

Method ID	Author	Date
MOA 010	AS	26.Jan.2006

Validated Method

1. Purpose of method

The method for identification of Ginger by HPTLC fingerprint is suitable to identify a given sample of plant material as Ginger (*Zingiber officinale*) based on its gingerol/shogaol fingerprint. Adulterants, such as Galangal (*Alpinia officinarum*) show a different profile.

The method may be used to identify an extract or finished product as derived from Ginger, provided that the material was made from a single herb and is intended to contain the constituent profile seen in Ginger.

2. Materials

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

2.1 Chemicals and solvents

Methanol, toluene, acetic acid, sulfuric acid, p-anisaldehyde, and ethyl acetate

2.2 Samples and reference materials (optional)

Botanically authenticated and freshly dried Ginger, and 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol (available from ChromaDex; a mixture of 6-gingerol and 6-shogaol is available from USP).

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 available]
- 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 10 mL of methanol 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 10 mL of methanol 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 6-gingerol in a flask. Add 1 mL of methanol. Individually dissolve 8-gingerol, 10-gingerol, and 6-shogaol in the same way.

3.3 Preparation of derivatizing reagent

10 mL of sulfuric acid are carefully added to a mixture of 170 mL ice-cold methanol and 20 mL acetic acid. To this solution 1 mL of anisaldehyde is added.

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 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 is lower than 40%RH, the plate may be conditioned to about 50%RH using a suitable device for optimal separation.

3.7 Chromatography

3.7.1 Developing solvent

Place 3 mL of toluene and 1 mL of ethyl acetate 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

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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

Not applicable

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 3 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

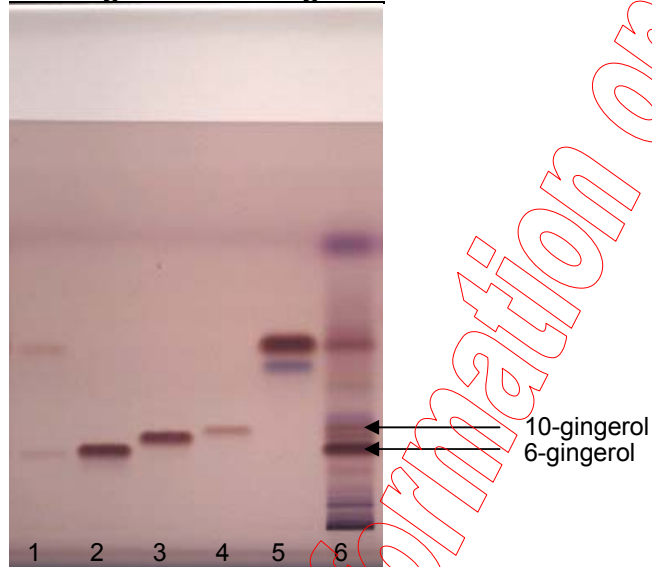
Compare the image(s) of the plate obtained under 3.8 with the image(s) provided under 4.1. The plate can only be evaluated if it passes the system suitability test (4.3).

Evaluate the results obtained with the test solution according to the description under 4.2. The test solution can be identified as Ginger if the fingerprint obtained is similar to that of the BRM. The intensity of the zones may vary, however, the zones corresponding to the four chemical references must be seen. In comparison to the BRM, the test solution must not show any additional intense zone.

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4. Results for comparison

4.1 Image of chromatograms



Track assignment:

Track 1: USP standard mixture (6-gingerol and 6-shogaol)

Track 2: 6-gingerol

Track 3: 8-gingerol

Track 4: 10-gingerol

Track 5: 6-shogaol

Track 6: Ginger BRM (*Zingiber officinale*)

4.2 Description of results:

White light after derivatization

The reference substances 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol appear as brown zones at $R_f = 0.23, 0.26, 0.28,$ and 0.51 (ΔR_f max 0.05) respectively. The test solution shows zones corresponding in color and position to those of the reference substances. The zones of 6-gingerol and 6-shogaol are most prominent. There are several brown or violet zones located below the zone due to 6-gingerol. Other violet zones may be present above the position of 6-shogaol. No dark blue zone is seen just above 6-shogaol (this zone is found in galangal, an adulterant of ginger).

4.3 System suitability test:

The result obtained in the test is suitable for evaluation if the following requirements are met: The two zones due to 6-gingerol and 10-gingerol are clearly separated (see arrows in Figure above). A zone due to 8-gingerol may or may not be seen in between.

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5. Approvals

Validation approved:

Date: 27. January 2006, **by:** ER

MOA 010 released:

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

Revision history

Creation date

27.Jan.2006/AS

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