

Method ID		Author	Date
MOA 001	Identification of Green Tea	AS	21.Oct.2005

## Validated Method

### 1. Purpose of method

The method for identification of Green Tea by HPTLC fingerprint is suitable to identify a given sample of plant material as Green Tea (*Camelia sinensis*) based on its polyphenol fingerprint.

The method may be used to identify an extract as derived from Green Tea (*Camelia sinensis*), provided that the material was made from a single herb and intended to contain the constituent profile seen in Green Tea.

### 2. Materials

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

#### 2.1 Chemicals and solvents

Ethanol, methanol, toluene, acetone, formic acid 98-100%, dichloromethane, Fast Blue salt B, all of "for analysis" or HPLC quality, distilled or demineralized water.

#### 2.2 Samples and reference materials (optional)

Botanically authenticated and freshly dried Green Tea leaves (*Camelia sinensis*), and (-)-epicatechin [Roth], (-)-epigallocatechin [Sigma], (-)-epicatechin gallate [Sigma], (-)-epigallocatechin gallate [Sigma].

#### 2.3 Plates

Glass plates HPTLC Si 60 F<sub>254</sub>, 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 is below 25%RT or 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 100 mg of powder in individual centrifuge tubes or flasks. Add 10 mL of an ethanol-water mixture (80:20) and mix well. Sonicate for 10 min. Centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. Store below -20°C.

##### **3.1.2 Dry extracts and dry finished products**

Weigh an amount of each extract powder or finished product equivalent to 100 mg of raw material in individual centrifuge tubes or flasks. Add 10 mL of an ethanol-water mixture (80:20) each and mix well. Sonicate for 10 min. Centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. Store below -20°C.

#### **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 (-)-epicatechin in a flask. Add 20 mL of methanol. Individually dissolve (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate in the same way. Store below -20°C.

#### **3.3 Preparation of derivatizing reagent**

Dissolve 140 mg of Fast Blue salt B in 10 mL of water, add 140 mL of methanol and 50 mL of dichloromethane. If stored at 4°C in the dark, the reagent can be used for one week.

#### **3.4 Stationary phase**

10x10 cm (or 20x10 cm) glass plates HPTLC silica gel 60 F<sub>254</sub> (Merck).

#### **3.5 Sample application**

Apply 1 µL of test solution, 1 µL of botanical reference solution, and 5 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 below 25%RH or exceeds 50%RH, condition the plate to about 30%RH using a suitable device.

#### **3.7 Chromatography**

##### **3.7.1 Developing solvent**

Place 9 mL of toluene, 9 mL of acetone, and 2 mL of formic acid 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 60 mm from lower edge of plate (52 mm from application position). Introduce the plate into the chamber 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

No documentation is needed.

### 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. Heat the plate at 100°C for 2 min. Place plate while still warm 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 and leave it to dry for 5 min in the fume hood.

### 3.8.3 Documentation of derivatized plate

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

## **3.9 Results**

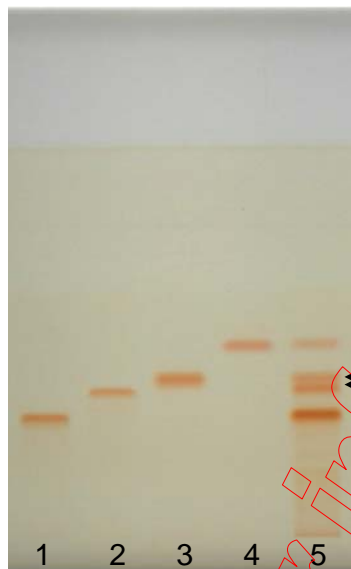
Compare the image of the plate obtained under 3.8 with the image provided under 4.1. The plate can only be evaluated if it passes the system suitability test (4.3).

Evaluate the results obtained with the test solution according to the description under 4.2. The test solution can be identified as Green Tea 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 doesn't show any additional intense zone.

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#### **4. Results for Comparison**

##### **4.1 Image of chromatogram**



##### **Track assignment**

- 1: (-)-epigallocatechin gallate
- 2: (-)-epigallocatechin
- 3: (-)-epicatechin gallate
- 4: (-)-epicatechin
- 5: Botanical reference material (BRM; Green Tea leaves)

##### **4.2 Description of results**

The chromatograms of the four standards (tracks 1-4 and 7) show a brownish-orange zone for each substance with the following  $R_F$  values: epigallocatechin gallate (track 1):  $R_F=0.38$ , epigallocatechin (track 2):  $R_F=0.48$ , epicatechin gallate (track 3):  $R_F=0.52$ , epicatechin (track 4):  $R_F=0.62$ .

The BRM shows four brownish-orange zones at the position of the reference substances. The lowest zone is the most intense. The upper zone is the faintest.

Note: The two upper zones may be significantly fainter than the two lower. For comparison with a wide range of samples see Appendix.

##### **4.3 System suitability test**

The result obtained in the test is suitable for evaluation if the fingerprint of the test solution shows two distinct bands for epigallocatechin and epicatechin gallate, see arrows in Fig. 4.1.

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### 5. Approvals

**Validation approved:**

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

**MOA 001 released:**

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

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### Revision history

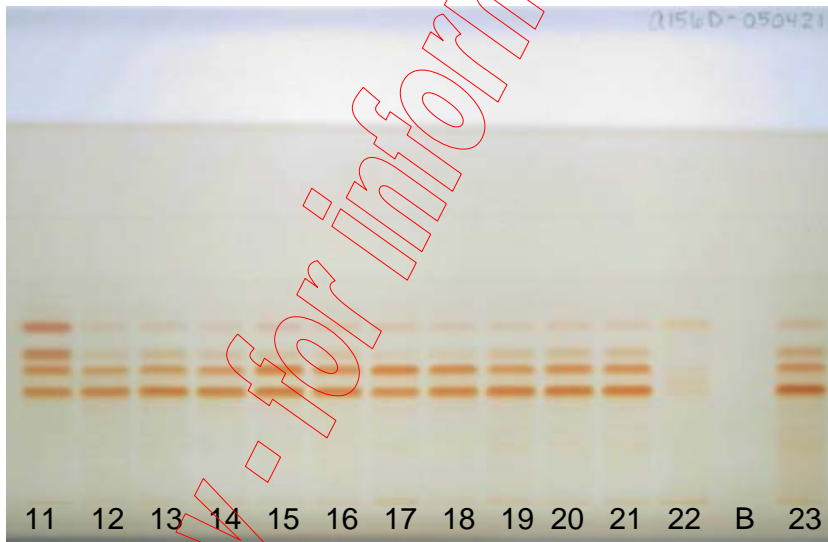
Creation date

21. October 2005/AS

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**Appendix**



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- 1, 11, 24: Epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin (increasing  $R_f$  value)  
2-10: Chinese Green tea  
12-22: Japanese Green tea (22: roasted green tea)  
23: Java Green tea  
25-27: Indian Green tea  
B: Blank

**Samples on tracks 9, 10, and 22 are not compliant.**