HPTLC Method

Detection of papaya seeds in black pepper samples by HPTLC.

Method Creator	Validated by	Final Authoriser	History	Date
Anchrom Enterprises (I) Pvt. Ltd. Mumbai.	NA			15-01-2019

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Objective: Detection of papaya seeds in black pepper samples.

Introduction: *Piper nigrum* (black pepper) is one of the most traded spice for its flavour and medicinal properties. As dried papaya seeds (*Carica papaya*) resemble black pepper, it is one of the major adulterants. They are often mixed in black pepper sample. Excessive consumption of papaya seeds may cause liver problems and stomach disorders. Therefore, HPTLC method was developed for identification of papaya seed adulteration in black pepper sample.

Method suitable for: Food Industry

Analysis method

Reagents and chemicals required: GR grade Methanol, Toluene, Chloroform and Ethanol.

TLC Al Si Gel 60 F_{254}, 20x10cm (Merck Catalogue No. 1.05554.0007). 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 $Mgcl_2.6H_2O$ saturated solution to keep 20x10 cm or 10x10cm sized plates.
Chromatogram Visualisation – Camag UV cabinet
Image documentation – Camag Visualiser
Scanning Densitometry – Camag Scanner 4

Standard Preparation: i) Piperine - 1mg/ml

ii) Caffeine (SST) – 1mg/ml

iii) Methyl paraben (SST)- 0.5mg/ml

Samples Preparation: Weigh 100mg powder in 1ml Methanol. Sonicate for 15 mins and centrifuge at 2500 rpm for 15 mins. Take the supernatant for analysis.

1. **CHROMATOGRAM LAYER:**

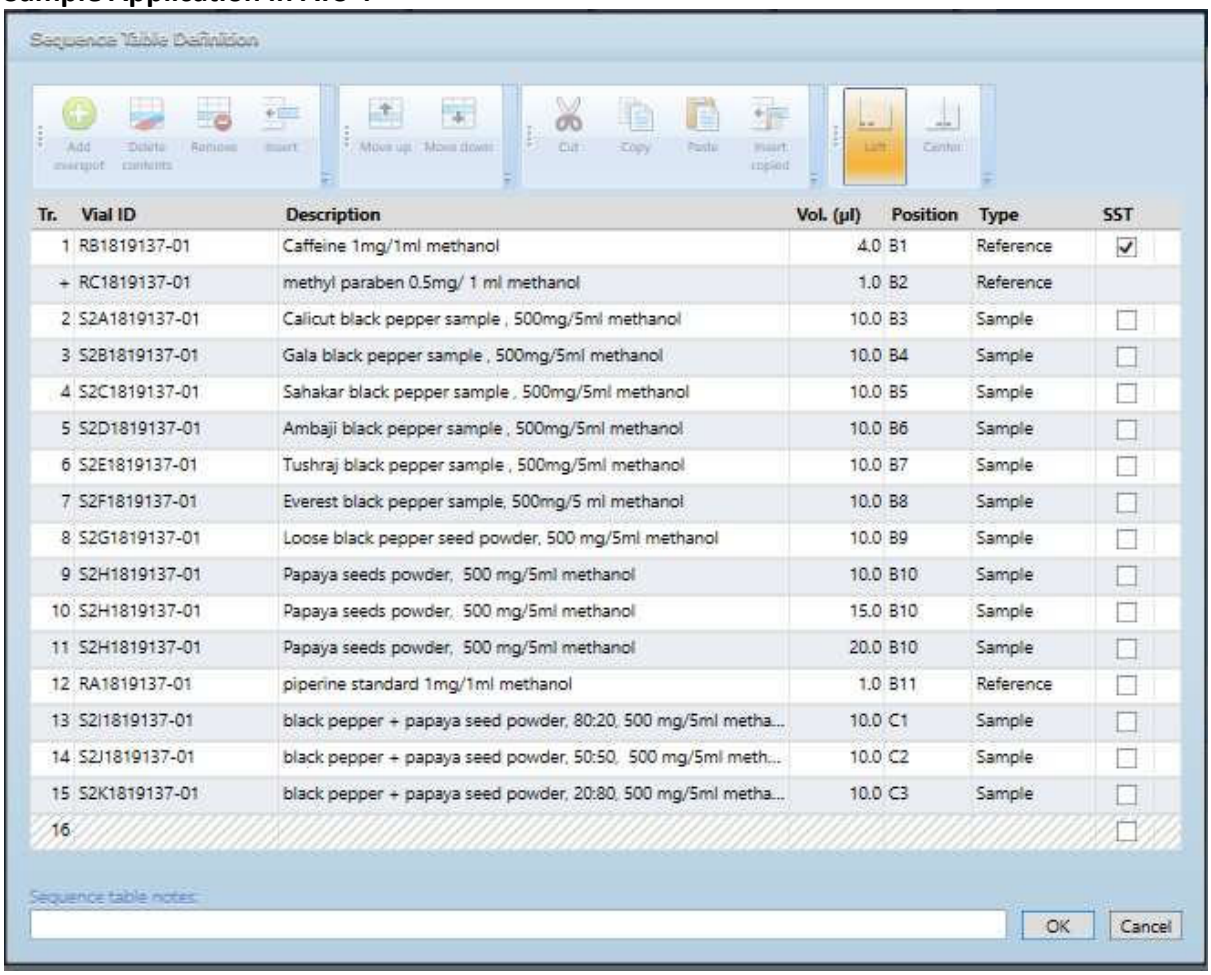
20x10 cm TLC Glass Silica gel $60F_{254}$ on plate (Merck 5554). If you have less than 10 tracks to apply (standard + sample), use a 10x10 cm silica gel $60F_{254}$ plate. Write ID on each plate. Mark with pencil at 70 mm, if no Automatic Development Chamber (ADC2) is available.

