ICS 79.080
B 70

LY

Forestry Industry Standard of the People's Republic of China

LY/T 2904—2017

# Agarwood

# 沉香

*(English Translation)*

Issue date: 2017-10-27 Implementation date: 2018-01-01

Issued by State Forestry and Grassland Administration of the People's Republic of China

# Foreword

SAC/TC 41 is in charge of this English translation. In case of any doubt about the contents of English translation, the Chinese original shall be considered authoritative.

This standard is drafted in accordance with the rules given in the GB/T 1.1—2009 *Directives for standardization — Part 1: Structure and drafting of standards*.

This standard was proposed by the Endangered Species Import and Export Management Office of the People's Republic of China.

This standard was prepared by SAC/TC 41 (National Standardization Technical Committee 41 on Timber, Standardization Administration of China).

# Introduction

*Aquilaria* spp. is included in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and *Aquilaria sinensis* is listed under protection level II in the Catalogue of the National Protected Key Wild Plants (the First Batch) (1999) in China.

This standard is developed to conserve and sustainably use agarwood resources, and to promote the healthy development of the agarwood industry in China.

# Agarwood

## 1 Scope

This standard specifies the terms and definitions, requirements, test method and determination of agarwood.

This standard is applicable to the inspection and identification of agarwood raw materials and agarwood products.

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 29894—2013 *General method of wood identification*

LY/T 1788—2008 *Standard terminology relationg to wood properties*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in LY/T 1788—2008 and the following apply.

3.1

*Aquilaria* species
plant species belonging to the *Aquilaria* genus of Thymelaeceae family as per plant taxonomy

3.2

*Aquilaria* wood
xylem tissue of *Aquilaria* species

3.3

agarwood
natural mixture composed of xylem tissue and its secretions, formed during the growth of tree *of Aquilaria* species

3.4

ethanol extractives of agarwood
substances extracted from agarwood with 95% ethanol, mainly including 2-(2-phenethyl) chromones, sesquiterpenes, aromatic compounds and fatty acids

3.5

reference substance of agarwood
standard material used for identification,test, and comparison by thin-layer chromatography(TLC) and high performance liquid chromatography( HPLC), prepared and calibrated by a national designated metrology or inspection agency

## 4 Requirements

The xylem structure and secretions characteristics of agarwood shall conform to the requirements given in Table 1.

Table 1 Requirements for xylem structure and secretions characteristics of agarwood

| Test items | | Requirements |
|---|---|---|
| Xylem structure | Macrostructure | Diffuse porous; growth rings are indistinct; the axial parenchyma is usually absent with a hand lens; the number of rays are medium, very fine to slightly fine; the number of the included phloem is large, visible to the naked eye, distinct with a hand lens |
| | Microstructure | Mainly radial multiple vessels, simple perforation plates, alternate intervessel pitting; vessel-ray pitting is similar to intervessel pitting; axial parenchyma is extremely rare, vasicentric; fibers are thin-walled; rays are mostly uniseriate, occasionally biseriate; rays are mostly one) rows of square or upright marginal cells homogeneous and uniseriate, with a small number of heterogeneous III or II type; included phloem is present in large amounts, foraminate or island type |
| Secretions | Ethanol extractives | ≥10.0 % |

| | | |
|---|---|---|
| | Chromogenic reaction | Cherry-red, purple blue, light red, or light purple is permitted; colorless or light yellow is not permitted |
| | TLC | Fluorescent spots shall appear, corresponding in position and color to those in chromatogram of agarwood reference substance |
| | HPLC characteristic chromatogram | 6 characteristic peaks shown in Figure 1 shall appear, corresponding to those in chromatogram of agarwood reference substance; retention time of peak 1 shall be consistent with that of agarwood reference substance |

