

HPTLC Method

HPTLC Fingerprint profile of Cocoa butter and detection of Palm oil in Cocoa butter.

Method Creator	Validated by	Final Authoriser	History	Date
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Method created at: **India - Specific HPTLC Application Research Lab**

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Objective: HPTLC Fingerprint profile of Cocoa butter and detection of Palm oil in Cocoa butter.

Introduction: Cocoa butter is a vegetable fat extracted from the ground beans of *Theobroma cacao*. Cocoa butter is an expensive fat and mostly used in the chocolates. Adulteration of chocolate is done by replacing cocoa butter with vegetable oil. Assessing the permissible limit of 5% vegetable oil in Chocolate. Cocoa butter being expensive is mostly adulterated with palm oil or vegetable fat.

Method suitable for: Food Industry

Analysis method

Reagents and chemicals required: GR grade Methanol, Toluene, Ethyl acetate.

TLC Al Si Gel 60 F₂₅₄, 20x10cm (Merck Catalogue No. 1.05554.0001). If no. of samples and standards to be applied is less than 10, then use a 10 x 10 cm sized plate.

HPTLC system requirements:

HPTLC system software – visionCATS

Sample Band Applicator – CAMAG Linomat 5 or ATS 4

Development Chamber – Automatic Developing Chamber with humidity control or Twin Trough Chamber, 20x10 cm and a desiccator filled with MgCl₂.6H₂O saturated solution to keep 20x10 cm or 10x10cm sized plates.

Chromatogram Visualisation – CAMAG UV cabinet

Image documentation – CAMAG Visualizer

Scanning Densitometry – CAMAG Scanner 4

Plate Cutter- CAMAG Smart Cut
Solvent Front Detector- CAMAG Smart Alert

Samples Preparation: Weigh 3g Cocoa butter in 4ml Methanol. Shake the sample vigorously for 5 mins. Refrigerate the sample for 24 hours. Take the supernatant for analysis.

1. CHROMATOGRAM LAYER:

20x10 cm TLC Al Silica gel 60F₂₅₄ on plate (Merck 5554). If you have less than 10 tracks to apply (standard + sample), use a 10x10 cm silica gel 60F₂₅₄ plate. Write ID on each plate. Mark with pencil at 70 mm, if no Automatic Development Chamber (ADC 2) is available.

Mobile phase: Toluene: Ethyl Acetate (93:7) v/v/v (Pipette out each volume separately in a 25ml stoppered conical flask, shake well.)

2. CAMAG visionCATS HPTLC software.

- a) Switch on and select instruments that will be used (Linomat or ATS for sample application, ADC 2, if available, CAMAG Visualizer, CAMAG Scanner etc.
- b) Sample Applicator parameters:

Parameters for Linomat 5	Parameters for ATS 4
No of bands – 11 Band length – 8mm Track distance – 16.4mm Distance from lower edge – 8mm Distance from left side edge – 15mm Application speed- Methanol (150 nl/s) Application volumes – ii. Sample: Cocoa butter-10 µl Palm oil-5 µl Groundnut oil-5 µl Olive oil-5 µl	No of bands – 11 Band length – 8mm Track distance – 16.4mm Distance from lower edge – 8mm Distance from left side edge – 15mm Application speed- Methanol (150 nl/s) Application volumes – ii. Sample: Cocoa butter-10 µl Palm oil-5 µl Groundnut oil-5 µl Olive oil-5 µl

