HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

PRINCIPLE, TECHNIQUES, APPLICATIONS

Dr. Kamran Javed Naquvi

CHROMATOGRAPHY AND ISOLATION TECHNIQUES

Course code: PHAR 333

Grade 3 Semester I

Lecture 5

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

- High-performance thin-layer Chromatography (HPTLC) is a chromatographic technique that is an advanced version of traditional thin-layer chromatography (TLC). It offers improved separation efficiency and precision.
- A number of enhancements can be made to the basic method of thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements.
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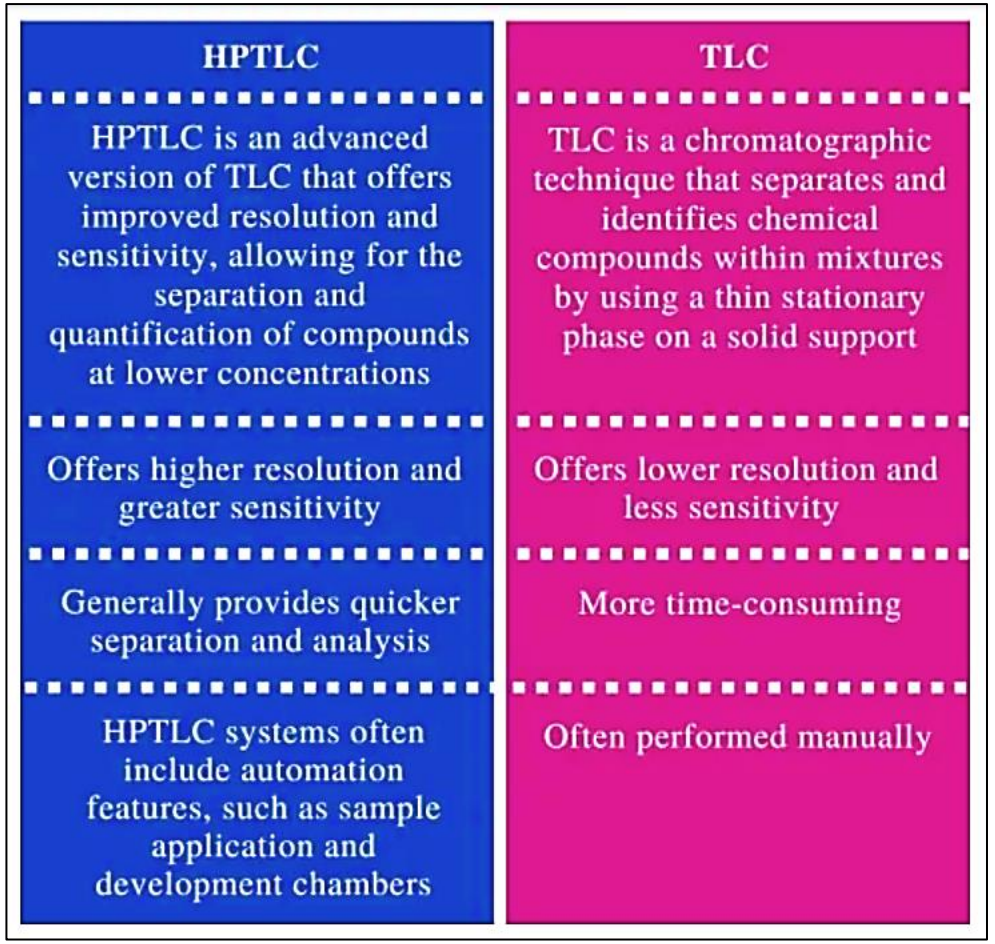
HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

- HPTLC (high-performance thin layer chromatography) is a sophisticated form of TLC, which provides superior separation efficiency.
- The HPTLC concept includes validated methods for qualitative and quantitative analysis, and fulfills all quality requirements for use in fully regulated environments.

Difference between HPTLC and TLC

- The main difference between HPTLC and TLC is that HPTLC offers **higher resolution** and **greater sensitivity** compared to TLC. In brief, HPTLC is an advanced form of TLC.



