



# 中华人民共和国出入境检验检疫行业标准

SN/T 4261—2015

## 出口中药材中苯并(*a*)芘残留量的测定

Determination of benzo(*a*)pyrene residue in Chinese herbal medicine for export

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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：中华人民共和国陕西出入境检验检疫局。

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## 出口中药材中苯并(a)芘残留量的测定

### 1 范围

本标准规定了中药材中苯并(a)芘残留量的气相色谱-串联质谱测定方法及高效液相色谱测定方法。

本标准第一法和第二法均适用于熟地黄、人参、黄芪、甘草、半夏、番泻叶、菊花、枸杞、苦杏仁、绞股蓝、茯苓、僵蚕、蜂胶等中药材中苯并(a)芘残留量的测定,第一法可以对苯并(a)芘进行确证。

### 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

#### 第一法 气相色谱-串联质谱法

### 3 方法提要

试样用水浸泡后,用正己烷萃取,硅胶固相萃取柱和聚苯乙烯-二乙烯基苯共聚物固相萃取柱净化,用气相色谱-串联质谱仪进行测定和确证,内标法定量。

### 4 试剂和材料

除另有规定外,所用试剂均为分析纯,水为符合 GB/T 6682 规定的一级水。

4.1 正己烷:HPLC 级。

4.2 二氯甲烷:HPLC 级。

4.3 正己烷-二氯甲烷(3+1,体积比):量取 300 mL 正己烷与 100 mL 二氯甲烷混合均匀。

4.4 苯并(a)芘标准物质(Benzo(a)pyrene,  $C_{20}H_{12}$ , CAS 号:50-32-8):纯度大于等于 99%。

4.5  $D_{12}$ -苯并芘内标物(Benzo(a)pyrene- $D_{12}$ ,  $C_{20}D_{12}$ , CAS 号:63466-71-7):纯度大于等于 98%。

4.6 苯并(a)芘标准储备溶液:准确称取适量的苯并(a)芘标准物质,用二氯甲烷配制成 1 000  $\mu\text{g/mL}$  的标准储备溶液,0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。

4.7  $D_{12}$ -苯并芘内标物储备溶液:准确称取适量的  $D_{12}$ -苯并芘标准物质,用正己烷配制成 100  $\mu\text{g/mL}$  的标准储备溶液,或市购  $D_{12}$ -苯并芘标准溶液,0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。

4.8  $D_{12}$ -苯并芘内标物工作溶液:将  $D_{12}$ -苯并芘内标物储备溶液用正己烷逐级稀释,配制成 0.05  $\mu\text{g/mL}$  的标准工作溶液,0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。

4.9 系列标准工作溶液:取一定量的苯并(a)芘标准溶液和  $D_{12}$ -苯并芘内标物溶液混合,用正己烷定容,配制成苯并(a)芘浓度为 2  $\text{ng/mL}$ ~100  $\text{ng/mL}$ ,  $D_{12}$ -苯并芘均为 10  $\text{ng/mL}$  的系列标准溶液,0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。

4.10 硅胶固相萃取柱:1 g/6 mL。

4.11 聚苯乙烯-二乙烯基苯共聚物固相萃取柱:500 mg/6 mL。

4.12 玻璃纤维滤纸:孔径 1.5  $\mu\text{m}$ 。

## 5 仪器和设备

5.1 气相色谱-串联质谱仪:配有 EI 电离源、串联四级杆质量分析器。

5.2 分析天平:感量为 0.01 mg 和 0.01 g。

5.3 高速粉碎机。

5.4 超声波提取仪。

5.5 离心机:4 500 r/min。

5.6 固相萃取装置。

5.7 具塞离心管:50 mL。

5.8 减压旋转蒸发器。

5.9 浓缩瓶:50 mL、100 mL。

## 6 试样制备与保存

人参、黄芪、甘草、半夏、番泻叶、菊花、苦杏仁、绞股蓝、茯苓、僵蚕等样品取不少于 200 g,用高速粉碎机粉碎,过孔径约 0.3 mm 的筛,取通过筛网的药材粉末装入洁净的容器内,密闭并标明标记,于 0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。熟地黄、枸杞、蜂胶等样品先切成小块,装入洁净样品袋中在 18  $^{\circ}\text{C}$  冷冻 2 h 以上,取出后迅速进行高速粉碎,粉碎后的样品再装入洁净容器内,密闭并标明标记,于 18  $^{\circ}\text{C}$  保存。

## 7 测定步骤

### 7.1 提取

称取 2 g(精确至 0.01 g)试样于 50 mL 离心管中,加入 200  $\mu\text{L}$  的  $\text{D}_{12}$ -苯并芘内标溶液(4.8),再加入 20 mL 水,超声浸泡 30 min,加入 15 mL 正己烷,振荡提取 10 min,4 000 r/min 离心 3 min,取上层正己烷溶液于 100 mL 浓缩瓶中,用 15 mL 正己烷再重复萃取 2 次,合并正己烷萃取液,于 40  $^{\circ}\text{C}$  减压浓缩至约 5 mL,待净化。

蜂胶样品浓缩后需要用玻璃纤维滤纸过滤,并用约 5 mL 正己烷淋洗玻璃纤维滤纸。

### 7.2 净化

将硅胶固相萃取柱串接在聚苯乙烯-二乙烯基苯共聚物固相萃取柱上方,依次用 5 mL 二氯甲烷、10 mL 正己烷活化,取样品溶液上样,并用约 5 mL 正己烷洗涤样品浓缩瓶,洗涤液合并上样,再用 15 mL 正己烷淋洗,弃去硅胶固相萃取柱,用 5 mL 正己烷淋洗聚苯乙烯-二乙烯基苯共聚物固相萃取柱,最后用 5 mL 正己烷-二氯甲烷溶液(4.3)洗脱,整个净化过程保持流速 2 mL/min~3 mL/min,收集洗脱液,于 40  $^{\circ}\text{C}$  减压浓缩至干,加入 1.0 mL 正己烷溶解定容,供气相色谱-串联质谱法测定和确证。