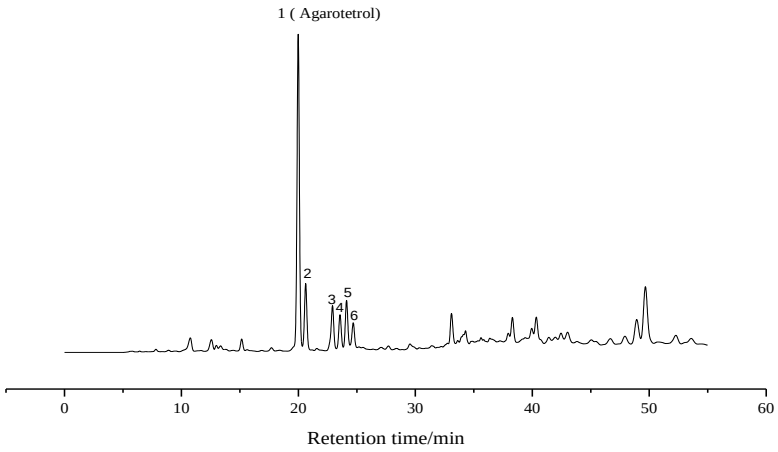



Figure 1 HPLC characteristic chromatogram of agarwood

## 5 Test method

### 5.1 Agarwood xylem structure

#### 5.1.1 Sampling

Sampling is carried out from perpendicular direction to the cross, radial and tangential section. Generally, the size shall be no less than 10 mm × 10 mm × 10 mm. When the requirements are not met, the sampling size should not be less than 5 mm × 5 mm × 5 mm.

#### 5.1.2 Macrostructure characteristics

Observe and record the color, odor, texture, and structure characteristics of the wood sample; observe the wood sample with the naked eye or hand lens of 10× magnification, and record the characteristics of heartwood, sapwood, growth rings, pores, axial parenchyma, rays, and included phloem from the cross section of the sample. Refer to Annex A for the macrostructure characteristics of *xylem of Aquilaria* species.

5.1.3 Microstructure characteristics

5.1.3.1 Softening

According to the provisions of 5.2.1 and 5.2.2 in GB/T 29894-2013, the samples shall be softened by the boiling method or the glycerol-ethanol method.

5.1.3.2 Preparing sections

Place the softened sample on a microtome and cut transverse, radial, and tangential sections with a thickness of 15~20 μm respectively; or use a suitable knife to prepare sections by hand. The microscopic sections shall be prepared following the steps of staining, dehydration, clearing and mounting.

5.1.3.3 Recording microscopic characteristics

The microscopic section shall be placed under a light microscope, and the microscopic features including vessels, axial parenchyma, wood fibers, rays, and included phloems shall be observed and recorded. Refer to Appendix A for microstructure characteristics of *xylem of Aquilaria* species.

5.2 Characteristics of Agarwood secretions

5.2.1 Sampling

5.2.1.1 Apparatus

5.2.1.1.1 Mill.

5.2.1.1.2 Screen, 24 mesh (850±29 μm).

5.2.1.1.3 Balance, accurate to 0.001g.

5.2.1.2 Testing procedure

Take about 10 g of representative sample, grind until the entire portions pass a 24-mesh screen, and mix thoroughly. Half of the sample is used for analysis, and the other half is reserved.

5.2.2 Determination of moisture

5.2.2.1 Reagents and apparatus

5.2.2.1.1 Phosphorus pentoxide, analytical reagent.

5.2.2.1.2 Petri dish, 12 cm in diameter.

5.2.2.1.3 Weighing bottle, 5 cm in diameter.

5.2.2.1.4 Vacuum desiccator, 30 cm in diameter.

5.2.2.1.5 Drying tube with anhydrous calcium chloride.

5.2.2.1.6 Balance, accurate to 0.0001g.

5.2.2.2 Testing procedure

Distribute 0.5~1.0 cm depth of phosphorus pentoxide in a petri dish, and put the dish in the vacuum desiccator.

Place a clean weighing bottle in the vacuum desiccator, remove the stopper of the bottle. Reduce the pressure of the desiccator by suction to less than 2.67 kPa and keep for 30 min, Maintain the vacuum at room temperature for 24 hours. Connect the drying tube with anhydrous calcium chloride to the air outlet, loosen the desiccator plunger to equalize the air pressure. Wait until the inside air pressure and outside pressure is consistent, switch off the plunger, open the desiccator, fit the stopper, take out the weighing bottle and weigh promptly.

Weigh duplicate samples of 0.5~1.0 g to an accuracy of 0.0001 g, put in the dried weighing bottle, dry and weigh in the same way as above. Caculate the moisture content in sample according to formula (1). Carry out two simultaneous measurements. The absolute error between the two measured values should not exceed 0.3 %, and the result shall be expressed as the average moisture of duplicate samples to the nearest 0.01 %.

$$W(\%) = \frac{m_1 - m_2}{m_s} \times 100 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (1)$$

where

$m_1$ —— original sample mass plus mass of weighing bottle, unit in grams (g);

$m_2$ —— dried sample mass plus mass of weighing bottle, unit in grams (g);

$m_s$ —— original sample mass, unit in grams (g).

