



# 中华人民共和国出入境检验检疫行业标准

SN/T 4777—2017

## 出口茶叶中蒽醌残留量的检测方法 气相色谱-质谱/质谱法

Determination of anthraquinone residues in tea for export—  
GC-MS/MS method

2017-05-12 发布

2017-12-01 实施

中 华 人 民 共 和 国  
国家质量监督检验检疫总局 发 布

## 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：浙江省检验检疫科学技术研究院、中华人民共和国上海出入境检验检疫局。

本标准主要起草人：谢文、陆勋元、黄超群、陈丽、申屠炎、楼成杰、于卓然、邓晓军、谢韵第。

## 出口茶叶中蒽醌残留量的检测方法

### 气相色谱-质谱/质谱法

#### 1 范围

本标准规定了出口绿茶、红茶、普洱茶、乌龙茶中 9,10-蒽醌残留量的气相色谱-质谱/质谱法测定方法。

本标准适用于绿茶、红茶、普洱茶、乌龙茶中 9,10-蒽醌残留量的定量测定和确证。

#### 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 方法提要

试样用正己烷-丙酮混合溶剂均质后提取,提取液浓缩后,再用弗罗里硅土柱净化,采用气相色谱-质谱/质谱仪检测和确证,内标法定量。

#### 4 试剂和材料

除非另有说明,所用试剂均为分析纯,所用水为符合 GB/T 6682 规定的一级水。

4.1 正己烷:色谱纯。

4.2 丙酮:色谱纯。

4.3 乙醚:色谱纯。

4.4 氯化钠。

4.5 无水硫酸钠:650℃灼烧4h,在干燥器内冷却至室温,贮于密封瓶中备用。

4.6 弗罗里硅土:粒度75μm~150μm(100目~200目),650℃灼烧4h,使用130℃活化4h在干燥器内冷却至室温,加2%的水脱活,备用。

4.7 正己烷-丙酮(1+1,体积比):取等体积的正己烷和丙酮混合均匀。

4.8 正己烷-乙醚(8+2,体积比):取正己烷和乙醚(8+2,体积比)混合均匀。

4.9 9,10-蒽醌、9,10-蒽醌-D8 标准物质(Anthraquinone, CAS:84-65-1,  $C_{14}H_8O_2$ ; Anthraquinone-D8, CAS:10439-39-1):纯度大于或等于99%。

4.10 标准储备溶液:准确称取适量标准物质(4.9),用丙酮配制成0.4 mg/mL 的标准储备液,于0℃~4℃冰箱内避光储存,有效期6个月。

4.11 蒽醌-D8 标准储备溶液:准确称取适量标准品(4.9),用丙酮配制成0.4 mg/mL 的标准储备液,于0℃~4℃冰箱内避光储存,有效期6个月。

4.12 标准中间液的配制:准确移取标准储备液适量(4.10),用丙酮配制成10 μg/mL 的标准溶液;准确移取标准储备液适量(4.11),用丙酮配制成0.2 μg/mL 的标准溶液于0℃~4℃冰箱内避光储存,有效

期 3 个月。

4.13 标准工作溶液的配制:根据需要将标准中间溶液(4.12)用丙酮稀释成适当浓度的标准工作溶液。

4.14 净化柱:200 mm×15 mm(内径)玻璃柱,底部垫约 5 mm 高脱脂棉和约 10 mm 高无水硫酸钠,10 g 弗罗里硅土(4.6),用正己烷湿法装柱,顶端加约 10 mm 高无水硫酸钠,弃去淋洗液(在使用前需先做淋洗曲线)。