### 7.3 测定

#### 7.3.1 气相色谱-串联质谱参考条件

气相色谱-串联质谱参考条件如下:

a) 色谱柱:DB-5 MS 弹性石英毛细管柱,30 m $\times$ 0.25 mm(内径),膜厚 0.25  $\mu\text{m}$ ,或相当者;



- b) 色谱柱温度:120 ℃保持 0.5 min,以 20 ℃/min 的速度升温至 250 ℃,保持 8 min,再以 10 ℃/min 的速度升温至 290 ℃,保持 5 min;
- c) 进样口温度:280 ℃;
- d) 色谱-质谱接口温度:290 ℃;
- e) 载气:氦气,纯度大于等于 99.999%,流速 1.0 mL/min;
- f) 进样量:1.0 μL;
- g) 进样方式:不分流进样,1 min 后打开分流阀;
- h) 电离方式:EI;
- i) 电离能量:70 eV;
- j) 离子源温度:250 ℃;
- k) 碰撞气:氩气,纯度大于等于 99.999%,压力 2.0 mTorr;
- l) 测定方式:多重反应监测模式(MRM);
- m) 多重反应监测条件:苯并(a)芘及同位素内标 D<sub>12</sub>-苯并芘的质谱检测条件见表 1;
- n) 溶剂延迟:15 min。

表 1 苯并(a)芘测定的 MRM 条件

化合物	定量离子对 <i>m/z</i>	碰撞能量 eV	定性离子对 <i>m/z</i>	碰撞能量 eV
苯并(a)芘	252/250	35	252/224	50
D <sub>12</sub> -苯并芘	264/260	40		

7.3.2 气相色谱-串联质谱测定及确证

7.3.2.1 定量测定

根据样液中苯并(a)芘含量情况,选定与样液浓度相近的标准工作溶液,标准工作溶液和样液中苯并(a)芘的响应值均应在仪器检测线性范围内,对标准工作溶液和样液等体积分段参插进样测定,内标法定量,在 7.3.1 规定的气相色谱-串联质谱条件下,苯并(a)芘标准溶液的气相色谱-串联质谱选择离子流图参见附录 A 中图 A.1。

7.3.2.2 定性测定

对标准工作液及样液按 7.3.1 规定的条件进行测定时,如果样液与标准工作液的选择离子图中,样液中目标物质的保留时间与相近浓度标准溶液的保留时间偏差在±2.5%以内;且定性离子对的相对丰度与相近浓度标准溶液对应定性离子对的相对丰度进行比较,若偏差不超过表 2 规定的范围,则可判定为样品中存在对应的待测物。

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

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#### 7.4 平行试验

按以上检测步骤,对同一试样进行平行试验测定。

#### 7.5 空白试验

除不加试样外,均按上述检测步骤进行。

#### 7.6 结果计算和表述

用色谱数据处理机或按式(1)计算试样中苯并(a)芘含量:

$$X = \frac{c_s \times c_i \times A \times A_{si} \times V}{c_{si} \times A_i \times A_s \times m} \times \frac{1\,000}{1\,000} \dots\dots\dots (1)$$

式中:

$X$  ——试样中苯并(a)芘的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );

$c_s$  ——标准工作液中苯并(a)芘的浓度,单位为纳克每毫升( $\text{ng}/\text{mL}$ );

$c_i$  ——样液中内标物  $D_{12}$ -苯并芘的浓度,单位为纳克每毫升( $\text{ng}/\text{mL}$ );

$A$  ——样液中苯并(a)芘的峰面积;

$A_{si}$  ——标准工作液中内标物  $D_{12}$ -苯并芘的峰面积;

$V$  ——样液定容体积,单位为毫升( $\text{mL}$ );

$c_{si}$  ——标准工作液中内标物  $D_{12}$ -苯并芘的浓度,单位为纳克每毫升( $\text{ng}/\text{mL}$ );

$A_i$  ——样液中内标物  $D_{12}$ -苯并芘的峰面积;

$A_s$  ——标准工作液中苯并(a)芘的峰面积;

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

计算结果应扣除空白值。

### 8 测定低限、回收率

#### 8.1 测定低限

本方法测定低限为  $1.0 \mu\text{g}/\text{kg}$ 。

#### 8.2 回收率

苯并(a)芘的添加水平及回收率数据参见附录 B 中表 B.1。

## 第二法 液相色谱法

### 9 方法提要

试样用水浸泡后,用正己烷萃取,硅胶固相萃取柱和聚苯乙烯-二乙烯基苯共聚物固相萃取柱净化,用液相色谱-荧光检测法进行测定,外标法定量。

### 10 试剂和材料

除另有规定外,所用试剂均为分析纯,水为符合 GB/T 6682 规定的一级水。

- 10.1 正己烷:同 4.1。
- 10.2 二氯甲烷:同 4.2。
- 10.3 乙腈:HPLC 级。
- 10.4 正己烷-二氯甲烷(3+1,体积比):同 4.3。
- 10.5 乙腈-水(85+15,体积比):量取 850 mL 乙腈与 150 mL 水混合均匀。
- 10.6 苯并(a)芘标准物质:同 4.4。
- 10.7 苯并(a)芘标准储备溶液:同 4.6。
- 10.8 苯并(a)芘标准工作溶液:将苯并(a)芘标准储备溶液根据需要用乙腈逐级稀释,配制成 0.05  $\mu\text{g/mL}$ ~0.2  $\mu\text{g/mL}$  的标准工作溶液,0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。
- 10.9 系列标准工作溶液:分别取一定量的苯并(a)芘标准工作溶液,用乙腈定容,配制成苯并(a)芘浓度为 2 ng/mL~100 ng/mL 的系列标准溶液,0  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  保存。
- 10.10 硅胶固相萃取柱:同 4.10。
- 10.11 聚苯乙烯-二乙烯基苯共聚物固相萃取柱:同 4.11。
- 10.12 玻璃纤维滤纸:孔径 1.5  $\mu\text{m}$ 。
- 10.13 微孔滤膜:0.2  $\mu\text{m}$ ,有机系。