HPTLC	TLC
HPTLC is an advanced version of TLC that offers improved resolution and sensitivity, allowing for the separation and quantification of compounds at lower concentrations	TLC is a chromatographic technique that separates and identifies chemical compounds within mixtures by using a thin stationary phase on a solid support
Offers higher resolution and greater sensitivity	Offers lower resolution and less sensitivity
Generally provides quicker separation and analysis	More time-consuming
HPTLC systems often include automation features, such as sample application and development chambers	Often performed manually

Difference between HPTLC and TLC

Factors	TLC	HPTLC
Type of chromatographic plates	Handmade/Pre coated	Pre coated
Adsorbent layer	200-250mm	100-150mm
Particle size	10-12µm	5-6µm
Application of sample	5-20µm	4-8µm
Shape of sample	Manual/ Semi automatic	Semi automatic
Spot shape	Spot	Band
Sample volume	1-10µl	0.1-2µl
No of sample/plate	15-20	40-45
Optimal development distance	10-16cm	6-8cm
Time of Development	Decided by mobile phase	40% less than TLC
Quantization	Manual	Manual/Instrumentation
Reproducibility of result	Difficult	Reproducible

HPTLC Benefits:

The following are some of the benefits of HPTLC.

- Quicker analysis, only 3 to 20 min for finest separation.
- 5 -10 times superior detection sensitivity than TLC.
- Extremely reproducible, sharp bands for quantitative analysis.
- In HPTLC, processing the sample and norm at the same time under identical conditions improves analytical precision and accuracy.
- HPTLC permits the use of mobile phases with corrosive and UV absorption.
- HPTLC is a simple procedure to learn, and the instrument is simple to use.

Principle

- Principles of HPTLC have similar approaches and employ the same physical principles of TLC (**adsorption chromatography**) i.e. the principle of separation is **adsorption**.
- The mobile phase solvent flows through because of **capillary action**.
- The components move according to their affinities towards the adsorbent.
- The component with **more affinity** towards the stationary phase travels **slower**.
- The component **with a lesser affinity** towards the stationary phase travels **faster**. Thus, the components are separated on a chromatographic plate.