4.15 微孔滤膜:0.22  $\mu\text{m}$ ,有机相型。

## 5 仪器和设备

5.1 气相色谱-质谱/质谱仪:配有(EI)离子源。

5.2 分析天平:感量 0.000 1 g 和 0.01 g。

5.3 涡旋混合器。

5.4 均质器。

5.5 离心机:最大转速 4 000 r/min。

5.6 旋转蒸发仪。

5.7 粉碎机。

## 6 试样制备与保存

取代表性样品约 250 g,经粉碎机粉碎并全部通过 20 目圆孔筛,混匀,装入洁净容器内密封,并标明标记。

在制样的操作过程中,应防止样品污染或发生残留物含量的变化。

## 7 测定步骤

### 7.1 提取

称取茶叶试样 2 g(精确至 0.01 g)于 50 mL 离心管中,加入 0.5 mL 蒽醌-D8(4.12),再加入 20 mL 正己烷-丙酮(4.7),以 10 000 r/min 均质 0.5 min,加入 2 g 氯化钠,涡旋 1 min,以 4 000 r/min 离心 3 min,将上层提取溶液转移入浓缩瓶中,用 20 mL 正己烷-丙酮(4.7)清洗均质器刀头,并将此提取液倒入残渣中,涡旋 1 min,以 4 000 r/min 离心 3 min,合并提取液,在 45  $^{\circ}\text{C}$  以下水浴减压浓缩至近干,待净化。

### 7.2 净化

用 3 mL 正己烷溶解残渣,并转移至净化柱(4.14),再用 50 mL 正己烷-乙醚(4.8)混合溶剂,流速为 3 mL/min,收集全部洗脱液,在 45  $^{\circ}\text{C}$  以下水浴减压浓缩至近干,加入 2.0 mL 丙酮溶解残渣,混匀,过 0.22  $\mu\text{m}$  滤膜(4.15),供气相色谱-质谱/质谱仪测定。

### 7.3 测定

#### 7.3.1 气相色谱-质谱/质谱条件

7.3.1.1 色谱柱:HP-5MS,30 m(长度)×0.25 mm(内径)×0.25  $\mu\text{m}$ (膜厚),或相当者。

7.3.1.2 程序柱温:100  $^{\circ}\text{C}$  保持 1 min,以 20  $^{\circ}\text{C}/\text{min}$  的速率升温至 300  $^{\circ}\text{C}$ ,保持 10 min。

7.3.1.3 载气:氦气,纯度 $\geq 99.999\%$ ,流速 1.0 mL/min。

- 7.3.1.4 进样口温度:300℃。
- 7.3.1.5 进样方式:不分流进样,1 min后开阀。
- 7.3.1.6 进样量:1 μL。
- 7.3.1.7 电离方式:EI。
- 7.3.1.8 电离能量:70 eV。
- 7.3.1.9 离子源温度:280℃。
- 7.3.1.10 接口温度:300℃。
- 7.3.1.11 测定方式:选择反应监测模式(SRM)。
- 7.3.1.12 监测离子对:见表1。

表1 定性离子对、定量离子对、碰撞气能量(CE)、采集时间窗

化合物名称	监测离子( $m/z$ )	碰撞气能量(CE)/V
蒎醌	208.0/180.0 *	10
	208.0/151.7	30
蒎醌-D8	215.9/188.0	10
注:“*”为定量离子对。		

### 7.3.2 定量测定

根据试样中被测物的含量,选取响应值适宜的标准工作溶液进行分析。标准工作液和待测样液中蒎醌的响应值均应在仪器线性响应范围内。在上述色谱条件下蒎醌的参考保留时间8.6 min,标准溶液的选择反应监测色谱图参见附录A中图A.1。

### 7.3.3 定性测定

在上述实验条件下,样品中待测物质的保留时间,与标准溶液的保留时间偏差在±0.5%之内;化合物的质谱定性离子至少应包括一个母离子和两个子离子,且样品中定性离子的相对丰度与标准品溶液对应的定性离子的相对丰度比进行比较,偏差不超过表2规定的范围,则可判断样品中存在被测物。

表2 相对离子丰度最大容许误差

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

## 7.4 空白试验

除不加试样外,均按上述操作步骤进行。

## 7.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中蒎醌的残留量,计算结果应扣除空白值:

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

式中:

X——试样中蒎醌的残留量,单位为毫克每千克(mg/kg);