Sample Application in ATS 4

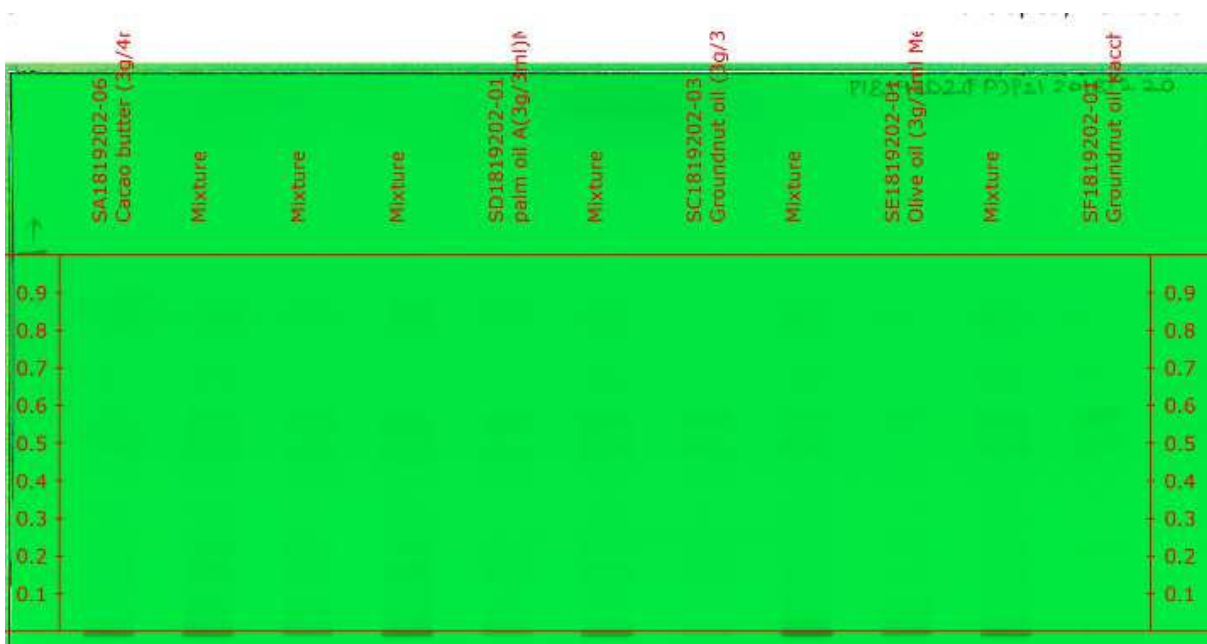
Track	Vial ID	Description	Volume	Position	Type
1	SA1819202-06	Cacao butter (3g/4ml) MeOH	10.0 µl	N/A	Sample
2	SA1819202-06	Cacao butter (3g/4ml) MeOH	10.0 µl	N/A	Sample
+	SB1819202-06	Palm oil (3g/3ml)MeOH	5.0 µl	N/A	Sample
3	SB1819202-06	Palm oil (3g/3ml)MeOH	5.0 µl	N/A	Sample
+	SE1819202-01	Olive oil (3g/3ml MeOH)	2.0 µl	N/A	Sample
4	SA1819202-06	Cacao butter (3g/4ml) MeOH	10.0 µl	N/A	Sample
+	SD1819202-01	palm oil A (3g/3ml)MeOH	5.0 µl	N/A	Sample
5	SD1819202-01	palm oil A(3g/3ml)MeOH	5.0 µl	N/A	Sample
6	SA1819202-06	Cacao butter (3g/4ml) MeOH	10.0 µl	N/A	Sample
+	SC1819202-03	Groundnut oil (3g/3ml MeOH)	5.0 µl	N/A	Sample
7	SC1819202-03	Groundnut oil (3g/3ml MeOH)	5.0 µl	N/A	Sample
8	SA1819202-06	Cacao butter (3g/4ml) MeOH	10.0 µl	N/A	Sample
+	SE1819202-01	Olive oil (3g/3ml MeOH)	5.0 µl	N/A	Sample
9	SE1819202-01	Olive oil (3g/3ml MeOH)	5.0 µl	N/A	Sample
10	SA1819202-06	Cacao butter (3g/4ml) MeOH	10.0 µl	N/A	Sample
+	SF1819202-01	Groundnut oil Kacchi ghani (3g/3ml MeOH)	5.0 µl	N/A	Sample
11	SF1819202-01	Groundnut oil Kacchi ghani (3g/3ml MeOH)	5.0 µl	N/A	Sample

3. **Chromatogram development:** If Auto development chamber is not available after sample application, keep the plate in a desiccator over saturated MgCl₂.6H₂O solution for 45 min, then quickly transfer to Twin trough chamber.

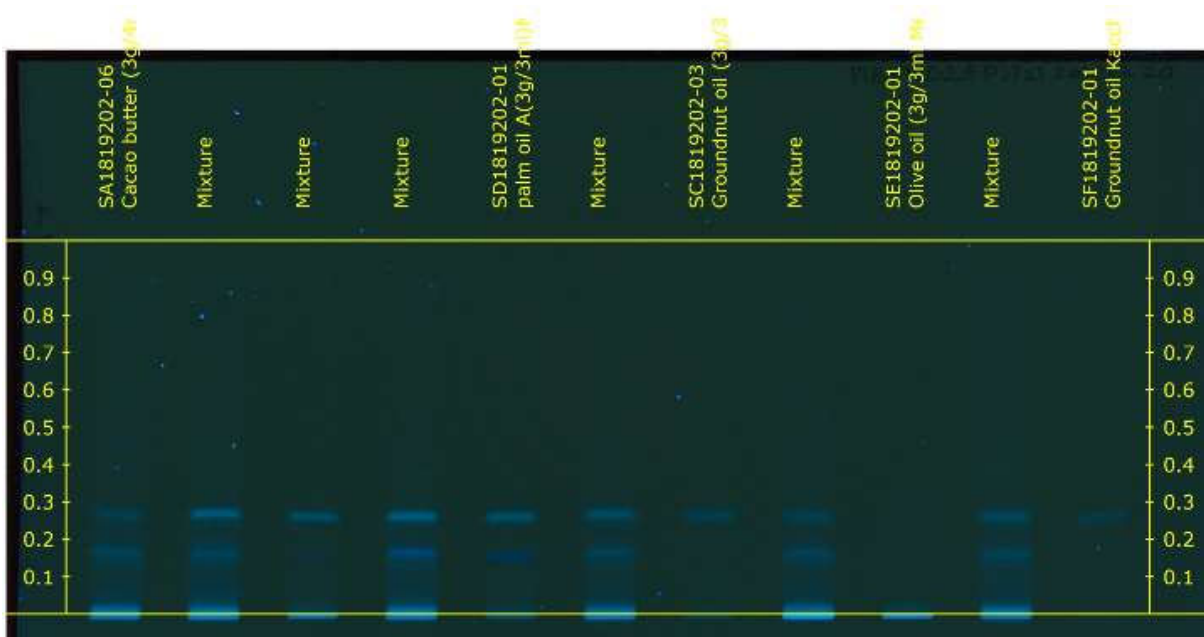
Using CAMAG Automatic Developing chamber 2	Using CAMAG Twin Trough Chamber 20x10 cm
Mobile phase saturation- 25ml MP for development - 10ml Paper lining – yes Layer preconditioning with 33% RH – Yes Development distance – 70mm	Mobile phase for saturation -5ml MP for development- 5ml Paper lining – yes After application, keep the plate in a desiccator over saturated MgCl ₂ .6H ₂ O solution for 45 min. Use Smart Alert to track the development of plate (Suitable for glass plates). Development distance – 70mm
Plate drying – 5 min	Plate drying – Hold plate vertically and the hair drier away for 10 mins. Use cool air only.