Mobile phase: Toluene: Chloroform: Ethanol (4:4.1) v/v/v

2. **Camag VisionCATS HPTLC software.**
   a) Switch on and select instruments that will be used (Linomat or ATS4 for sample application, ADC-2, if available, Camag Visualiser, Camag Scanner etc.
   b) Sample Applicator parameters:

Parameters for Linomat 5	**Parameters for ATS 4**
No of bands – 15 Band length – 8mm Track distance – 11.4mm Distance from lower edge – 8mm Distance from left side edge – 20mm Application speed- Methanol (150 nl/s) Application volumes – i. Standard: Piperine- 1 µl Caffeine (SST)- 4 µl Methyl paraben (SST)- 1 µl ii. Sample: 10 µl	No of bands – 15 Band length – 8mm Track distance – 11.4mm Distance from lower edge – 8mm Distance from left side edge – 20mm Application speed- Methanol (150 nl/s) Application volumes – i. Standard: Piperine- 1 µl Caffeine (SST)- 4 µl Methyl paraben (SST)- 1 µl ii. Sample: 10 µl

Sample Application in ATS 4



Tr.	Vial ID	Description	Vol. (µl)	Position	Type	SST
1	RB1819137-01	Caffeine 1mg/1ml methanol	4.0	B1	Reference	☑
+	RC1819137-01	methyl paraben 0.5mg/ 1 ml methanol	1.0	B2	Reference	
2	S2A1819137-01	Calicut black pepper sample , 500mg/5ml methanol	10.0	B3	Sample	☐
3	S2B1819137-01	Gala black pepper sample , 500mg/5ml methanol	10.0	B4	Sample	☐
4	S2C1819137-01	Sahakar black pepper sample , 500mg/5ml methanol	10.0	B5	Sample	☐
5	S2D1819137-01	Ambaji black pepper sample , 500mg/5ml methanol	10.0	B6	Sample	☐
6	S2E1819137-01	Tushraj black pepper sample , 500mg/5ml methanol	10.0	B7	Sample	☐
7	S2F1819137-01	Everest black pepper sample, 500mg/5 ml methanol	10.0	B8	Sample	☐
8	S2G1819137-01	Loose black pepper seed powder, 500 mg/5ml methanol	10.0	B9	Sample	☐
9	S2H1819137-01	Papaya seeds powder, 500 mg/5ml methanol	10.0	B10	Sample	☐
10	S2H1819137-01	Papaya seeds powder, 500 mg/5ml methanol	15.0	B10	Sample	☐
11	S2H1819137-01	Papaya seeds powder, 500 mg/5ml methanol	20.0	B10	Sample	☐
12	RA1819137-01	piperine standard 1mg/1ml methanol	1.0	B11	Reference	☐
13	S2I1819137-01	black pepper + papaya seed powder, 80:20, 500 mg/5ml metha...	10.0	C1	Sample	☐
14	S2J1819137-01	black pepper + papaya seed powder, 50:50, 500 mg/5ml meth...	10.0	C2	Sample	☐
15	S2K1819137-01	black pepper + papaya seed powder, 20:80, 500 mg/5ml metha...	10.0	C3	Sample	☐
16						☐

3. **Chromatogram development:** If Auto development chamber is not available after sample application, keep the plate in a desiccator over saturated $Mgcl_2.6H_2O$ solution for 45 min, then quickly transfer to Twin trough chamber.