5.2.3 Determination of ethanol extractives content

5.2.3.1 Reagents and apparatus

5.2.3.1.1 95% ethanol, analytical reagent.

5.2.3.1.2 Conical flask, 250 mL.

5.2.3.1.3 Condenser.

5.2.3.1.4 Pipette, 25 mL and 100 mL.

5.2.3.1.5 Evaporating dish, 9 cm in diameter.

5.2.3.1.6 Desiccator, 30 cm in diameter.

5.2.3.1.7 Balance, accurate to 0.0001g.

5.2.5.1.4 Temperature-controlled drying oven, room temperature~200 ℃, accurate to 0.1 ℃.

5.2.3.2 Testing procedure

Weigh duplicate samples of 2 g to an accuracy of 0.0001 g, and put in a 250-mL conical flask. When the requirement is not met, the sample size should be no less than 0.5 g. Add 100 mL of 95 % ethanol with pipette, fit the stopper, weigh and allow to stand for 1 h. Connect with reflux condenser, heat to boiling and simmer for 1 h.

Cool, remove the flask, stopper it, and weigh again. Add 95 % ethanol to restore its original weight, shake thoroughly and filter with filter paper. Measure 25 mL of the filtrate with a pipette and transfer into an evaporating dish which has been previously dried to constant weight. Evaporate to dryness on water bath, and then dry in an oven for 3 h at $103\pm2$℃. Cool in a desiccator for 30 min and weigh promptly. Caculate the content of ethanol extractives in sample according to formula (2). Carry out two simultaneous measurements. The absolute error between the two measured values should not exceed 0.3 %, and the result shall be expressed as the average ethanol extractives of duplicate samples to the nearest 0.01 %.

$$X(\%) = \frac{m_1 - m_2}{m_s \times (1 - W)} \times 400 \quad \text{(2)}$$

where

$m_1$ —— mass of ethanol extractives plus mass of evaporating dish, unit in grams (g);

$m_2$ —— mass of evaporating dish, unit in grams (g);

$m_s$ —— original sample mass, unit in grams (g);

$W$ —— moisture content of sample, %.

5.2.4 Chromogenic reaction

5.2.4.1 Reagents and apparatus

5.2.4.1.1 95% ethanol, analytical reagent.

5.2.4.1.2 37% concentrated hydrochloric acid, analytical reagent.

5.2.4.1.3 Vanillin, analytical reagent.

5.2.4.1.4 Alcohol burner.

5.2.4.1.5 Watch glass, 5 cm in diameter.

5.2.4.1.6 Evaporating dish, 9 cm in diameter.

5.2.4.1.7 Graduated pipette, 5 mL.

5.2.4.2 Testing procedure

Take 2~3 mL filtrate of ethanol extractives prepared in 5.2.3 to the evaporating dish, heat the bottom of evaporating dish with alcohol burner until the filtrate is evaporated to dryness, cover the evaporating dish with a watch glass promptly, and continue to heat until oily substances appear on the watch glass. Remove the watch glass, add 1 drop of concentrated hydrochloric acid, about 0.05 g of vanillin, and 1~2 drops of 95% ethanol to the oily substances, stand, and observe the color change.

5.2.5 Thin-layer chromatography

5.2.5.1 Reagents and apparatus

5.2.5.1.1 Diethyl ether, analytical reagent.

5.2.5.1.2 Trichloromethane, analytical reagent.

5.2.5.1.3 Graduated scale, the measuring range is 0 ~20 cm, and the minimum scale is no more than 0.5 mm.

5.2.5.1.4 Temperature-controlled drying oven, room temperature~200 ℃, accurate to 0.1 ℃.

5.2.5.1.5 Balance, accurate to 0.001g.

5.2.5.1.6 Thin-layer plate, Silica gel G, usually activated at 110 ℃ for 0.5 h before use.

5.2.5.1.7 Sample applicator, quantitative capillary, manual, semi-automatic, or full-automatic.

5.2.5.1.8 Chromatographic chamber, glass chamber with a flat bottom or twin trough and a tightly fitted lid.

5.2.5.1.9 Detection device, a camera obscura equipped with ultraviolet light (UV) of 365 nm and corresponding filter. Additional camera equipment could be used to take picture. The light source should have enough intensity of illumination.

