3~96.3	87.1~96.3	8.0	85.7~98.6	8.0	85.7~99.4	89.4~100.6	90.3~100.3
	1.6	85.1~104.3	90.4~103.1	90.1~101.7	16.0	90.7~103.2	16.0	86.3~101.5	93.5~102.2	92.1~101.7
	3.2	86.3~103.8	91.1~104.3	89.8~103.9	32.0	91.4~102.1	32.0	88.6~102.4	94.1~101.9	91.7~103.9
Bupropion	0.5	81.3~94.3	81.1~89.6	82.1~93.3	5.0	82.6~95.5	5.0	80.5~89.1	80.5~87.6	83.5~95.4
	1.0	82.6~95.6	81.7~91.5	83.1~96.5	10.0	83.6~98.7	10.0	82.1~92.5	82.3~90.3	86.3~98.7
	2.0	83.2~96.2	80.6~92.2	82.4~97.2	20.0	83.1~98.2	20.0	81.2~93.6	82.5~91.1	84.5~97.9

Table 4 (continued)

Compound	add concentration mg/kg	Recovery %			add concentration µg/L	Recovery %	add concentration mg/kg	Recovery %		
		Tablet	Powder	Cookies				Tea	Coffee	Capsule
Sertraline	0.5	84.3~97.5	89.4~96.1	87.3~96.8	5.0	87.3~98.6	5.0	82.4~97.5	85.3~98.7	85.2~98.7
	1.0	87.8~ 104.3	92.1~ 103.1	89.5~ 101.5	10.0	90.6~ 101.2	10.0	83.6~ 101.3	89.7~ 101.5	85.0~99.3
	2.0	89.4~ 106.2	91.7~ 102.3	90.3~ 106.3	20.0	92.3~ 105.1	20.0	83.3~ 105.2	90.4~ 99.3	93.3~ 101.5
Benfluorex	0.5	82.3~96.5	83.7~96.6	83.1~97.8	5.0	88.8~104.6	5.0	85.3~ 102.7	91.2~98.4	90.6~ 102.4
	1.0	86.4~ 102.3	89.5~ 104.1	86.5~ 104.7	10.0	92.3~ 103.5	10.0	90.3~ 101.1	93.1~ 100.2	91.4~ 101.2
	2.0	85.5~ 104.8	88.9~ 103.3	87.4~ 103.9	20.0	90.7~ 106.2	20.0	88.7~ 102.3	92.7~ 102.3	92.5~ 105.1
Sibutramine	0.1	85.7~96.5	86.1~97.4	86.6~98.4	1.0	85.4~98.1	1.0	85.1~96.3	86.4~96.6	89.5~97.3
	0.2	85.2~ 103.3	87.4~ 101.5	89.4~ 103.8	2.0	89.7~ 99.4	2.0	87.3~ 101.1	89.3~ 97.1	90.1~ 101.8
	0.4	86.5~ 102.5	89.2~ 102.4	90.7~ 102.9	4.0	90.1~98.3	4.0	88.1~99.2	90.4~ 101.4	89.4~ 103.9

Annex A
(Normative Annex)
Information of 8 chemical compounds

Table A.1—Information of 8 appetite suppressants

Name	CAS NO	Structural formula	Molecular formula	Molecular weight
Phenylpropanolamine	14838-15-4		$C_9H_{13}NO$	151.10
Fenfluramine	458-24-2		$C_{12}H_{16}F_3N$	231.12
Citalopram	59729-33-8		$C_{20}H_{21}FN_2O$	324.16
Fluoxetine	54910-89-3		$C_{17}H_{18}F_3NO$	309.13
Bupropion	34911-55-2		$C_{13}H_{18}ClNO$	239.11
Sertraline	79617-96-2		$C_{17}H_{17}Cl_2N$	305.07
Benfluorex	23602-78-0		$C_{19}H_{20}F_3NO_2$	351.14
Sibutramine	106650-56-0		$C_{17}H_{26}ClN$	279.18

