

Method ID		Author	Date
MOA 007	Identification of Kava	AS	24.Jan.2006

Validated Method

1. Purpose of method

The method for identification of Kava by HPTLC fingerprint is suitable to identify a given sample of plant material as Kava (*Piper methysticum*) based on its kavalactone fingerprint.

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

2. Materials

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

2.1 Chemicals and solvents

Methanol, *tert.*-butyl methyl ether, hexane, glacial acetic acid, anisaldehyde, sulfuric acid, caffeine, dichloromethane.

2.2 Samples and reference materials (optional)

Botanically authenticated and freshly dried Kava roots, and kawain, dihydrokawain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin (available from ChromaDex).

2.3 Plates and plate impregnation

Caffeine-impregnated HPTLC plates are available from Merck (EM-Science) or Macherey&Nagel. The impregnation can also be performed on standard 10x10 cm (or 20x10 cm) glass plates HPTLC silica gel 60 as follows:

8 g of caffeine are dissolved in 200 mL of dichloromethane. Plates are immersed in the solution for 1 second and allowed to dry at room temperature in a fume hood for 5 minutes. Drying of plates is then completed in an oven for 5 minutes at 80°C.

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 kavain in a flask. Add 2 mL of toluene. Individually dissolve dihydrokawain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin in the same way.

3.3 Preparation of derivatizing reagent

10 mL of sulfuric acid are carefully added to an ice-cooled 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

Caffeine-impregnated silica gel 10x10 cm (or 20x10 cm) glass plates, prepared as described under 2.3.

3.5 Sample application

Apply 2 μ L of test solution, 2 μ L of botanical reference solution, and 2 μ L 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.

3.7 Chromatography

3.7.1 Developing solvent

Place 7 mL of *tert*-butyl methyl ether and 3 mL of hexane 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

Use an unsaturated 10x10 cm Twin Trough Chamber. Prior to introducing the plate pour 5 mL of developing solvent in the front trough of the chamber, close the lid. If using a 20x10 cm chamber, use 10 mL of developing solvent. If using a Flat Bottom Chamber, use enough

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solvent to cover the bottom to a height of 5 mm. 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 5 min and document it immediately.

3.8.3 Documentation of derivatized plate

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

Document the plate using illumination with UV366 nm while still hot.

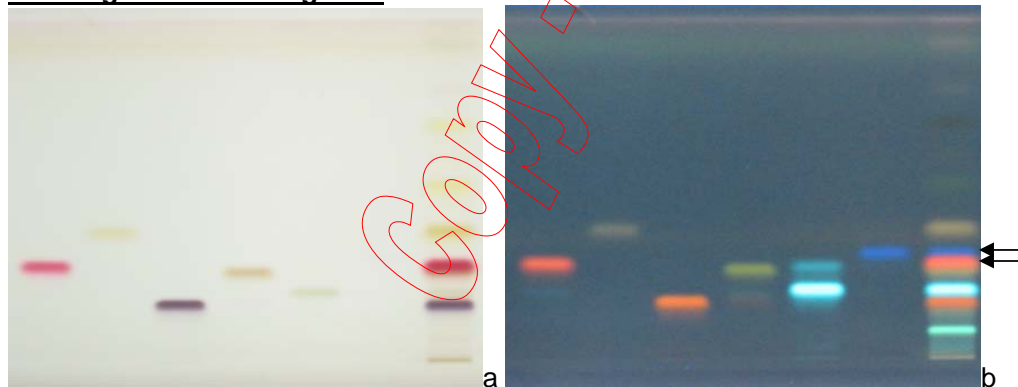
3.9 Results

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 Kava if the fingerprint obtained is similar to that of the BRM. The intensity of the zones may vary, however, the zones corresponding to the six chemical references must be seen. In comparison to the BRM, the test solution must not show any additional intense zone.

4. Results for comparison

4.1 Image of chromatograms



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Track assignment:

1. Kawain
2. Dihydrokawain
3. Methysticin
4. Dihydromethysticin (yellowish main zone)
5. Yangonin (main zone)
6. Desmethoxyyangonin
7. Kava (*Piper methysticum*) BRM

4.2 Description of results:

a) White light after derivatization

The standards kawain (track 1), dihydrokawain (track 2), methysticin (track 3), dihydro-methysticin (track 4), and yangonin (track 5) show colored bands. Desmethoxyyangonin (track 6) is not detected.

There are bands in the BRM corresponding in color and position to the standards. Kawain and dihydromethysticin are not well resolved and the brown band of the latter is difficult to see in the sample. Additional yellow or violet bands in the upper R_F region are seen.

b) UV 366 nm after derivatization

The standards kawain (track 1), dihydrokawain (track 2), methysticin (track 3), dihydro-methysticin (track 4), yangonin (track 5), and desmethoxyyangonin (track 6) show fluorescing bands of characteristic color. Some of the standards used here are not pure and show additional weaker bands due to impurities. There are bands in the BRM corresponding in color and position to the standards. The BRM shows additional weak bands in the upper R_F region, and a greenish-blue band is seen between the application and the band of methysticin.

4.3 System suitability test:

The result obtained in the test is suitable for evaluation if the following requirement is met. After derivatization the zones of kawain and desmethoxyyangonin (black arrows) are separated in the fingerprint of the test solution.

5. Approvals

Validation approved:

Date: 25. January 2006, **by:** ER

MOA 007 released:

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

Revision history

Creation date:

27. January 2006/AS