5.2.5.2 Testing procedure

Weigh 0.2 g of ground agarwood reference substance to an accuracy of 0.0001 g, add 30 mL of diethyl ether, ultrasonicate in a water bath for 60 min, then filter. Evaporate the diethyl ether to dryness, and dissolve the residue in 1 mL of trichloromethane as reference solution. Prepare sample solution in the same manner as the reference solution.

Apply separately the above two solutions to the same plate with sample applicator. The distance between sample zone and lower edge of thin-layer plate is 1.5~2.0 cm. The applied volume of solution is usually 4 μL, adjusted according to the separation resolution.

Add a proper amount of trichloromethane-ether (10:1) mobile phase to the chromatographic chamber, place the plate loaded with sample into the chromatographic chamber, keep the solvent level about 5 mm below the sample zone, and cover the chamber tightly. When the mobile phase moves over the prescribed development distance, remove the plate from the chamber, and allow the plate to dry. Generally, 8~15 cm shall be developed for normal thin-layer plate, and 5~8 cm for high performance thin-layer plate.

Examine under ultraviolet light at 365 nm, and compare the chromatograms of sample with reference solution. Refer to Annex B for representative TLC chromatogram of agarwood.

5.2.6 HPLC characteristic chromatogram

5.2.6.1 Reagents and apparatus

5.2.6.1.1 95% ethanol, analytical reagent.

5.2.6.1.2 Acetonitrile, chromatographically pure.

5.2.6.1.3 Formic acid, guaranteed reagent.

5.2.6.1.4 Water, Grade 1.

5.2.6.1.5 Centrifuge tube with stopper, 30 mL.

5.2.6.1.6 Pipette,10 mL.

5.2.6.1.7 0.1% solution of formic acid, prepared before use. Measure 1 mL of formic acid with a pipette, make up to 1000 mL with water, and shake thoroughly. The solution shall be passed through a membrane filter (pore size 0.45 μm).

5.2.6.1.8 Balance, accurate to 0.001 g.

5.2.6.1.9 Ultrasonic cleaner, with a power of 250 W and a frequency of 40 kHz.

5.2.6.1.10 HPLC, equipped with a UV spectrophotometric detector and a gradient elution device.

5.2.6.1.11 Chromatographic column, Diamonsil C18 or Phenomenex luna C18 (particle size 5 μm, column length 25 cm, inner diameter 4.6 mm).

5.2.6.2 Testing procedure

Weigh 0.2 g of ground agarwood reference substance to an accuracy of 0.001 g, put in stoppered centrifuge tube, add 10 mL of 95% ethanol with a pipette,weigh, ultrasonicate in a water bath for 1 h, cool, and weigh again. Replenish the loss of weight with 95% ethanol, mix well, stand,

filter the supernatant through a 0.45-μm membrane filter, and use as reference solution. Prepare sample solution in the same manner as the reference solution, or take 2 mL filtrate of ethanol extractives prepared in 5.2.3, pass through a 0.45-μm membrane filter, and use as sample solution.

Chromatographic conditions and system suitability shall be performed. Use acetonitrile as mobile phase A, 0.1% solution of formic acid as mobile phase B, elute in gradient at 0.7 mL per minute as specified in Table 2 with column temperature 31 ℃ and spectrophotometer set at 252 nm. The number of theoretical plates of column is no less than 6000, calculated with reference to the peak of agarotetrol.

Table 2 Gradient elution conditions

| Time (min) | Mobile phase A (%) | Mobile phase B (%) |
|---|---|---|
| 0～10 | 15→20 | 85→80 |
| 10～19 | 20→23 | 80→77 |
| 19～28 | 23→33 | 77→67 |
| 28～40 | 33 | 67 |
| 40～41 | 33→35 | 67→65 |
| 41～50 | 35 | 65 |
| 50.1～60 | 95 | 5 |

Inject separately 10 μL of the above two solutions into HPLC, and compare the chromatograms of sample with reference solution. Refer to Annex C for representative HPLC chromatogram of agarwood.