Annex B
(Informative Annex)
MS/MS condition

MS/MS reference conditions are as follows:

- a) Capillary voltage;3.5 kV;
- b) Radiofrequency voltage;0.5 V;
- c) Source temperature;150 ℃ ;
- d) Desolvation temperature;500 ℃ ;
- e) Desolvation gas flow ;Nitrogen,800 L/h;
- f) Cone gase flow ;Nitrogen,50 L/h;
- g) Photoaugereon voltage;650 V;
- h) Collision gas pressure ;Argon, 2.6×10^{-4} Pa;
- i) Monitoring ion pairs,Cone voltage,Collision energy see Table B.1.

Table B.1—Monitoring ion pairs,Cone voltage,Collision energy

Compound	Ionization mode	Monitoring ion paris <i>m/z</i>	Cone Voltage V	Collision Energy eV
Phenylpropanolamine	ESI ⁺	152.1/134.0 ^a	12	8
		152.1/116.9		15
Fenfluramine	ESI ⁺	232.1/159.0	25	20
		232.1/108.9 ^a		40
Citalopram	ESI ⁺	325.1/262.0	30	15
		325.1/108.9 ^a		20
Fluoxetine	ESI ⁺	310.1/148.1	13	6
		310.1/43.9 ^a		10
Bupropion	ESI ⁺	240.0/184.1 ^a	18	10
		240.0/131.0		25
Sertraline	ESI ⁺	305.9/274.9 ^a	12	10
		305.9/158.9		25

Table B.1 (continued)

Compound	Ionization mode	Monitoring ion pairs <i>m/z</i>	Cone Voltage V	Collision Energy eV
Benfluorex	ESI ⁺	352.1/230.1 ^a	25	15
		352.1/149.0		15
Sibutramine	ESI ⁺	280.1/139.0	18	15
		280.1/124.9 ^a		20
^a the ion for quantification				