## 11 仪器和设备

- 11.1 液相色谱仪:配有荧光检测器。
- 11.2 其他仪器和设备同 5.2~5.9。

## 12 试样制备与保存

同 6。

## 13 测定步骤

### 13.1 提取

除不加入 200  $\mu\text{L}$  的  $\text{D}_{12}$ -苯并芘内标溶液(4.8)外,其余同 7.1。

### 13.2 净化

将硅胶固相萃取柱串接在聚苯乙烯-二乙烯基苯共聚物固相萃取柱上方,依次用 5 mL 二氯甲烷、10 mL 正己烷活化,取样品溶液上样,并用约 5 mL 正己烷洗涤样品浓缩瓶,洗涤液合并上样,再用 15 mL 正己烷淋洗,弃去硅胶固相萃取柱,用 5 mL 正己烷-二氯甲烷溶液(4.3)洗脱聚苯乙烯-二乙烯基苯共聚物固相萃取柱,整个净化过程保持流速 2 mL/min~3 mL/min,收集洗脱液,于 40  $^{\circ}\text{C}$  减压浓缩至干,加入 1.0 mL 乙腈溶解定容,过 0.2  $\mu\text{m}$  微孔滤膜,供液相色谱-荧光检测法检测。

### 13.3 测定

#### 13.3.1 液相色谱条件

液相色谱条件如下:

- a) 色谱柱:多环芳烃专用液相色谱柱,长 250 mm,内径 4.6 mm,粒径 5  $\mu\text{m}$ ,或相当者;
- b) 流动相:乙腈-水(85+15,体积比)等度洗脱;



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- c) 柱温:35℃;
- d) 激发波长:384 nm;发射波长:406 nm;
- e) 流速:1.0 mL/min;
- f) 进样量:20 μL。

## 13.3.2 液相色谱测定

根据样液中苯并(a)芘含量情况,选定与样液浓度相近的标准工作溶液,标准工作溶液和样液中苯并(a)芘的响应值均应在仪器检测线性范围内,如果样液中苯并(a)芘含量超出检测的线性范围,则稀释后再进样。对标准工作溶液和样液等体积分时段参插进样测定,以保留时间定性,外标法定量,在13.3.1 给定的液相色谱条件下,苯并(a)芘标准溶液的液相色谱图参见附录 A 中图 A.2。

## 13.4 平行试验

按以上检测步骤,对同一试样进行平行试验测定。

## 13.5 空白试验

除不加试样外,均按上述检测步骤进行。

## 13.6 结果计算和表述

用色谱数据处理机或按式(2)计算试样中苯并(a)芘含量:

$$X = \frac{A \times c_s \times V}{A_s \times m} \times \frac{1\,000}{1\,000} \dots\dots\dots (2)$$

式中:

- $X$  ——试样中苯并(a)芘的含量,单位为微克每千克(μg/kg);
- $A$  ——样液中苯并(a)芘的色谱峰面积;
- $c_s$  ——标准工作液中苯并(a)芘的浓度,单位为纳克每毫升(ng/mL);
- $V$  ——样液最终定容体积,单位为毫升(mL);
- $A_s$  ——标准工作液中苯并(a)芘的色谱峰面积;
- $m$  ——最终样液所代表的试样量,单位为克(g)。

计算结果应扣除空白值。

## 14 测定低限、回收率

## 14.1 测定低限

本方法的测定低限为 1.0 μg/kg。

## 14.2 回收率

苯并(a)芘的添加水平及回收率数据参见附录 B 中表 B.1。



附 录 A  
(资料性附录)