## 6 Determination

If all the results of xylem structure and secretions characteristics conform to requirements given in Table 1, the sample shall be considered as agarwood. If one inspection item fails, the sample shall not be considered as agarwood.

# Annex A

(informative)

## Main characteristics of Xylem in *Aquilaria* species

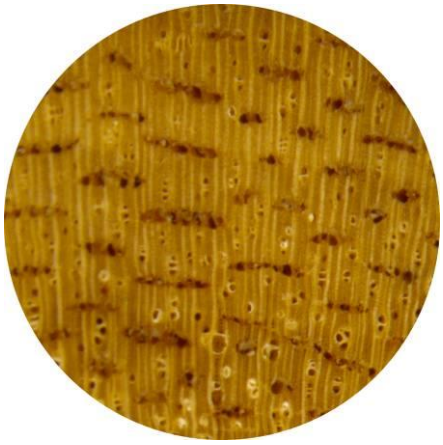
*Aquilaria* genus (Thymelaeaceae family)

Foreign trade names: Agarwood, Eaglewood

Trees and distribution: Evergreen trees. Approx. 22 species; distributed in Indonesia, Malaysia, Vietnam, Cambodia, Laos, Thailand, Myanmar, India, the Philippines, Singapore, New Guinea, Brunei, Bhutan and China. In China, there are two native *Aquilaria* species — *Aquilaria sinensis* and *Aquilaria yunnanensis*, mainly distributed in Guangdong, Hainan, Guangxi, Yunnan, and Fujian province.

Macro-structural features (take *Aquilaria sinensis* as an example): Diffuse-porous wood. The wood color is yellowish white. Once the wood is exposed to the air for a long term, its surface will turn dark. The heartwood color and sapwood color are indistinguishable. The wood is glossy and has a mild fragrant and sweet odor; there is no special taste. Growth rings are indistinct, and there exist dark lines between the rings. The number of vessels is rare, slightly small to medium, visible with a hand lens. The size of vessels is consistent and evenly distributed in a dispersive arrangement; tyloses are absent. The axial parenchyma is usually absent. The number of rays are medium, very fine to slightly fine, and visible with a hand lens; there are ray stripes on the radial section. Ripple marks and intercellular canal are absent. The number of included phloem is large, visible with the naked eye, foraminate or island type, distributed evenly in the secondary xylem (Figure A.1). The color of where aromatic resin is produced turns darker, and is yellowish brown or dark brown, in black lines or plaques.

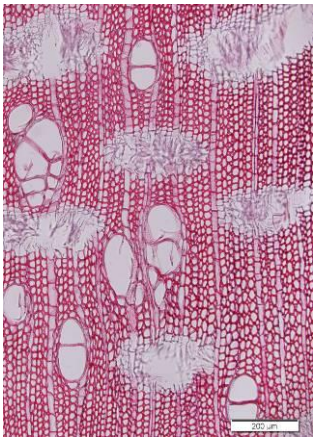
a) Longitudinal section of solid wood



b) Cross section of solid wood (12X)

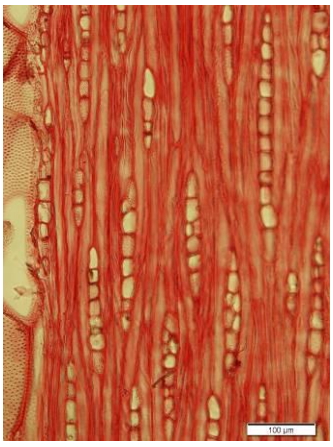
Figure A.1 Xylem macrostructure picture

Microstructural characteristics (take *Aquilaria sinensis* as an example): Vessels are circular to oval in outline as viewed in cross section, mostly 4 to 6 /mm$^2$; they are in radial multiples mainly of 2 to 4 and in clusters, occasionally solitary; diffuse; the diameters of most vessels are from 85 to 135 μm; tyloses are absent. Simple perforations with slightly inclined perforation plate. The intervessel pits are alternate, vestured with included lenticular apertures. The vessel-ray pits are similar to intervessel pits in size and shape. The axial parenchyma cells are scarce and vasicentric. They have nodular end walls that are distinct. Gums and crystals are absent. Thin-walled fibers have simple pits with narrow border; part of the simple pits are slightly circular, with slit-like or X-shaped apertures. Rays are nonstoried, 5 to 10/mm, mostly uniseriate with occasional biseriate rays. Rays have 7 to 20 cells in height; ray tissues are mostly heterogeneous and with occasionally heterogeneous III or II type. Ray cell contents are usually present. Crystals are absent. Included phloem is present in large amounts, foraminate or island type, and evenly distributed (Figure A.2).



a) Cross section

b) Radial section



c) tangential section

Figure A.2 Xylem microstructure picture

Air-dry density (12% moisture content): about 0.40 g / cm$^3$.

