

Method ID		Author	Date
MOA 006	Identification of Licorice	AS	20.Aug.07

Validated Method

1. Purpose of method

The method for identification of Licorice by HPTLC fingerprint is suitable to identify a given sample of plant material as Licorice (*Glycyrrhiza glabra* Linne or *Glycyrrhiza uralensis* Fischer).

The method may be used to identify an extract, or finished product as derived from Licorice (*Glycyrrhiza glabra* Linne or *Glycyrrhiza uralensis* Fischer), provided that the material was made from a single herb and intended to contain the constituent profile seen in Licorice.

2. Materials

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

2.1 Chemicals and solvents

Ethanol, methanol, ethyl acetate, sulfuric acid 95-97%, formic acid 98-100%, glacial acetic acid 99%, all of "for analysis" or HPLC quality, distilled or demineralized water.

2.2 Samples and reference materials (optional)

Botanically authenticated and freshly dried Licorice root (*Glycyrrhiza glabra* Linne or *Glycyrrhiza uralensis* Fischer).

Ammonium glycyrrhizate [Roth].

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]
- 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 0.5 g each of powder in individual centrifuge tubes or flasks. Add 10 mL of an ethanol-water mixture (70:30) 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 0.5 g of raw material in individual centrifuge tubes or flasks. Add 10 mL of an ethanol-water mixture (70:30) 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.

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 ammonium glycyrrhizate in a flask. Add 10 mL of an ethanol-water mixture (70:30).

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 2 µL of test solution, 2 µL of botanical reference solution, and 10 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 60%RH, condition the plate to about 30-40%RH using a suitable device.]

3.7 Chromatography

3.7.1 Developing solvent

Place 15 mL of ethyl acetate, 1 mL of formic acid, 1 mL of acetic acid, and 2 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

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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 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 10 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 images 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 Licorice if the fingerprint obtained is similar to that of the BRM. The intensity of the zones may vary; however, the zone corresponding to the standard ammonium glycyrrhizate must be seen. In comparison to the BRM, the test solution does not show any additional intense zone neither prior nor after derivatization.

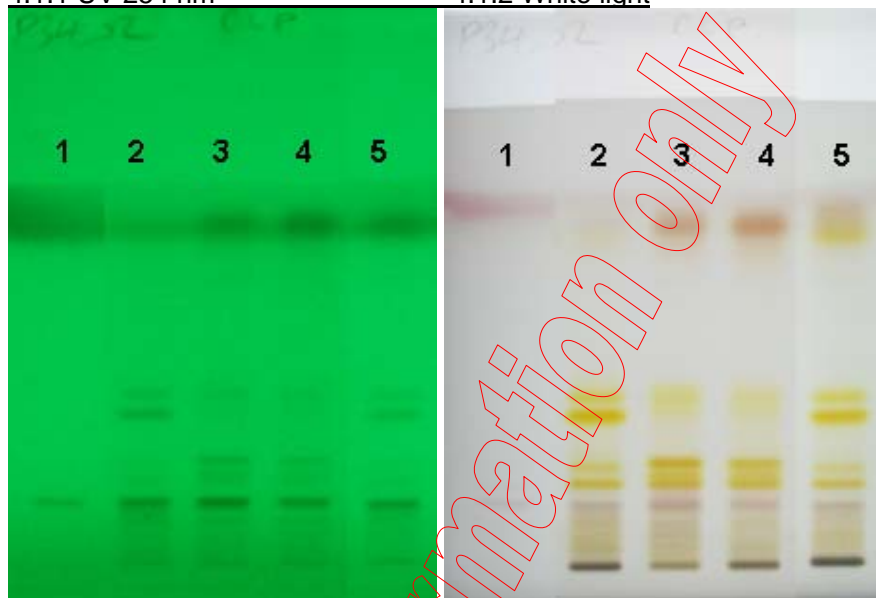
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4. Results for comparison

4.1 Images of chromatograms

4.1.1 UV 254 nm

4.1.2 White light



Track assignment

- 1: Ammonium glycyrrhizate
- 2: *Glycyrrhiza glabra*
- 3: *Glycyrrhiza glabra*
- 4: *Glycyrrhiza glabra*
- 5: *Glycyrrhiza uralensis*

4.2 Description of results:

4.2.1 UV 254 nm

The chromatogram of the standard (track 1) shows a quenching zone around $R_F=0.16$. All samples show a quenching zone at the same position. Four additional quenching zones are seen at middle R_F in the samples. The pattern of samples on tracks 2 and 5 are similar and so are those on tracks 3 and 4.

4.2.2 White light

The chromatogram of the standard (track 1) shows a violet zone around $R_F=0.16$. All samples show a violet zone at the same position. Four yellow zones are seen at middle R_F in the samples. The intensity of those zones varies in the samples. Additional zones are seen near the front and between application position and ammonium glycyrrhizate. The pattern of samples on tracks 2 and 5 are similar and so are those on tracks 3 and 4.

4.3 System suitability test:

The result obtained in the test is suitable for evaluation if the following requirement is met. The fingerprint of the test solution shows a zone corresponding to that of ammonium glycyrrhizate at $R_F=0.16$ (± 0.05).

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5. Approvals

Validation approved:

Date: 07.October 2005, **by:** ER

MOA 006 released:

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

Revision history

Creation date

25. October 2005/AS

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