苯并(a)芘标准溶液的气相色谱-串联质谱选择离子流图及液相色谱图

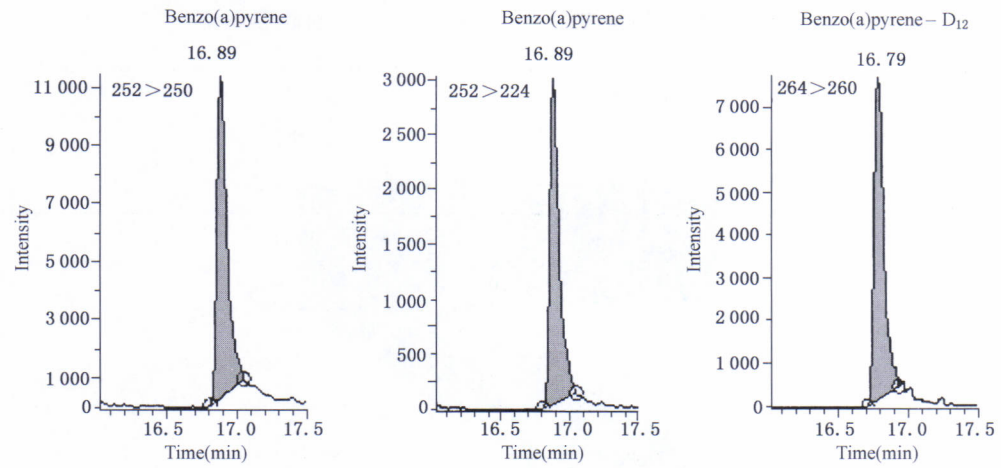


图 A.1 苯并(a)芘标准溶液的气相色谱-串联质谱选择离子流图

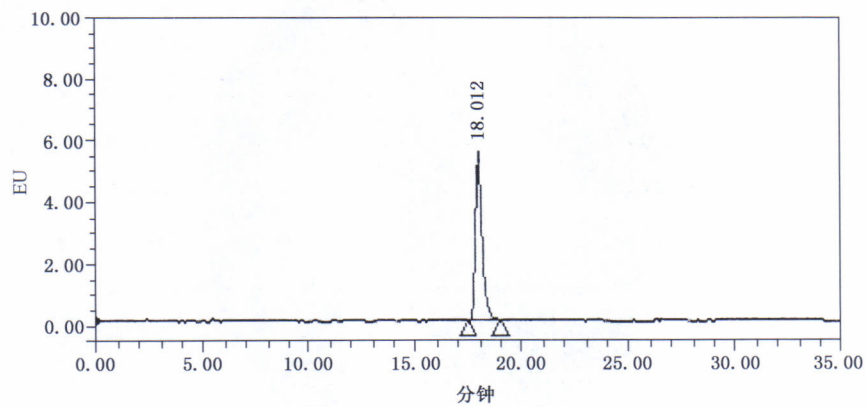


图 A.2 苯并(a)芘标准溶液的液相色谱图

**附 录 B**  
(资料性附录)

苯并(a)芘残留量检测的添加回收率数据

表 B.1 中药材中苯并(a)芘检测的添加回收率数据

样品	气相色谱-串联质谱法		液相色谱法	
	加标水平 $\mu\text{g/kg}$	回收率范围 %	加标水平 $\mu\text{g/kg}$	回收率范围 %
熟地黄	1.0	82.0~105.0	1.0	75.0~103.0
	2.0	85.0~104.0	2.0	80.5~99.5
	5.0	86.4~101.2	5.0	81.6~101.2
人参	1.0	80.0~101.0	1.0	78.0~99.0
	2.0	84.5~101.5	2.0	81.0~98.5
	5.0	88.2~100.0	5.0	82.0~102.6
黄芪	1.0	82.0~104.0	1.0	79.0~97.0
	2.0	85.5~102.5	2.0	80.0~98.0
	5.0	87.2~103.6	5.0	81.4~100.6
甘草	1.0	79.0~106.0	1.0	76.0~96.0
	2.0	81.0~103.5	2.0	78.0~99.0
	5.0	83.8~105.0	5.0	80.0~99.8
半夏	1.0	83.0~103.0	1.0	78.0~101.0
	2.0	85.2~100.0	2.0	78.5~102.5
	5.0	88.2~102.6	5.0	80.0~100.2
番泻叶	1.0	83.0~102.0	1.0	78.0~96.0
	2.0	86.5~106.0	2.0	79.5~100.5
	5.0	87.2~104.4	5.0	81.2~102.2
菊花	1.0	81.0~101.0	1.0	78.0~102.0
	2.0	80.5~101.5	2.0	79.0~98.5
	5.0	86.8~102.6	5.0	79.6~105.8
枸杞	1.0	83.0~103.0	1.0	79.0~100.0
	2.0	83.5~102.5	2.0	79.5~98.0
	5.0	88.2~104.2	5.0	80.4~99.6
苦杏仁	1.0	82.0~107.0	1.0	79.0~101.0
	2.0	86.0~102.0	2.0	80.0~101.5
	5.0	88.8~104.2	5.0	80.6~102.8

表 B.1 (续)

样品	气相色谱-串联质谱法		液相色谱法	
	加标水平 $\mu\text{g/kg}$	回收率范围 %	加标水平 $\mu\text{g/kg}$	回收率范围 %
绞股蓝	1.0	83.0~102.0	1.0	78.0~101.0
	2.0	86.5~102.0	2.0	79.5~100.0
	5.0	88.6~101.0	5.0	82.0~101.2
茯苓	1.0	84.0~103.0	1.0	78.0~101.0
	2.0	85.0~105.5	2.0	75.0~100.0
	5.0	88.8~102.2	5.0	77.6~98.6
僵蚕	1.0	84.0~104.0	1.0	77.0~97.0
	2.0	85.5~103.5	2.0	78.5~99.0
	5.0	87.4~101.4	5.0	82.0~97.2
蜂胶	1.0	82.0~104.0	1.0	75.0~99.0
	2.0	84.0~102.5	2.0	80.0~97.5
	5.0	86.2~103.0	5.0	79.4~99.0

## Foreword

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

Please note that this document may involve some of the contents of the patent, The standard file publishing institutions does not assume the responsibility of identifying these patents.

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

This standard is drafted by Shaanxi Entry-Exit Inspection and Quarantine bureau of the People's Republic of China.

The standard is mainly drafted by He Qiang, Kong Xianghong, Li Jianhua, Li Gaohua, Wu Shuangmin, Zhangying, Zou Yang, Zhang lu, Li Ying, Fu Chengyu.



# Determination of benzo(a)pyrene residue in Chinese herbal medicine for export

## 1 Scope

This standard specifies the determination of benzo(a)pyrene residues in Chinese herbal medicine by GC-MS/MS or HPLC.

This standard is application to the determination and confirmation of benzo(a)pyrene residues in Radix Rehmanniae Praeparata, Ginseng, Astragalus, Licorice, Pinellia, Senna, Chrysanthemum, Medlar, Bitter almond, Gynostemma, Poria, Stiff silkworm, Propolis, etc. The first method can be used for confirmation the benzo(a)pyrene.