4. **CAMAG UV Cabinet – Inspect the plate under 366 nm for 30 seconds and then at 254nm to make sure that chromatography is performed well.**
5. **Image documentation by industrial camera with no image recording variables or user settings.**
Document images at

UV 254 nm

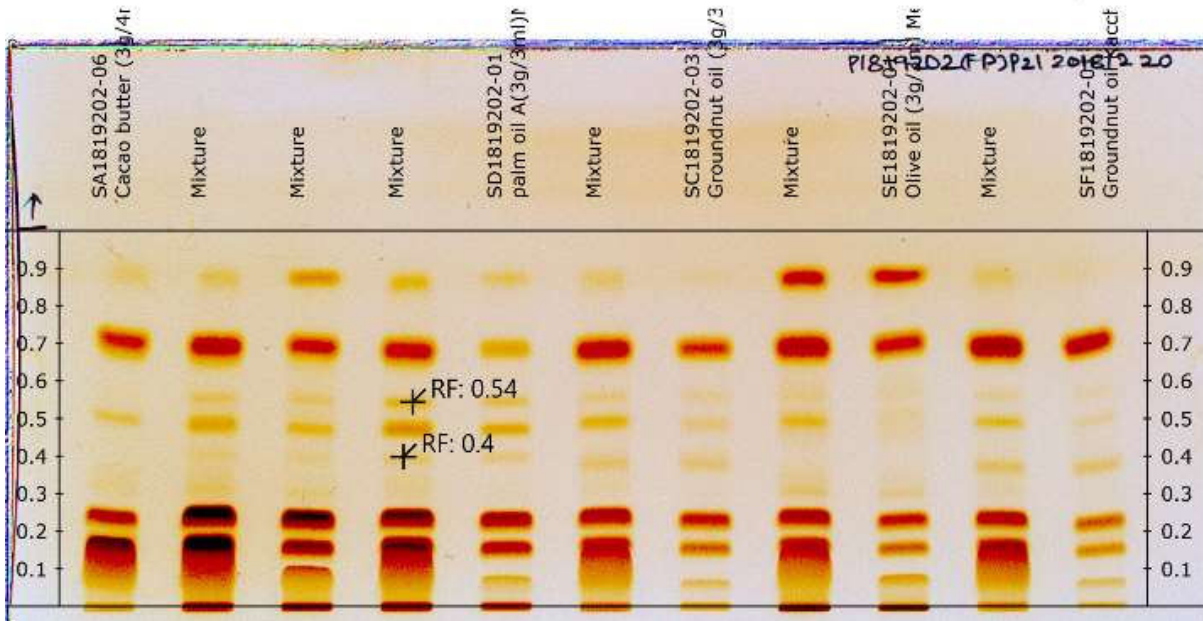


UV 366 nm



Derivatisation reagent: keep the plate in Iodine fumes for 5mins. Immediately document the image under white light.

White light, from top after derivatization with Iodine fumes



In the above image in Track 4, Palm oil is detected as it shows distinctive bands which are absent in cocoa butter. So, if cocoa butter is adulterated with Palm oil these bands can identify.

A method was established to check adulteration of cocoa butter with Palm oil, Olive oil and groundnut oil.

Analysis Done:
By Ankita Sarnath
On 24-12-2018

Approved:
By Dr. Saikat Mallick
On 24-12-2018