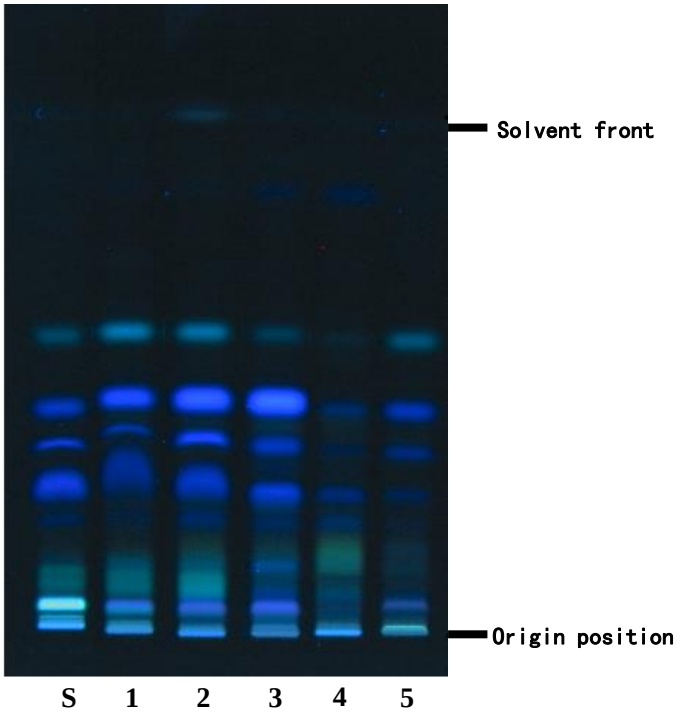
Specimen materials: CAFW8810 (Guangxi), CAFW9581 (Hainan), CAFW12164 (Hainan), CAFW14326 (Hainan), CAFW14804 (Guangdong), CAFW22143 (Hainan).

Information on regulation and protection: *Aquilaria* spp. is included in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES),and *Aquilaria sinensis* is listed under protection level II in the Catalogue of the National Protected Key Wild Plants (the First Batch) (1999) in China. In case of any change, the latest version shall prevail.

# Annex B

(informative)

## Representative TLC chromatogram of agarwood




Notes:

S——Agarwood reference substance, purchased form National Institutes for food and drug control, PRC;

1——Agarwood, of Cambodian provenance;

2——Agarwood, of Hainan provenance;

3——Agarwood, of Vietnamese provenance;

4——Agarwood, of Indonesian provenance;

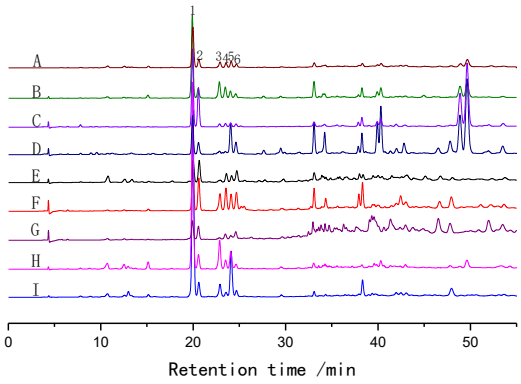
5——Agarwood, of Vietnamese provenance.

Figure B.1 Thin-layer chromatogram of agarwood

# Annex C

(informative)

## Representative HPLC characteristic chromatogram of agarwood




Notes:

A ——Agarwood reference substance, purchased form National Institutes for food and drug control, PRC;

B ——Agarwood, of Hainan provenance;

C~D——Agarwood, of Guangdong provenance;

E ——Agarwood, of Hainan provenance;

F ——Agarwood, of Hong Kong provenance;

G ——Agarwood, of Laotian provenance;

H ——Agarwood, of Malaysian provenance;

I ——Agarwood, of Vietnamese provenance.

Figure C.1 HPLC characteristic chromatogram of agarwood