$c$  ——从标准曲线上得到的蒽醌的溶液浓度,单位为微克每毫升( $\mu\text{g/mL}$ );

$V$  ——样液最终定容体积,单位为毫升( $\text{mL}$ );

$m$  ——最终样液所代表的试样质量,单位为克( $\text{g}$ )。

## 8 测定低限和回收率

### 8.1 测定低限

本方法的测定低限均为  $0.02 \text{ mg/kg}$ 。

### 8.2 回收率

不同茶叶中添加不同浓度水平蒽醌标准溶液的回收率范围参见附录 B 中表 B.1。

附录 A  
(资料性附录)

标准溶液的气相色谱-质谱/质谱选择反应监测色谱图

蒽醌、蒽醌-D8 混合标准溶液选择反应监测色谱图见图 A.1。

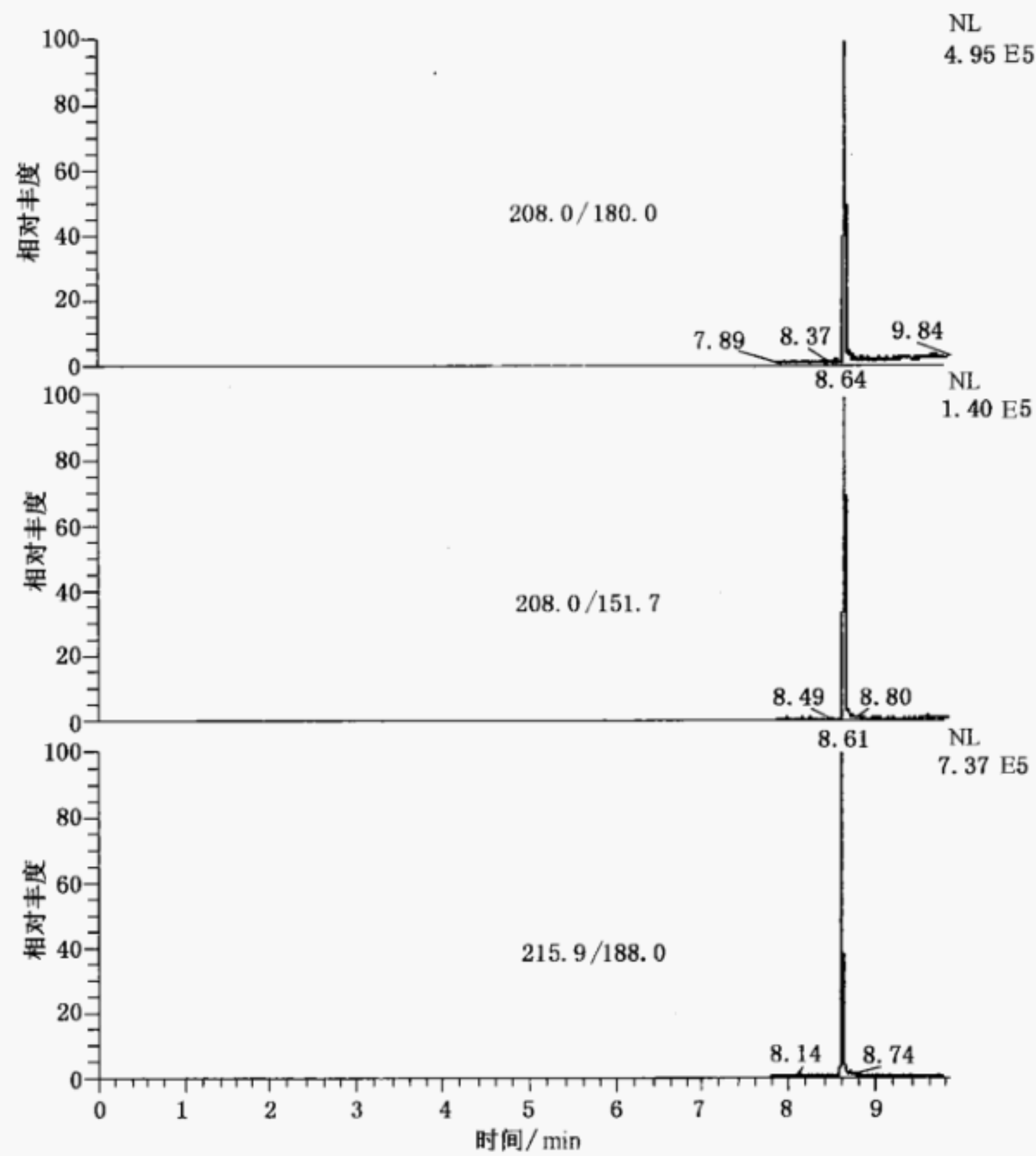


图 A.1 蒽醌、蒽醌-D8 混合标准溶液选择反应监测色谱图(0.02 mg/kg)

附 录 B  
(资料性附录)

不同茶叶中蒽醌添加浓度及回收率

不同茶叶中蒽醌添加浓度及回收率见表 B.1。

表 B.1 不同茶叶中蒽醌添加浓度及回收率

茶叶名称	添加水平/(mg/kg)	回收率范围/%
绿茶	0.02	85.0~105.0
	0.04	75.0~107.5
	0.08	87.5~110.0
红茶	0.02	80.0~105.0
	0.04	72.5~105.0
	0.08	87.5~103.7
普洱茶	0.02	75.0~105.0
	0.04	80.0~107.5
	0.08	77.5~107.5
乌龙茶	0.02	70.0~95.0
	0.04	80.0~107.5
	0.08	78.7~102.5



## Foreword

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

Please note that some of the elements of this standard may involve patents, but the standards organization does not assume responsibility for identifying these patents.

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

This standard was drafted by Zhejiang Academy of Science & Technology for Inspection & Quarantine, Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Xie Wen, Lu Xunyuan, Huang Chaoqun, Chen Li, Shen Tuyan, Lou Chengjie, Yu Zhuoran, Deng Xiaojun, Xie Yundi.

---

1) Note: This english version, a translation from the chinese text, is solely for guidance.

## Determination of anthraquinone residues in tea for export—GC-MS/MS method

### 1 Scope

This standard specifies the method of sample preparation and determination by GC-MS/MS of 9,10-anthraquinone residues in green tea, black tea, pu'er tea and oolong for export.