## 2 Normative references

The following document is essential for the application of this document. For dated references, only dated version apply to this document. For undated references, the latest edition (including all the amendments) apply to this document.

GB/T 6682 water for analytical laboratory use-Specification and test methods

## Method 1: Gas chromatography-tandem mass spectrometry method

## 3 Method summary

The sample was soaked in water, than extracted with n-hexane, following by a cleanup step with silica solid phase extraction column and Polystyrene-divinyl benzene copolymer solid phase extraction column. Then determined and confirmed by gas chromatography-tandem mass spectrometry (GC-MS/MS) using internal method.

## 4 Reagents and materials

Unless otherwise specified, all the reagents used should be analytical grade, and water for a water provisions in accordance with GB/T 6682.

4.1 n-hexane: HPLC grade.

4.2 Dichloromethane: HPLC grade.

4.3 n-hexane-dichloromethane (3+1, V/V): Measure 300 mL n-hexane and 100 mL dichloromethane, mixed uniformly.

4.4 Benzo(a)pyrene standard substance (Benzo(a)pyrene,  $C_{20}H_{12}$ , CAS: 50-32-8): purity  $\geq 99\%$ .

4.5  $D_{12}$ -benzopyrene internal standard (Benzo(a)pyrene- $D_{12}$ ,  $C_{20}D_{12}$ , CAS: 63466-71-7): purity  $\geq 98\%$ .

4.6 Benzo(a)pyrene standard stock solution: Accurately weigh an adequate amount benzo(a)pyrene standard and dissolve in volume of dichloromethane, the stock solution is 1 000  $\mu\text{g/mL}$  in concentration, and stored at  $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$ .

4.7 Benzo(a)pyrene- $D_{12}$  internal standard stock solution: Accurately weigh an adequate amount benzo(a)pyrene- $D_{12}$  standard and dissolve in volume of n-hexane, the stock solution is 100  $\mu\text{g/mL}$  in concentration, or buy the benzo(a)pyrene- $D_{12}$  standard solution, the stock solution is stored at  $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$ .

4.8 Benzo(a)pyrene- $D_{12}$  internal standard work solution: The benzo(a)pyrene- $D_{12}$  standard stock solution is diluted with n-hexane to 0.05  $\mu\text{g/mL}$ , the work solution is stored at  $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$ .

4.9 Series of standard work solution: Accurately mix an adequate volume benzo(a)pyrene standard work solution and benzo(a)pyrene- $D_{12}$  internal standard work solution, add to volume by n-hexane, the benzo(a)pyrene concentrations are ranged from 2  $\text{ng/mL}$  to 100  $\text{ng/mL}$  and the benzo(a)pyrene- $D_{12}$  concentrations was 10  $\text{ng/mL}$ , the standard solution is stored at  $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$ .

4.10 Silica solid phase extraction column: 1 g/6 mL.

4.11 Polystyrene-divinyl benzene copolymer solid phase extraction column: 500 mg/6 mL.

4.12 Glass fiber filter paper: Aperture 1.5  $\mu\text{m}$ .

## 5 Instruments and equipment

5.1 Gas chromatography-tandem mass spectrometry: EI ionization source with a tandem quadrupole mass analyzer.

5.2 Analysis Balance: 0.01 mg and 0.01 g.

- 5.3 High-speed grinder.
- 5.4 Ultrasonic extraction instrument.
- 5.5 Centrifuge: 4 500 r/min.
- 5.6 Solid phase extraction device.
- 5.7 Centrifuge tube: 50 mL.
- 5.8 Vacuum rotary evaporator.
- 5.9 Concentrated bottle: 50 mL, 100 mL.

## 6 Sample preparation and preservation

For Ginseng, Astragalus, Licorice, Pinellia, Senna, Chrysanthemum, Bitter almond, Gynostemma, Poria, Stiff silkworm: take not less than 200 g samples and crush with a high-speed grinder, then through a diameter about 0.3 mm sieve, take the herb powder into a clean container, sealed and marked, and stored at 0 °C ~ 4 °C. For Radix Rehmanniae Praeparata, Medlar, Propolis: samples were cut into small pieces, and frozen at -18 °C for more than 2 h, then high-speed grind quickly, crushed samples were taken into a clean container, sealed and marked, and stored at -18 °C.

## 7 Procedure

### 7.1 extract

weigh about 2 g (accurate to 0.01 g) test sample in 50 mL centrifuge tube, add 200 µL benzo(a) pyrene-D<sub>12</sub> internal standard work solution (4.8), then add 20 mL water. Soak and ultrasonic wave extracted 30 min, then add 15 mL n-hexane, oscillation extracted 10 min, centrifuged at 4 000 r/min for 3 min, n-hexane solution was transferred to a 100 mL bottle, the residue is extracted with 15 mL and 15 mL n-hexane again. And combined the n-hexane extracts, concentrated to about 5 mL at 40 °C, and waiting for cleanup operation.

Propolis samples need to filter with glass fiber filter paper filter after concentrated, and elution the glass fiber filter with about 5 mL n-hexane.

### 7.2 Cleanup

The silica gel solid phase extraction column is connected to the top of polystyrene-divinyl benzene copolymer solid phase extraction column, washed with 5 mL dichloromethane and 10 mL n-hexane



successively. Transfer the sample solution to the series solid phase extraction columns, and use 5 mL n-hexane to wash the sample bottle, the wash solution is transferred to the SPE columns too. Then use 15 mL n-hexane to wash the SPE columns. Get rid of the silica gel solid phase extraction column, use 5 mL n-hexane to wash the polystyrene-divinyl benzene copolymer solid phase extraction column. Use 5 mL n-hexane-dichloromethane(4.3) to elute the polystyrene-divinyl benzene copolymer SPE column and collect the eluent. All the process control the flow rate between 2 mL/min and 3 mL/min. Evaporate the eluent to almost dry under 40 °C, add 1 mL n-hexane, wait for gas chromatography-tandem mass spectrometry determine and confirm.