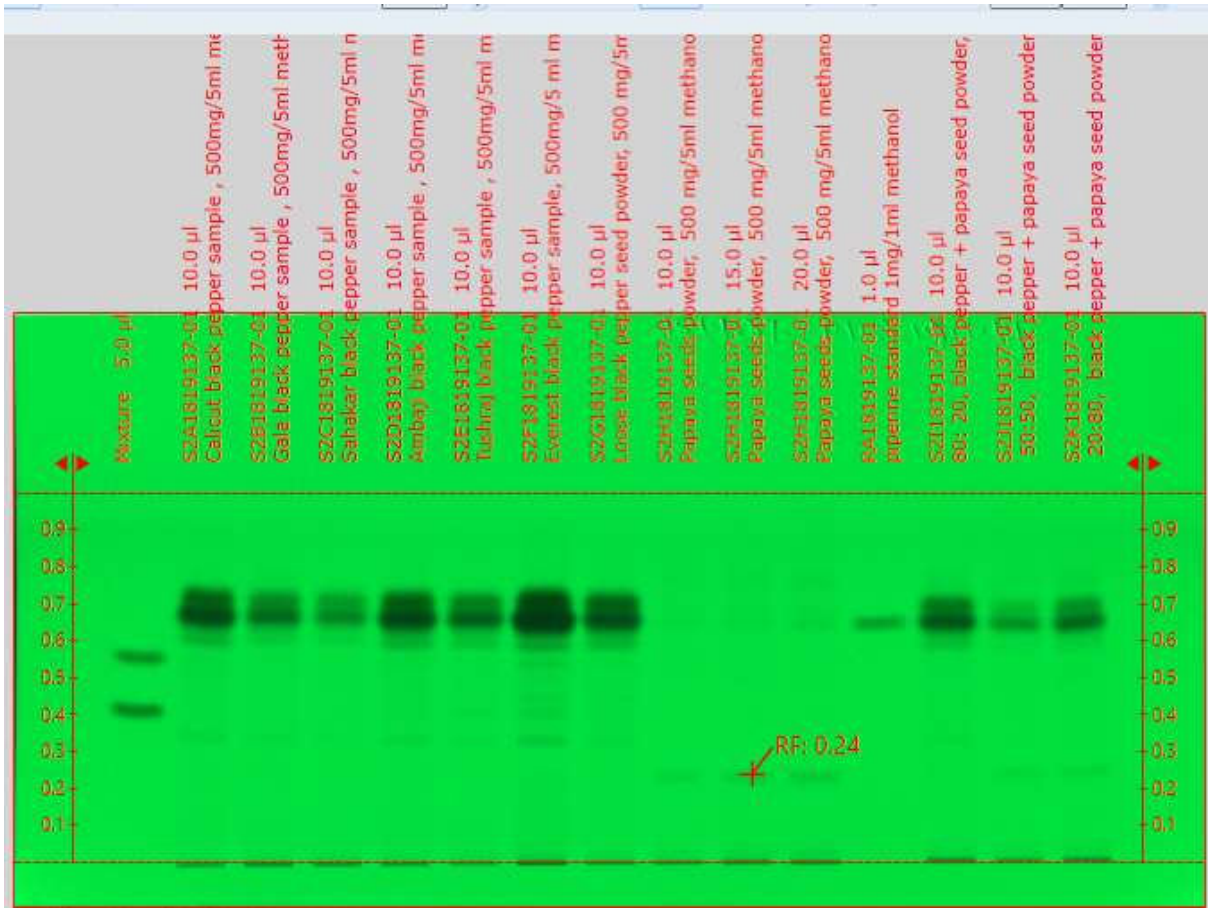
Using Camag Automatic Developing chamber 2	**Using Camag Twin Trough Chamber 20x10 cm**
Mobile phase saturation- 25ml MP for development - 10ml Paper lining – yes Layer preconditioning with 33% RH – Yes Development distance – 70mm	Mobile phase for saturation -10 ml MP for development- 10 ml Paper lining – yes After application, keep the plate in a desiccator over saturated $Mgcl_2.6H_2O$ 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.