This standard specifies the method of determination 9,10-anthraquinone residues in green tea, black tea, pu'er tea and oolong by GC-MS/MS.

### 2 Normative references

Following documents are 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

Anthraquinone residues are extracted from the sample with *n*-hexane-acetone. It is cleaned up with florisil column. The residues are determined by GC-MS/MS and quantified by internal standard method.

### 4 Reagents and materials

Unless otherwise specified, all reagents used should be analytical reagent grade. “Water” is first-grade water prescribed by GB/T 6682.

4.1 *n*-hexane: HPLC grade.

4.2 Acetone: HPLC grade.

4.3 Ethyl ether: HPLC grade.

4.4 Sodium chloride.

4.5 Anhydrous sodium sulfate: Ignite for 4 h at 650 °C, and keep in a tightly closed container.

4.6 Florisil: 75 μm—150 μm granule size (100 mesh—200 mesh), ignite for 4 h at 650 °C, then heat for 4 h at 130 °C in an oven before using a day, and keep in a tightly closed container, cool to room, add 2% water inside.

4.7 n-hexane: Acetone(1+1, V/V).

4.8 n-hexane: Ethyl ether (8+2, V/V).

4.9 Standards: 9,10-anthraquinone ( $C_{14}H_8O_2$ , CAS NO. 84-65-1), 9,10-anthraquinone-D8 (CAS NO. 10439-39-1), purity of standards are  $\geq 99\%$ .

4.10 9,10-anthraquinone stock solution: Accurately weigh an adequate amount of standard (4.9) (accurate to 0.1 mg), dissolve in acetone, and prepare a solution of 0.4 mg/mL as the standard stock solution respectively. It should be stored in brown volumetric flask at 0 °C—4 °C, 6 months.

