

Method ID		Author	Date
MOA 002	Identification of Ginseng	AS	24.Oct.2005

## Validated Method

### 1. Purpose of method

The method for the identification of Ginseng species by HPTLC fingerprint is suitable to identify a given sample of plant material either as *Panax ginseng*, *P. quinquefolium*, or *P. notoginseng* (syn. *P. pseudoginseng*).

Adulterants, such as *Eleutherococcus senticosus*, show a different profile.

### 2. Materials

Wear lab coat, protective goggles and gloves at all times when handling chemicals.

#### 2.1 Chemicals and solvents

Ethanol (absolute), methanol, ethyl acetate, chloroform, sulfuric acid 95-97%: all of "for analysis" or HPLC quality, distilled or demineralized water.

#### 2.2 Samples and reference materials (optional)

Botanically authenticated and freshly dried *Panax ginseng*, *P. quinquefolium*, *P. notoginseng*, various ginsenosides (for example Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rh1, Rh2, and pseudoginsenoside F11 [ChromaDex]).

#### 2.3 Plates

Glass plates HPTLC Si 60 F<sub>254</sub>, 10x10 or 20x10 cm, Merck (Darmstadt, Germany), or others if equivalence was shown.

#### 2.4 Lab ware and instruments

- Analytical mill or mortar,
- ultrasonic bath,
- centrifuge with centrifuge tubes, or suitable set-up for filtration with beakers or small flasks (10 or 20 mL)
- analytical balance,
- graduated pipettes (1, 5, and 10 mL),
- graduated cylinder (50 mL),
- glass bottles (with tightly closing lid, 100 mL and 200 mL),
- TLC Twin Trough Chamber or Flat Bottom Chamber 20x10 cm, alternatively automatic developing chamber,
- sample application device using the spray-on technique (such as Linomat, ATS [CAMAG] or AS 30 [Desaga]),
- chromatogram immersion device [CAMAG],
- plate heater or oven,
- documentation system consisting of an illumination device for UV 254 nm, UV 366 nm, and white light and a video or digital camera,
- suitable TLC software,
- thermometer and hygrometer
- device for humidity control of plates if humidity of lab exceeds 50%RH
- lab coat, protective goggles and gloves.

### 3. Description of method

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### **3.1 Preparation of test solutions**

#### **3.1.1 Raw materials**

Mill each sample to a fine powder. Weigh 1 g each of powder in individual centrifuge tubes or flasks. Add 10 mL of absolute ethanol each and mix well. Sonicate for 10 min. Centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.

### **3.2 Preparation of reference solutions (optional)**

#### **3.2.1 Botanical reference solution**

As 3.1.1

#### **3.2.2 Chemical reference solutions**

Weigh individually 1 mg of each ginsenoside into suitable vials. Add 5 mL of methanol to each vial and dissolve by mixing.

### **3.3 Preparation of derivatizing reagent**

Place 180 mL of methanol in a 200 mL glass bottle and cool it down in a water-ice cubes-salt bath or in a freezer. To the ice-cold methanol add slowly and carefully 20 mL of sulfuric acid and mix well. Allow the mixture to cool to room temperature.

### **3.4 Stationary phase**

10x10 cm (or 20x10 cm) glass plates HPTLC silica gel 60 F<sub>254</sub> (Merck).

### **3.5 Sample application**

Apply 10 µL of test solution, 10 µL of botanical reference solution, and 5 µL of each chemical reference solution each as 8 mm band, at least 2 mm apart, 8 mm from the lower edge and at least 15 mm from left and right edges of the plate.

### **3.6 Temperature and humidity**

Record temperature and humidity in the laboratory. If the relative humidity exceeds 50%RH, condition the plate to about 30-35%RH using a suitable device.

### **3.7 Chromatography**

#### **3.7.1 Developing solvent**

Place 15 mL of chloroform, 40 mL of ethyl acetate, 22 mL of methanol, and 9 mL of water in a bottle, close lid tightly and mix content by shaking. Larger or smaller amounts of solvent can be prepared once a day.

#### **3.7.2 Chamber**

Line one side of a 10x10 cm Twin Trough Chamber with filter paper. Pour 10 mL of developing solvent over the paper, and tilt the chamber to equilibrate solvent level in both troughs, close the lid. Allow the chamber to saturate for 20 min. If using a 20x10 cm chamber, use 20 mL of developing solvent. If using a Flat Bottom Chamber, use enough solvent to cover the bottom with a 5 mm level. If using an automatic chamber, refer to the manufacturer's instructions.

#### **3.7.3 Development**

Measure and mark on the plate the developing distance of 80 mm from lower edge of plate (72 mm from application position). Open the saturated chamber and introduce the plate with the layer facing the inside, close the chamber and wait for the solvent to reach the mark. Remove the plate from the chamber.

#### **3.7.4 Drying**

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Dry the plate for 5 min with cold air (hair dryer).