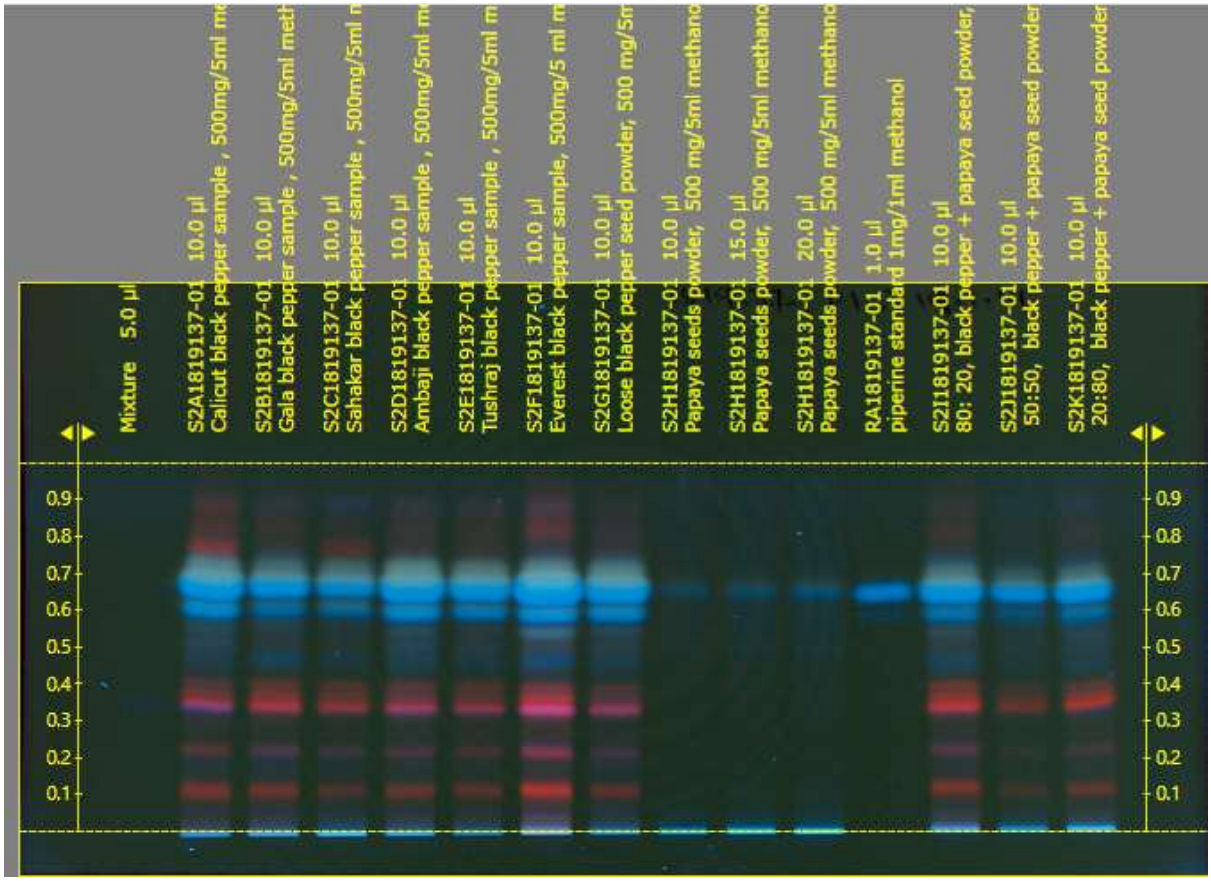
4. **Camag UV Cabinet – Inspect the plate under 366 nm for 30 seconds and then at 254nm to make sure that chromatography is performed well.**

5. **Image documentation by industrial camera with no image recording variables or user settings.**
   Document images at