### 7.3 Determination

#### 7.3.1 Gas chromatography-tandem mass spectrometry reference conditions

Gas chromatography-tandem mass spectrometry reference conditions are as following:

- a) Chromatographic column. DB-5MS column elastic quartz capillary column, 30 m × 0.25 mm(i.d.), film thickness 0.25 μm, or equivalent.
- b) Column temperature: 120 °C keep 0.5 min, with 20 °C/min speed up to 250 °C, keep 8 min, with 10 °C/min speed up to 290 °C and keep 5 min.
- c) Inlet temperature: 280 °C.
- d) Interface temperature: 290 °C.
- e) Carrier gas: Helium, purity is greater than or equal to 99.999%, the flow is 1.0 mL/min.
- f) Injection volume: 1.0 μL.
- g) Injection mode: splitless, purge after 1 min.
- h) Ionization mode: EI.
- i) Ionization energy: 70 eV.
- j) Ion source temperature: 250 °C.
- k) Collision gas: argon, purity is greater than or equal to 99.999%, the pressure is 2.0 mTorr.
- l) measure mode: multiple reaction monitoring(MRM).



- m) multiple reaction monitoring conditions: The MS detection conditions of benzo(*a*)pyrene and isotope internal standard benzo(*a*)pyrene-D<sub>12</sub> see Table 1.
- n) solvent delay time: 15 min.

Table 1—MRM conditions of benzo(*a*)pyrene and benzopyrene-D<sub>12</sub>

compound	quantificational ion pair <i>m/z</i>	collision energy eV	Qualitative ion pair <i>m/z</i>	collision energy eV
benzo( <i>a</i> )pyrene	252/250	35	252/224	50
benzo( <i>a</i> )pyrene-D <sub>12</sub>	264/260	40		

### 7.3.2 GC-MS/MS determination and confirmation

#### 7.3.2.1 Quantification determination

According to the approximate concentration of the benzo(*a*)pyrene in the sample solution, select the standard working solution with similar concentration of the sample solution. The response of the standard solution and the sample solution should be within the linear range of the instrument detection. The standard working solution should be injected in between the injections of the sample solution with one common volume. Use internal method to quantitative. Under the above GC-MS/MS operating conditions, the MRM chromatogram of benzo(*a*)pyrene see Fig. A.1 in Annex A.

#### 7.3.2.2 Qualitative determination

Analyse the working standard solution and sample solution according the condition of 7.3.1. If the mass chromatography retention time deviation of the peak of sample and peak of the standard is less than  $\pm 2.5\%$ , and the relative abundance of the qualitative ion pair is consistent to the similar concentration standard solution, and relative abundance deviation is in the range of Table 2, then to determine that there is corresponding compound.

Table 2—Qualitative confirmatory relative ion abundance maximum allowable deviation

relative ion intensities/%	>50	>20~50	>10~20	≤10
permitted tolerances/%	± 20	± 25	± 30	± 50

### 7.4 parallel test

Use the above detection step to analysis the same sample again.

## 7.5 Blank test

The operation is same as the above detection step, but with omission of sample addition.

## 7.6 Calculation and expression of the result

Calculate Benzo(a)pyrene content in the sample with chromatographic data processor or using the formula(1):

$$X = \frac{c_s \times c_i \times A \times A_{si} \times V}{c_{si} \times A_i \times A_s \times m} \times \frac{1\ 000}{1\ 000} \dots\dots\dots (1)$$

Where:

$X$  —benzo(a)pyrene content in the test sample,  $\mu\text{g}/\text{kg}$ ;

$c_s$  —the concentration of benzo(a)pyrene in the standard solution,  $\text{ng}/\text{mL}$ ;

$c_i$  —the concentration of benzo(a)pyrene- $\text{D}_{12}$  in the test sample solution,  $\text{ng}/\text{mL}$ ;

$A$  —the peak area of benzo(a)pyrene in the test sample solution;

$A_{si}$  —the peak area of benzo(a)pyrene- $\text{D}_{12}$  in the standard solution;

$V$  —the constant volume of the sample solution,  $\text{mL}$ ;

$c_{si}$  —the concentration of benzo(a)pyrene- $\text{D}_{12}$  in the standard solution,  $\text{ng}/\text{mL}$ ;

$A_i$  —the peak area of benzo(a)pyrene- $\text{D}_{12}$  in the test sample solution;

$A_s$  —the peak area of benzo(a)pyrene in the standard solution;

$m$  —the corresponding mass of test sample in the final sample solution,  $\text{g}$ .

The calculation results shall be deducted the blank value.

## 8 LOQ and recovery

### 8.1 LOQ

LOQ of this method is  $1.0\ \mu\text{g}/\text{kg}$ .

## 8.2 Recovery

The data of recovery see Table B.1 in Annex B.

## Method 2: High Performance liquid chromatography method

## 9 Methods Summary

The sample was soaked in water, then extracted with n-hexane, following by a cleanup step with silica solid phase extraction column and Polystyrene-divinyl benzene copolymer solid phase extraction column. Then determined by liquid chromatography-fluorescence detection using external method.

## 10 Reagents and materials

Unless otherwise specified, all the reagents used should be analytical grade, and water for a water provisions in accordance with GB/T 6682.