### **3.8 Documentation and derivatization**

#### 3.8.1 Documentation of non-derivatized plate

No documentation is needed.

#### 3.8.2 Derivatization

Turn on plate heater or oven and select temperature (100°C). Charge the tank of the immersion device with 200 mL of reagent. Place plate in holder of immersion device, set parameters (speed:5, time:0) and press start. Let excess reagent drip off the plate, wipe off the back of the plate with a paper towel. Remove plate from plate holder. Place plate onto plate heater or in oven. Remove hot plate after 5 min and let it cool down to room temperature.

#### 3.8.3 Documentation of derivatized plate

Document the plate using illumination with white light (reflection and transmission).

Document the plate using illumination with UV366 nm.

### **3.9 Results**

Compare the images of the plate obtained under 3.8 with the image provided under 4.1. The plate can only be evaluated if it passes the system suitability test (4.3).

Evaluate the results obtained with the test solution according to the description under 4.2. The test solution can be identified as one of the three *Panax* species if the fingerprint obtained is similar to that of the corresponding BRM. In comparison to the BRM, the test solution doesn't show any additional intense zone after derivatization neither in white light nor under UV 366 nm light. None of the described zone is missing.

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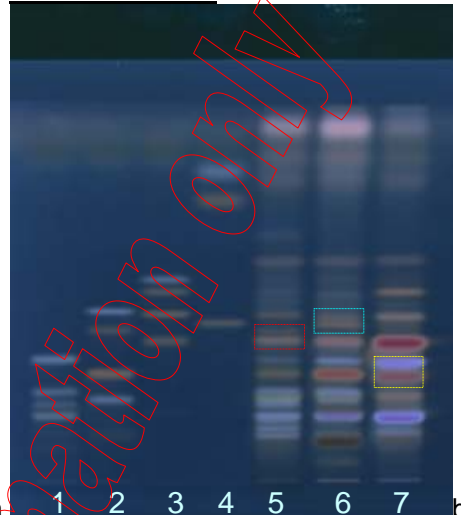
#### 4. Results for comparison

##### 4.1 Image of chromatograms

###### 4.1.1 White light



###### 4.1.2 UV 366 nm



##### Track assignment

- 1: Ginsenosides Rb1, Rb2, Rc, Rd
- 2: Ginsenosides Rb3, Re, Rf, Rg3
- 3: Ginsenosides Rg1, Rg2, Rh1, Rh2
- 4: Pseudoginsenoside F11, panaxatriol, panaxadiol (increasing  $R_F$  values)
- 5: *Panax ginseng* (Asian ginseng)
- 6: *Panax quinquefolium* (American ginseng), cultivated
- 7: *Panax notoginseng* (syn. *Panax pseudoginseng*)

##### 4.2 Description of results:

###### 4.2.1 White light

All standards on tracks 1-4 appear as brownish bands. Bands of different intensities matching ginsenoside Rb1, Rc, Rd, Re, Rg1, and Rg2 are seen in all Ginseng samples (ginsenoside Rc is not seen in *P. notoginseng*).

- A band corresponding to ginsenoside Rf is seen in *P. ginseng* only.
- Pseudoginsenoside F11 is only detected in *P. quinquefolium*.
- *P. notoginseng* shows an intense band about the position of ginsenoside Rh1 (probably notoginsenoside R1). *P. ginseng* and *P. quinquefolium* have only a weak zone below this point.
- In *P. ginseng*, the intensity of all bands is similar, whereas *P. quinquefolium* and *P. notoginseng* show prominent bands for ginsenosides Rb1, Re, and Rg1.

Additional brown to green bands are seen above ginsenoside Rh2 and below Rb1 in the samples.

###### 4.2.2 UV 366 nm

A similar picture is seen, but better differentiation of the compounds can be made due to the coloration of the zones. Ginsenosides Rb1, Rb2, Rc, Rd, Rb3, Rg3, and Rh2 are blue (panaxadiol derivatives), and ginsenosides Re, Rf, Rg1, Rg2, Rh1, and pseudoginsenoside F11 are brownish (panaxatriol derivatives).

##### 4.3 System suitability test:

The result obtained in the test is suitable for evaluation if the following requirements are met:

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In *P. ginseng*, the ginsenosides Rg1 and Rf must be seen as two distinct zones (red box in Image 4.1).

In *P. quinquefolium*, pseudoginsenoside F11 and ginsenoside Rg2 must be seen as two distinct zones (blue box in Image 4.1).

In *P. notoginseng*, the ginsenosides Rd and Re must be seen as two distinct zones (yellow box in Image 4.1).

### 5. Approvals

**Validation approved:**

**Date:** 12. August 2005, **by:** ER

**MOA 002 released:**

**Date:** , **by:** , **Signature:** Signature removed

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### Revision history

Creation date

24. October 2005/AS

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