UV 254 nm



UV 366 nm

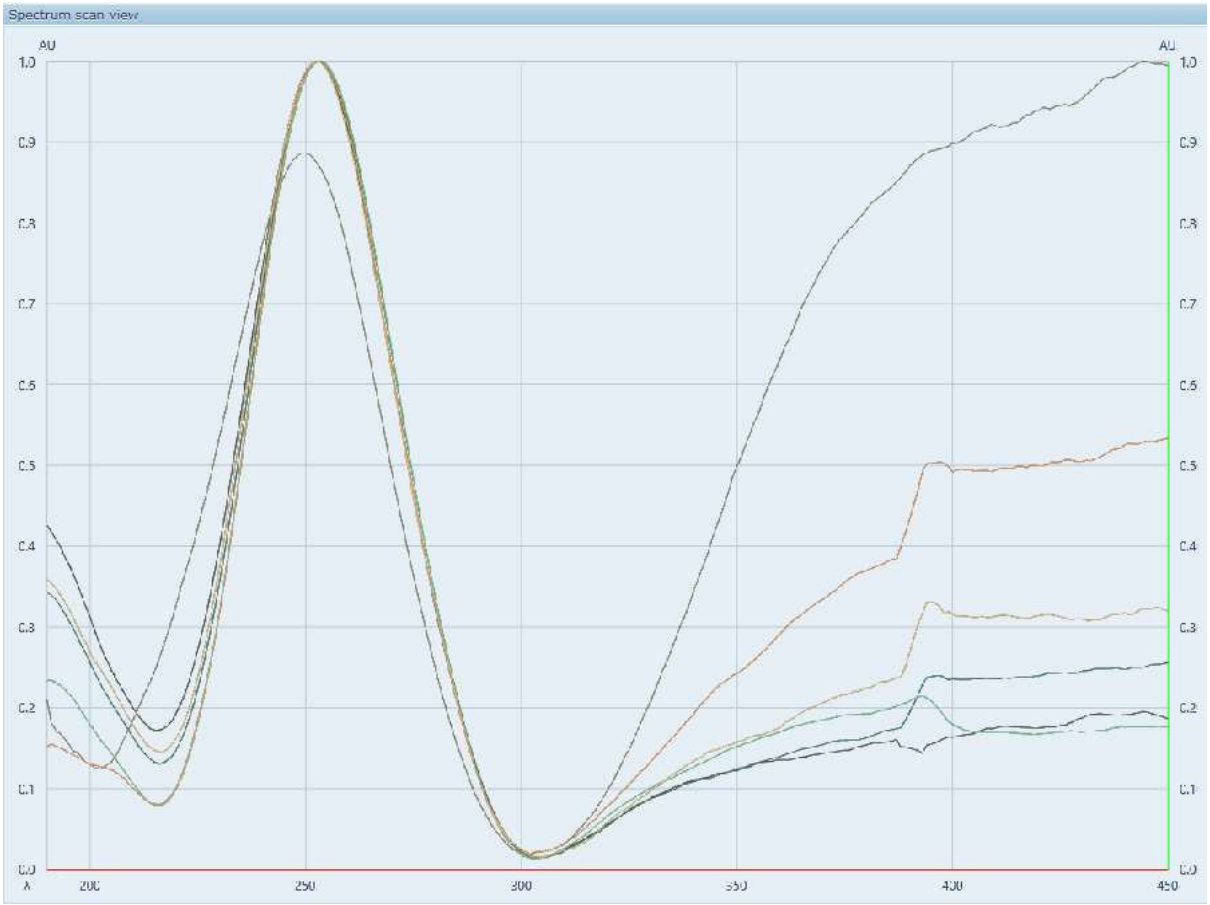


In the above image at 254nm, band at Rf 0.24 was observed in papaya sample only. Band at same Rf value was also observed in different blends in the ratios of 80:20, 50:50 and 20:80 of black pepper and papaya seed powder respectively. The band at Rf 0.24 was not observed in all market samples of black pepper. Hence, this fingerprint method can be used for detection of papaya seeds in black pepper samples.

6. **Scanning densitometry:**
   Scanner Model – Camag Scanner 4
   Scan Slit dimension: 6 x 0.45mm
   Scan speed – 20 mm/sec
   Spectrum recording UV (190-400 nm); Band Width -5nm (spectrum)

Scanning positions – Automatic
Scan wavelength – 254nm, 366nm

7. **Overlay of the papaya band at Rf 0.24 in all blended samples of black pepper and papaya seed (in the ratio of 80:20, 50:50 and 20:80)**



A method was established to detect papaya seeds in black pepper samples.
A HPTLC Fingerprint profile has been developed to detect presence of papaya seed in black pepper samples.

Results:

Acceptance Criteria	Sample ID	Value Found	Pass	Fail
Band of papaya observed at Rf 0.24	S2A1819137-01	NA	No	-
	S2B1819137-01		No	-
	S2C1819137-01		No	-
	S2D1819137-01		No	-
	S2E1819137-01		No	-
	S2F1819137-01		No	-
	S2G1819137-01		No	-

Analysis Done:
By Manjusha Phanse
On 15-01-2019

Approved:
By Dr. Saikat Mallick
On 15-01-2019