4.11 9,10-anthraquinone-D8 stock solution: Accurately weigh an adequate amount of standard (4.9) (accurate to 0.1 mg), dissolve in acetone, and prepare a solution of 0.4 mg/mL as the standard stock solution respectively. It should be stored in brown volumetric flask at 0 °C—4 °C, 6 months.

4.12 Intermediate standard working solution: Diluted the standard stock solution (4.10) by acetone to 10 μg/mL, diluted the standard stock solution (4.11) by acetone to 0.2 μg/mL. It should be stored in brown volumetric flask at 0 °C—4 °C, 3 months.

4.13 Standard working solution: Diluted the standard stock solution (4.12) by acetone to appropriate concentration.

4.14 Column: 200 mm × 15 mm(id) glass column, pack with ca 5 mm absorbent cotton at the bottom of the column and fill in 10 mm anhydrous sodium sulfate, 10 g florisil(4.6) with n-hexane, fill in 10 mm anhydrous sodium sulfate at top. (eluted curve should be done before using the column).

4.15 Membrane filter: 0.22 μm, organic type.

## 5 Apparatus and equipment

5.1 Gas chromatography-tandem mass spectrometry: Equipped with electrospray ionization source (EI).

5.2 Analytical balances: Accuracy 0.000 1 g and 0.01 g.

5.3 Vortex mixer.

5.4 Homogenizer.

5.5 Centrifuge:  $\geq 4\,000$  r/min, respectively.

5.6 Rotary vacuum evaporator.

5.7 Pulverizer.

## 6 Sample preparation and storage

Take about 250 g of representative sample. Grind in a pulverizer for granule with size of 20 mesh. Keep the prepared sample into a clean container, seal and label.

In the course of sample preparation, precautions shall be taken to avoid contamination or any factors which may cause the change of residue content.

## 7 Analysis procedure

### 7.1 Extraction

Weigh ca 5 g test sample (accurate to 0.01 g) into a 50 mL centrifuge tube. Add 0.5 mL 9,10-anthraquinone-D8 (4.12) and 20 mL n-hexane:acetone(1+1, V/V) (4.7). Homogenize for 0.5 min 10 000 r/min. Add 2 g sodium chloride, vortex for 1 min, centrifuge at 4 000 r/min for 3 min. Transfer the supernatant layer into flask. Repeat the extraction of the residues in the same way with 20 mL n-hexane:Acetone(1+1, V/V) (4.7), and combined the solution. The solution evaporate to nearly dryness in water bath below 45 °C. It is ready for the next clean up.

### 7.2 Clean up

The residues is reconstituted in 3 mL n-hexane, transfer the solution into the column (4.14), elute the column with 50 mL n-hexane: Ethyl ether (8+2, V/V) (4.8), flow rate is 3 mL/min. Collect eluted solution, it is evaporated to nearly dryness in a water bath below 45 °C. The residues is reconstituted in 2.0 mL acetone, mix it. The solution is passed through a 0.22  $\mu\text{m}$  filter (4.15). The filtrate is ready for GC-MS/MS determination.

### 7.3 Determination

#### 7.3.1 GC-MS/MS operating condition

7.3.1.1 GC column: HP-5MS, 30 m  $\times$  0.25 mm (i.d.)  $\times$  0.25  $\mu\text{m}$  (film thickness), or equivalent.

7.3.1.2 Column temperature: 100 °C (1 min), 20 °C/min 300 °C (10 min).

7.3.1.3 Carrier gas: High purity Helium, purity  $\geq 99.999\%$ . Flow rate of carrier gas: 1.0 mL/min.

7.3.1.4 Injection port temperature: 300 °C.

7.3.1.5 Injection mode: Splitless, purge after 1 min.

7.3.1.6 Injection volume: 1  $\mu$ L.

7.3.1.7 Ion source: Electron impact ion source (EI).

7.3.1.8 Electron Energy: 70 eV.

7.3.1.9 Source temperature: 280 °C.

7.3.1.10 Mass transfer line temperature: 300 °C.

7.3.1.11 Detection mode: SRM.

7.3.1.12 SRM ions and energy are shown in table 1.

Table 1—SRM ions and energy

Compound	MRM( <i>m/z</i> )	CE/V
anthraquinone	208.0/180.0 *	10
	208.0/151.7	30
anthraquinone-D8	215.9/188.0	10
Note: “*” The product ion is used for quantification.		

