

Method ID		Author	Date
MOA 003	Identification of Eleuthero	AS	24.Oct.2005

Validated Method

1. Purpose of method

The identification method for Eleuthero by HPTLC fingerprint is suitable to identify a given sample of plant material as Eleuthero root (*Eleutherococcus senticosus* syn. *Acanthopanax senticosus*) based on the separation of Eleutherosides E, B, and E1 (lignan fingerprint). Adulterants, such as *Periploca sepium*, show a different profile.

The method is also applicable for identification of extracts and finished products as derived from Eleuthero root (*Eleutherococcus senticosus*), provided that the material was made from a single herb and intended to contain the constituent profile seen in Eleuthero.

2. Materials

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

2.1 Chemicals and solvents

Ethanol, methanol, 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 Eleuthero root, *Periploca sepium* root. Eleutheroside B, E, and E1 [ChromaDex].

2.3 Plates

Glass plates HPTLC Si 60 F₂₅₄, 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.

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3. Description of method

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 5 mL of an ethanol-water mixture (1:1) each and mix well. Sonicate for 10 min. Centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.

3.1.2 Dry extracts and dry finished products

Weigh an amount of each extract powder or finished product equivalent to 1 g of raw material in individual centrifuge tubes or flasks. Add 5 mL of an ethanol-water mixture (1:1) each and mix well. Sonicate for 10 min. Centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.

3.1.3 Liquid extracts and liquid finished products

Dilute the liquid samples with the same solvent (as on the label) to obtain a solution with the same concentration as that of a test solution from raw material as described under 3.1.1. Liquid extracts containing glycerin may not perform in this test.

3.2 Preparation of reference solutions (optional)

3.2.1 Botanical reference solution

As 3.1.1

3.2.2 Chemical reference solutions

Weigh 1 mg of eleutheroside B in a flask. Add 1.5 mL of an ethanol-water mixture (1:1). Individually dissolve eleutheroside E and E1 in the same way.

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 F₂₅₄ (Merck).

3.5 Sample application

Apply 10 µL of test solution, 10 µL of botanical reference solution, and 2 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%RH using a suitable device.

3.7 Chromatography

3.7.1 Developing solvent

Place 70 mL of chloroform, 30 mL of methanol, and 4 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

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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 70 mm from lower edge of plate (62 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

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

3.8 Documentation and derivatization

3.8.1 Documentation of non-derivatized plate

Document the plate using UV 254 nm illumination.

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).

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).

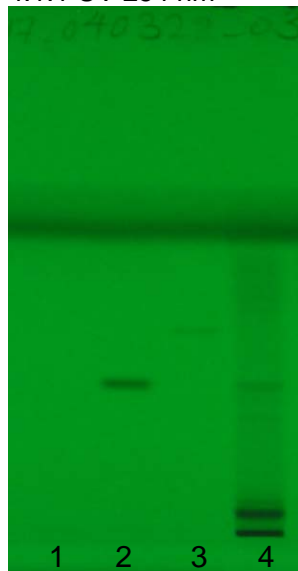
Evaluate the results obtained with the test solution according to the description under 4.2. The test solution can be identified as Eleuthero if the fingerprint obtained is similar to that of the BRM. The intensity of the zones may vary, however, the zones corresponding to the three standards (Eleutheroside E, B, and E1) must be seen. In comparison to the BRM, the test solution does not show any additional intense zone neither prior nor after derivatization.

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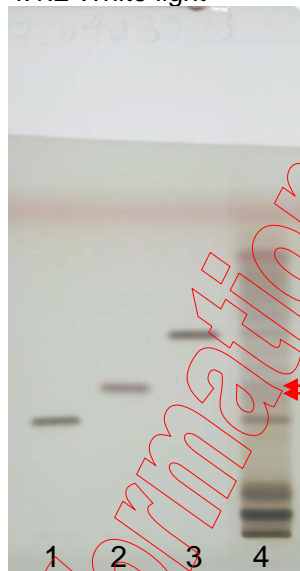
4. Results for comparison

4.1 Image of chromatograms

4.1.1 UV 254 nm



4.1.2 White light



Track assignment

1. Eleutheroside E
2. Eleutheroside B
3. Eleutheroside E1
4. Eleuthero root (BRM)

4.2 Description of results

4.2.1 UV 254 nm

The standard eleutheroside B (track 2, $R_f=0.45$) appears as a dark band. A fainter band for eleutheroside E1 is also seen (track 3, $R_f=0.60$). Eleutheroside E (track 1) is not seen using this method of detection. A band matching eleutheroside B is seen the BRM. A dark band just above the application is also seen in the BRM.

The test solution can be identified as Eleuthero root if the fingerprint obtained is similar to that of the BRM.

4.2.2 White light

The standards eleutheroside E (track 1, $R_f=0.34$), eleutheroside B (track 2, $R_f=0.45$), and eleutheroside E1 (track 3, $R_f=0.60$) appear as brown bands. The BRM shows bands corresponding to eleutherosides E, B, and E1, two very strong bands near the application, and reddish zones near the front. Additional brownish-purple zones are seen between Eleutheroside E and the solvent front.

The test solution can be identified as Eleuthero root if the fingerprint obtained is similar to that of the BRM.

4.3 System suitability test

The result obtained in the test is suitable for evaluation if the following requirement is met. After derivatization the fingerprint of the test solution shows a zone corresponding to eleutheroside B and another zone directly below. These two zones appear as two distinct bands, see arrows in Fig. 4.1.2.

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5. Approvals

Validation approved:

Date: 21. June 2005, **by:** ER

MOA 003 released:

Date: _____, **by:** _____, **Signature:** _____

Revision history

Creation date

24. October 2005/AS

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