10.1 n-hexane; see 4.1.

10.2 Dichloromethane; see 4.2.

10.3 Acetonitrile; HPLC grade.

10.4 n-hexane-dichloromethane(3+1, V/V); see 4.3.

10.5 Acetonitrile-water(85+15, V/V): Measure 850 mL acetonitrile and 150 mL water, mixed uniformly.

10.6 Benzo(a)pyrene standard material; see 4.4.

10.7 Benzo(a)pyrene standard stock solution; see 4.6.

10.8 Benzo(a)pyrene standard work solution: The benzo(a)pyrene standard stock solution is diluted with acetonitrile to 0.05 µg/mL~0.2 µg/mL, the work solution is stored at 0 °C ~4 °C.

10.9 series of standard working solution: Accurately remove an adequate volume benzo(a)pyrene standard work solution, add to volume by acetonitrile, the benzo(a)pyrene concentrations are ranged from 2 ng/ mL to 100 ng/mL, the standard solution is stored at 0 °C ~4 °C.

10.10 Silica solid phase extraction column: see 4.10.

10.11 Polystyrene-divinyl benzene copolymer solid phase extraction column: see 4.11.

10.12 Glass fiber filter paper: Aperture 1.5  $\mu\text{m}$ .

10.13 Filter: 0.2  $\mu\text{m}$ , organic phase.

## 11 Instruments and equipment

11.1 Liquid chromatography: with fluorescence detector.

11.2 Other instruments and equipment: see 5.2 to 5.9.

## 12 Specimen preparation and preservation

See 6.

## 13 Procedure

### 13.1 Extract

In addition to not add 200  $\mu\text{L}$  benzopyrene- $\text{D}_{12}$  internal standard(4.8), others same to 7.1.

### 13.2 Cleanup

The silica gel solid phase extraction column is connected to the top of polystyrene-divinyl benzene copolymer solid phase extraction column, washed with 5 mL dichloromethane and 10 mL n-hexane successively. Transfer the sample solution to the series solid phase extraction columns, and use 5 mL n-hexane to wash the sample bottle, the wash solution is transferred to the SPE columns too. Then use 15 mL n-hexane to wash the SPE columns. Get rid of the silica gel solid phase extraction column, Use 5 mL n-hexane-dichloromethane(4.3) to elute the polystyrene-divinyl benzene copolymer SPE column and collect the eluent. All the process control the flow rate between 2 mL/min and 3 mL/min. Evaporate the eluent to dry under 40  $^{\circ}\text{C}$ , use 1.00 mL acetonitrile to dissolve the residue, then filter it with 0.2  $\mu\text{m}$  membrane, wait for HPLC-FLD determine.



### 13.3 Determination

#### 13.3.1 Liquid chromatography conditions

Liquid chromatography conditions are as following:

- a) Column: polycyclic aromatic hydrocarbons (PAHs) dedicated liquid chromatographic column, length 250 mm, inner diameter of 4.6 mm, particle size 5  $\mu\text{m}$ , or equivalent;
- b) Mobile phase: acetonitrile-water (85 + 15, V/V);
- c) Column temperature: 35  $^{\circ}\text{C}$ ;
- d) Excitation wavelength: 384 nm; Emission wavelength: 406 nm;
- e) Flow rate: 1.0 mL/min;
- f) Injection volume: 20  $\mu\text{L}$ .

#### 13.3.2 Liquid chromatography determination

According to the approximate concentration of the benzo(a)pyrene in time sample solution, select the standard working solution with similar concentration of the sample solution. The response of the standard solution and the sample solution should be within the linear range of the instrument detection. If the response of the sample solution is out the linear range of the instrument detection, should dilute the sample solution and inject again. The standard working solution should be injected in between the injections of the sample solution with one common volume. Use external method to quantitative. Under the 13.3.1 operating conditions, the HPLC chromatogram of benzo(a)pyrene see Fig. A.2 in Annex A.

### 13.4 Parallel trial

Use the above detection step to analysis the same sample again.

### 13.5 Blank test

The operation is same as the above detection step, but with omission of sample addition.

### 13.6 Calculation and expression of the result

Calculate Benzo(a)pyrene content in the sample with chromatographic data processor or using the formula(2)

$$X = \frac{A \times c_s \times V}{A_s \times m} \times \frac{1\ 000}{1\ 000} \dots\dots\dots (2)$$

Where:

$X$  —benzo(*a*)pyrene content in the test sample,  $\mu\text{g}/\text{kg}$ ;

$A$  —the peak area of benzo(*a*)pyrene in the test sample solution;

$c_s$  —the concentration of benzo(*a*)pyrene in the standard solution,  $\text{ng}/\text{mL}$ ;

$V$  —the constant volume of the sample solution,  $\text{mL}$ ;

$A_s$  —the peak area of benzo(*a*)pyrene in the standard solution;

$m$  —the corresponding mass of test sample in the final sample solution,  $\text{g}$ .

The calculation results shall be deducted the blank value.

## 14 LOQ and recovery

### 14.1 LOQ

LOQ of this method is  $1.0\ \mu\text{g}/\text{kg}$ .

### 14.2 Recovery

The data of recovery see Table B.1 in Annex B.