Annex C
(Informative Annex)
Standard MRM chromatogram of appetite suppressants

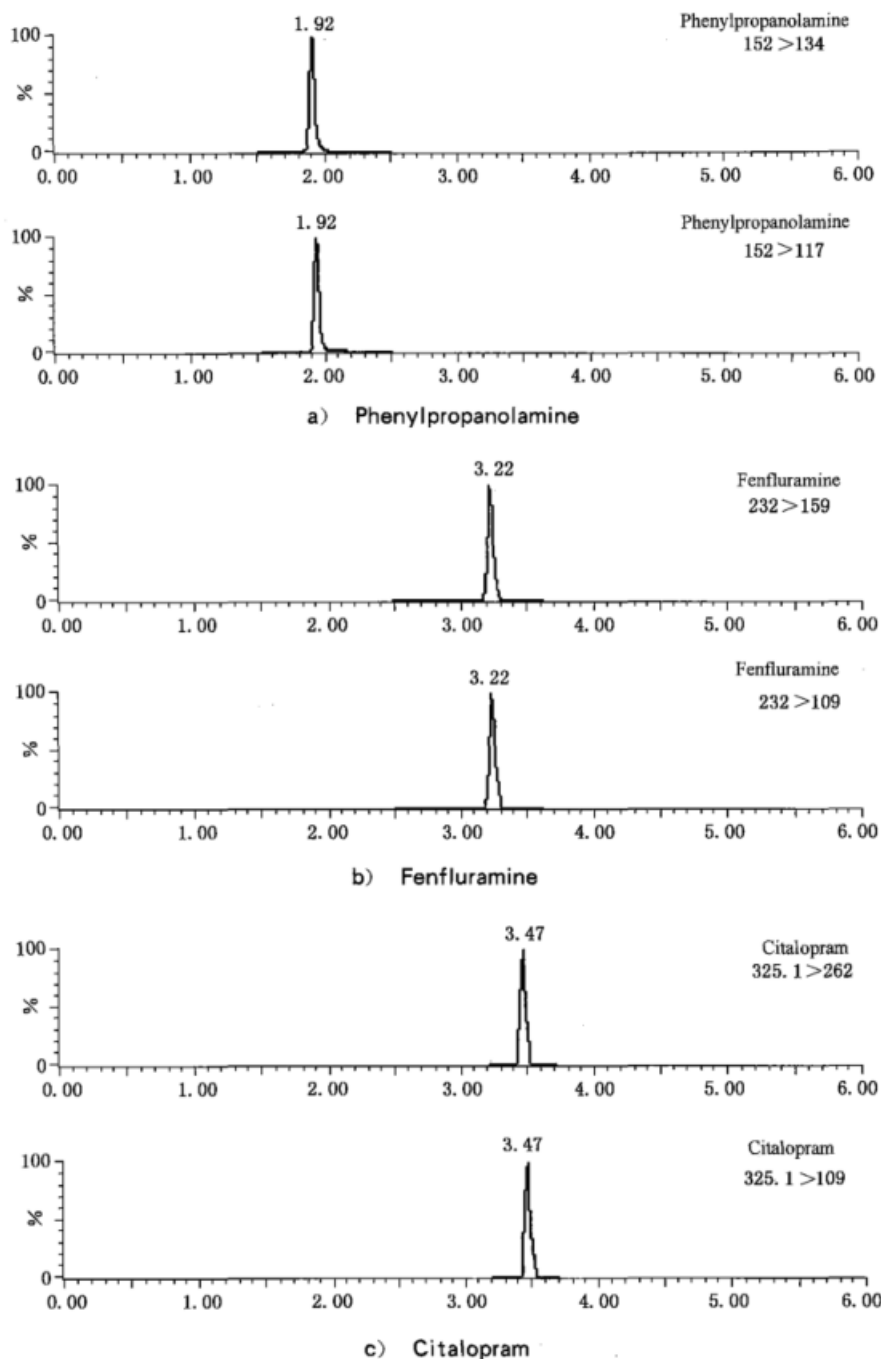
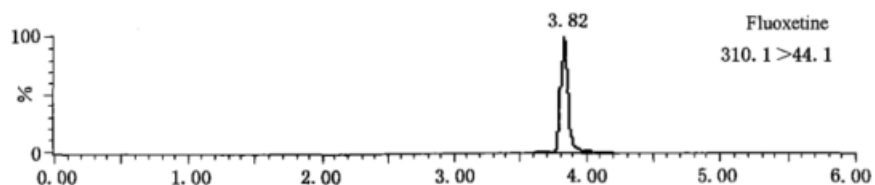
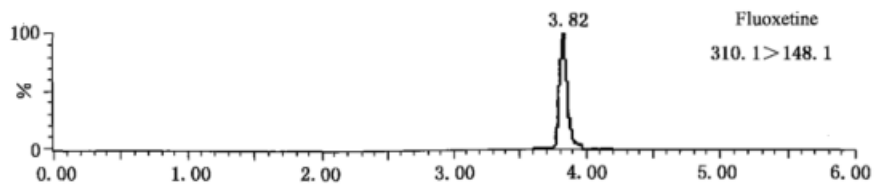
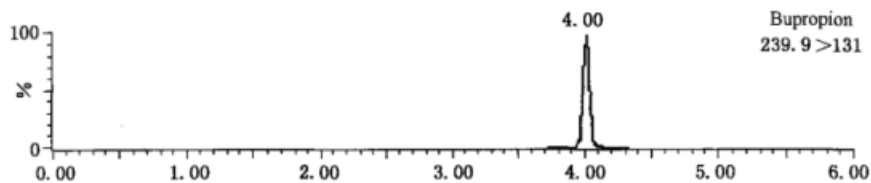
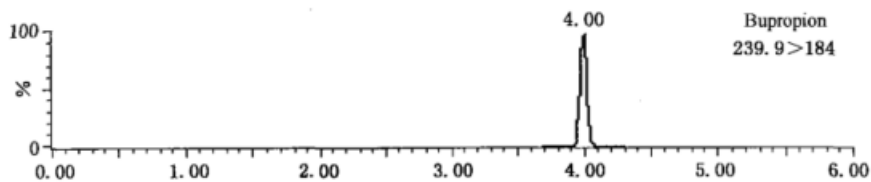


