

Method ID		Author	Date
MOA 009	Identification of Feverfew	AS	25.Jan.2006

Validated Method

1. Purpose of method

The method for identification of Feverfew by HPTLC fingerprint is suitable to identify a given sample of plant material as Feverfew (*Tanacetum parthenium*) based on its fingerprint, using parthenolide as a reference.

Adulterants, such as Mexican Feverfew, Chamomile, and Roman Chamomile show a different profile.

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

2. Materials

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

2.1 Chemicals and solvents

Methanol, ethyl acetate, acetic acid, cyclohexane, sulfuric acid, and p-anisaldehyde; all of "for analysis" or HPLC quality

2.2 Samples and reference materials (optional)

Botanically authenticated and freshly dried Feverfew, and parthenolide (available from 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
- 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 parthenolide in a flask. Add 1 mL of methanol.

3.3 Preparation of derivatizing reagent

Anisaldehyde Sulfuric acid Reagent: 10 mL of sulfuric acid are carefully added to an ice-cold mixture of 170 mL of methanol and 20 mL of 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 5 µL of test solution, 5 µL of botanical reference solution, and 5 µL of 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.

3.7 Chromatography

3.7.1 Developing solvent

Place 30 mL of cyclohexane and 30 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 chamber, use 20 mL of developing solvent. If using a Flat Bottom Chamber, use enough

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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), within 10 min after the completion of derivatization.

3.9 Results

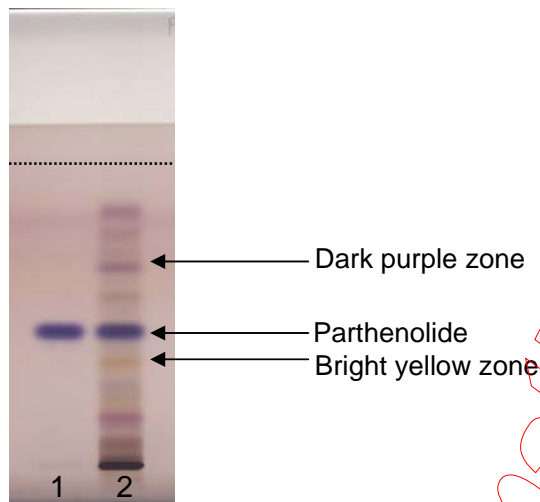
Compare the image(s) of the plate obtained under 3.8 with the image(s) provided under 4.1. The plate can only be evaluated if it passes the system suitability test (4.3).

Evaluate the results obtained with the test solution according to the description under 4.2. The test solution can be identified as Feverfew if the fingerprint obtained is similar to that of the BRM. The intensity of the zones may vary, however, the zones corresponding to the chemical reference parthenolide 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:

1: Parthenolide
2: Feverfew (BRM)

4.2 Description of results:

a) White light after derivatization

The chemical reference standard parthenolide (track 1) appears as a dark blue zone at $R_F=0.43$. The major zone in the sample corresponds in color and position to that of parthenolide. There is a dark purple zone at $R_F=0.63$ and a bright yellow at $R_F=0.33$. Several additional zones are seen as well present but no blue zone is seen below parthenolide at $R_F=0.3-0.4$.

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 parthenolide at $R_F=0.43$ (± 0.05).

5. Approvals

Validation approved:

Date: 27.Jan.06, by: ER

MOA 009 released:

Date: _____, by: _____, Signature: _____

Revision history

Creation date

27. January 2006/AS