## Annex A

### (Informative Annex)

#### GC-MS/MS chromatogram and HPLC chromatogram of benzo(a)pyrene standard

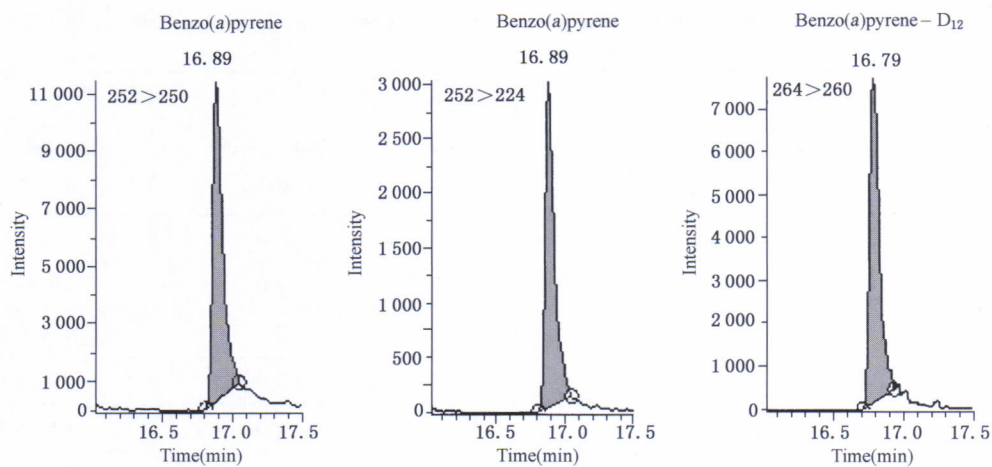


Figure A.1—GC-MS/MS chromatogram of benzo(a)pyrene standard solution

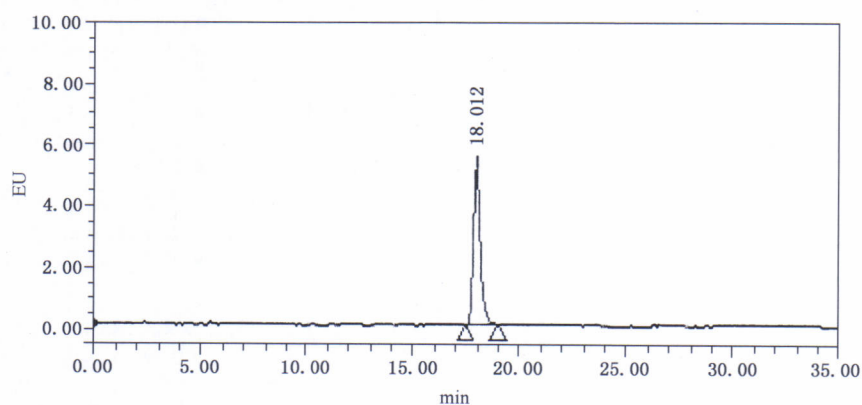


Figure A.2—HPLC chromatogram of benzo(a)pyrene standard solution

**Annex B**  
(Informative Annex)

**The add recoveris data of Benzo(a)pyrene residue detection**

**Table B.1—The add recoveries data of benzo(a)pyrene in Chinese herbal medicines**

sample	GC-MS/MS		HPLC	
	Spiked level μg/kg	Range of recovery %	Spiked level μg/kg	Rage of recovery %
Radix Rehmanniae Praeparata	1.0	82.0~105.0	1.0	75.0~103.0
	2.0	85.0~104.0	2.0	80.5~99.5
	5.0	86.4~101.2	5.0	81.6~101.2
Ginseng	1.0	80.0~101.0	1.0	78.0~99.0
	2.0	84.5~101.5	2.0	81.0~98.5
	5.0	88.2~100.0	5.0	82.0~102.6
Astragalus	1.0	82.0~104.0	1.0	79.0~97.0
	2.0	85.5~102.5	2.0	80.0~98.0
	5.0	87.2~103.6	5.0	81.4~100.6
Licorice	1.0	79.0~106.0	1.0	76.0~96.0
	2.0	81.0~103.5	2.0	78.0~99.0
	5.0	85.8~105.0	5.0	80.0~99.8
Pinellia	1.0	83.0~103.0	1.0	78.0~101.0
	2.0	85.2~100.0	2.0	78.5~102.5
	5.0	88.2~102.6	5.0	80.0~100.2
Senna	1.0	83.0~102.0	1.0	78.0~96.0
	2.0	86.5~106.0	2.0	79.5~100.5
	5.0	87.2~104.4	5.0	81.2~102.2
Chrysanthemum	1.0	81.0~101.0	1.0	78.0~102.0
	2.0	80.5~101.5	2.0	79.0~98.5
	5.0	86.8~102.6	5.0	79.6~105.8
Medlar	1.0	83.0~103.0	1.0	79.0~100.0
	2.0	83.5~102.5	2.0	79.5~98.0
	5.0	88.2~104.2	5.0	80.4~99.6
Bitter almond	1.0	82.0~107.0	1.0	79.0~101.0
	2.0	86.0~102.0	2.0	80.0~101.5
	5.0	88.8~104.2	5.0	80.6~102.8



Table B.1 (Continued)

sample	GC-MS/MS		HPLC	
	Spiked level μg/kg	Range of recovery %	Spiked level μg/kg	Range of recovery %
Gynostemma	1.0	83.0~102.0	1.0	78.0~101.0
	2.0	86.5~102.0	2.0	79.5~100.0
	5.0	88.6~101.0	5.0	82.0~101.2
Poria	1.0	84.0~103.0	1.0	78.0~101.0
	2.0	85.0~105.5	2.0	75.0~100.0
	5.0	88.8~102.2	5.0	77.6~98.6
Stiff silkworm	1.0	84.0~104.0	1.0	77.0~97.0
	2.0	85.5~103.5	2.0	78.5~99.0
	5.0	87.4~101.4	5.0	82.0~97.2
Propolis	1.0	82.0~104.0	1.0	75.0~99.0
	2.0	84.0~102.5	2.0	80.0~97.5
	5.0	86.2~103.0	5.0	79.4~99.0