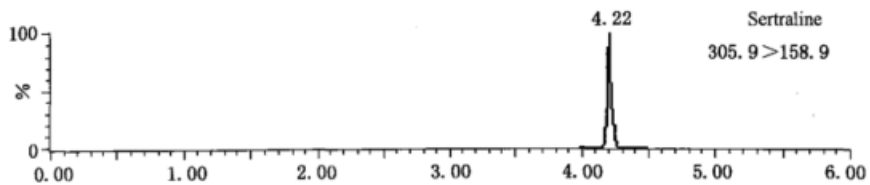
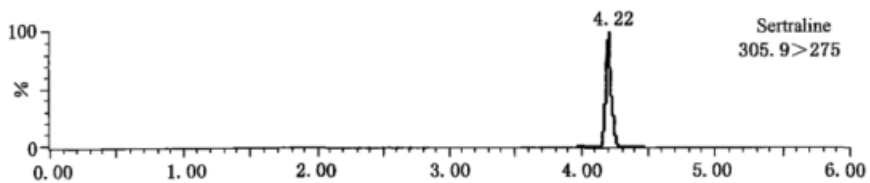
Figure C.1—Chromatogram of 8 chemical compounds



d) Fluoxetine

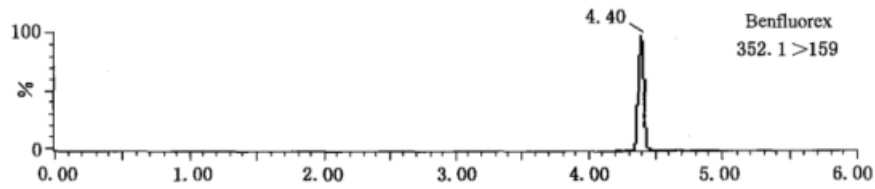
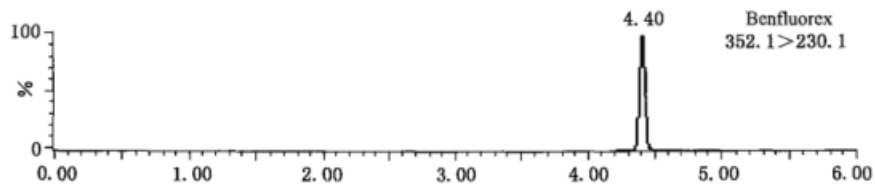


e) Bupropion

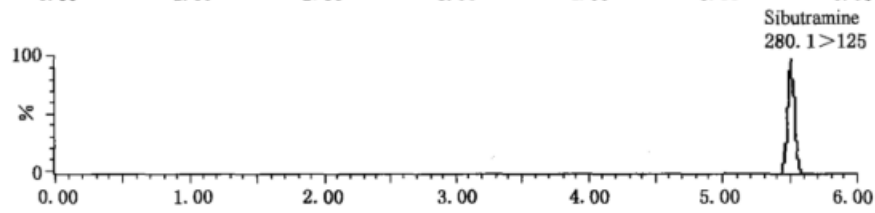
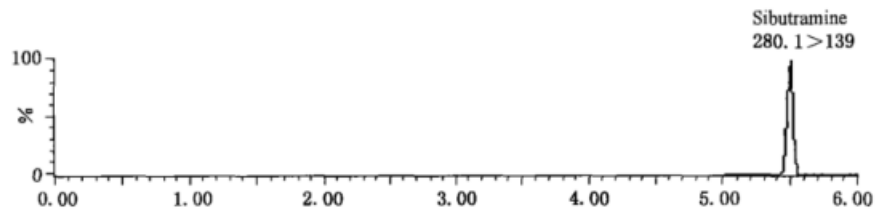


f) Sertraline

Figure C.1 (continued)



g) Benfluorex



h) Sibutramine

Figure C.1 (continued)