### 7.3.2 Quantitative determination

According to the concentrations of compounds in sample solution, select the standard working solution of similar concentration to that of sample solution. The responses of anthraquinone in the sample solution should be within the linear range of the calibration curve. Under the above GC-MS/MS operating condition, the retention times of anthraquinone is 8.6 min, respectively. The SRM chromatogram of anthraquinone is shown in figure A.1 of annex A.

### 7.3.3 Qualitative determination

Under above determination condition, the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of  $\pm 0.5\%$ . 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 similar concentration can not 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

#### 7.4 Blank test

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

#### 7.5 Calculation and expression of result

Calculate the content of anthraquinone residues in the test sample by GC-MS/MS data processor or using the followed formula (1), the blank value should be subtracted from the about result of calculation.

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

where:

$X$  —the anthraquinone residue content of analyte in the test samples (mg/kg);

$c$  —the concentration of anthraquinone 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).

### 8 Limit of quantification (LOQ) and recovery

#### 8.1 Limit of quantification

The limit of quantification is 0.02 mg/kg.

#### 8.2 Recovery

The recoveries of anthraquinone residues in different tea samples with different spike levels is shown in table B.1 of annex B.

Annex A  
(Informative annex)  
SRM chromatogram of standard

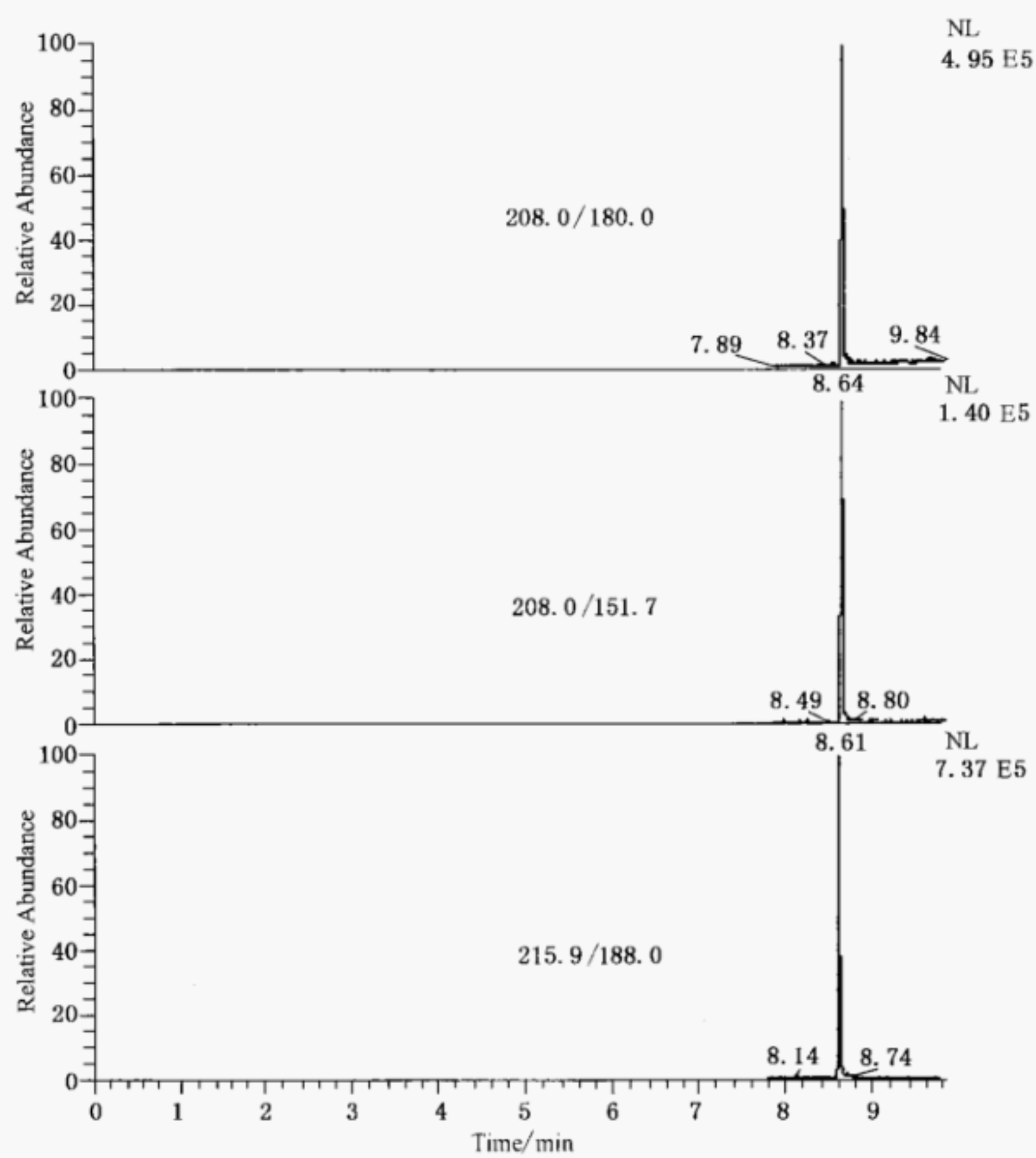


Figure A.1—SRM chromatogram of anthraquinone and anthraquinone-D8 standards solution (0.02 mg/kg)

## Annex B

(Informative annex)

The recoveries of anthraquinone residues in different tea samples  
with different spike levels

Table B.1—The recoveries of anthraquinone residues in different tea samples with  
different spike levels

Sample	spike levels /(mg/kg)	recoveries /%
green tea	0.02	85.0—105.0
	0.04	75.0—107.5
	0.08	87.5—110.0
black tea	0.02	80.0—105.0
	0.04	72.5—105.0
	0.08	87.5—103.7
pu'er tea	0.02	75.0—105.0
	0.04	80.0—107.5
	0.08	77.5—107.5
oolong	0.02	70.0—95.0
	0.04	80.0—107.5
	0.08	78.7—102.5



中华人民共和国出入境检验检疫  
行 业 标 准  
出口茶叶中蒽醌残留量的检测方法  
气相色谱-质谱/质谱法  
SN/T 4777—2017

\*

中国标准出版社出版  
北京市朝阳区和平里西街甲2号(100029)  
北京市西城区三里河北街16号(100045)  
总编室:(010)68533533

网址 [www.spc.net.cn](http://www.spc.net.cn)

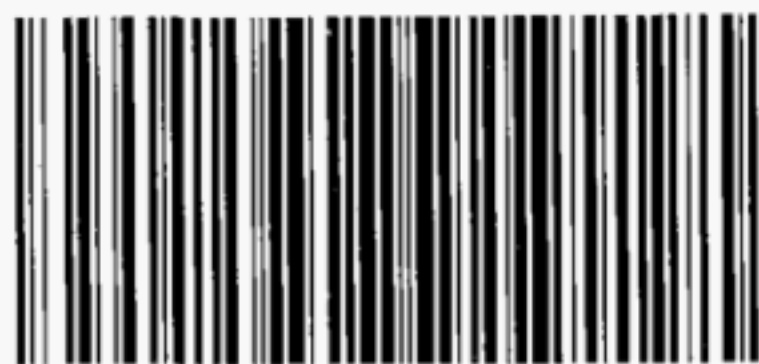
中国标准出版社秦皇岛印刷厂印刷

\*

开本 880×1230 1/16 印张 1.25 字数 32 千字  
2018年5月第一版 2018年5月第一次印刷  
印数 1—500

\*

书号: 155066·2-33050 定价 21.00 元



SN/T 4777-2017