Steps in HPTLC

Sample and standard preparation

Banding was performed using an automated TLC applicator

Selection of chromatic layer

Layers pre washing

Layer pre conditioning

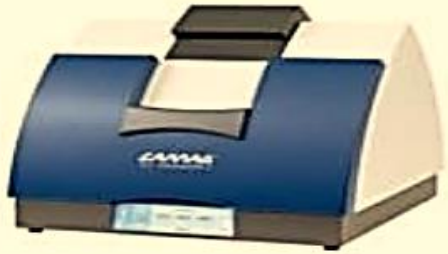
application of sample and standard

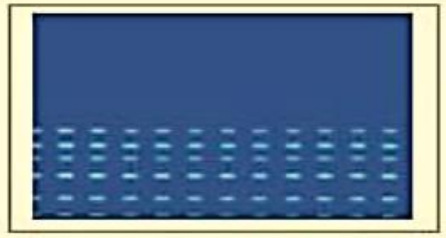
Twin trough

Chromatographic development

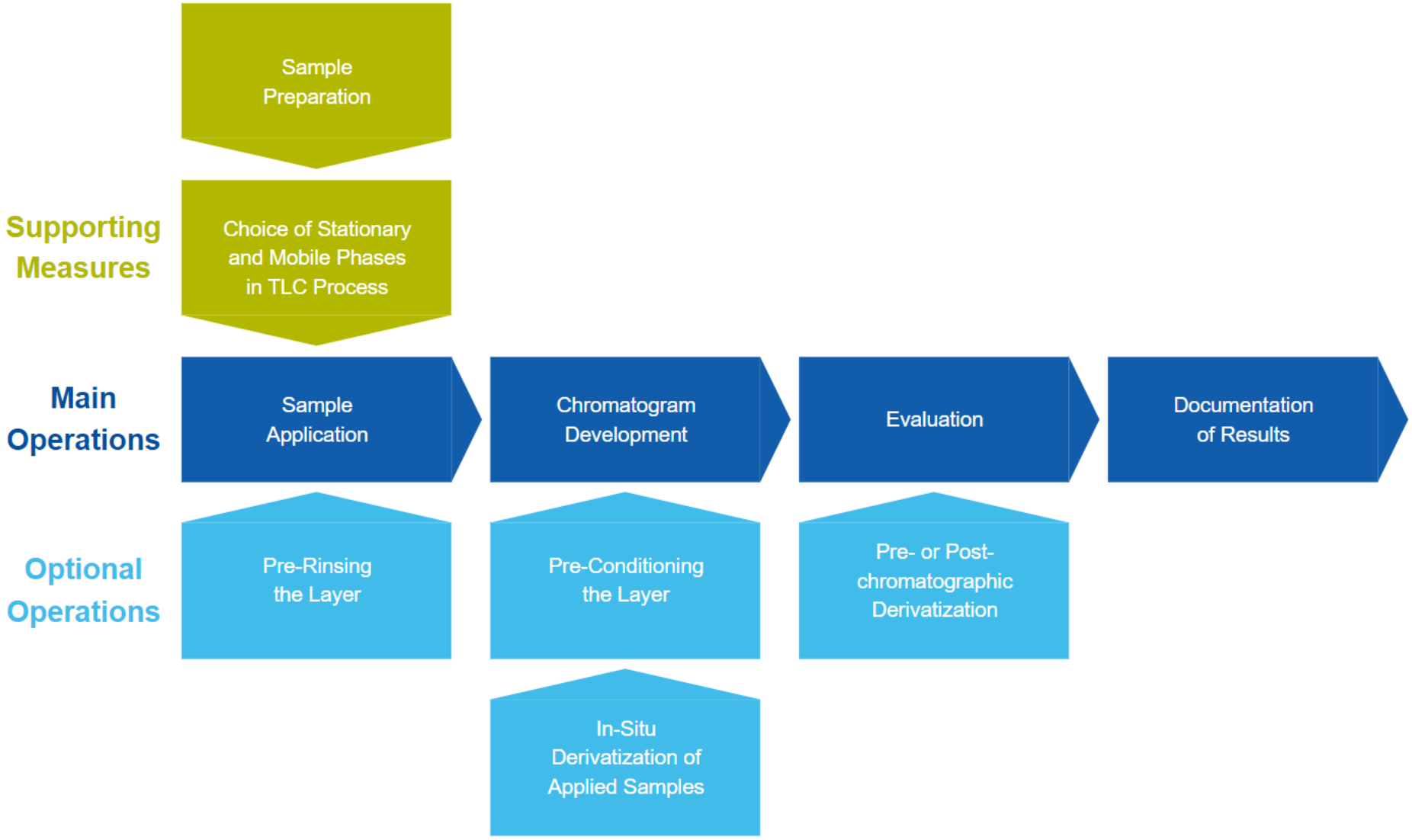
Detection of spots,

Scanning and documentation

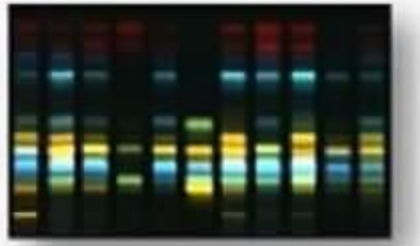
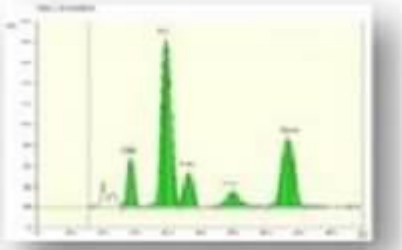
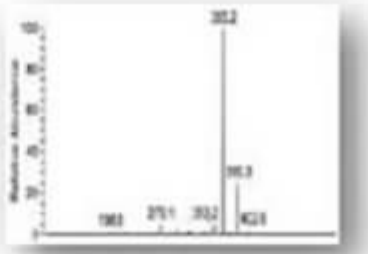
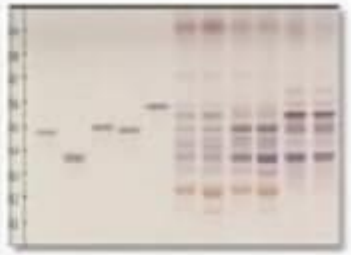




Process


Sample Preparation
Supporting Measures
Choice of Stationary and Mobile Phases in TLC Process
Main Operations
Sample Application
Chromatogram Development
Evaluation
Documentation of Results
Optional Operations
Pre-Rinsing the Layer
Pre-Conditioning the Layer
Pre- or Post-chromatographic Derivatization
In-Situ Derivatization of Applied Samples

Instrumentation


Applicator


Developer


Visualizer


Scanner


MS Interface


Derivatizer


visionCATS Software

HPTLC Plates for Quantitative Analysis

HPTLC plates (from Merck) are the perfect choice for quantitative separation using instrumental HPTLC. The optimized, smaller particles enable significantly higher speed, more efficiency, and better sensitivity than classical TLC plates. HPTLC plates are available with glass or aluminum backing in a variety of formats to suit many separation needs.

The fluorescent indicators used are **green fluorescent F254** or **blue fluorescent acid-stable F254s**. Both indicators fluoresce in UV light at an excitation wavelength of 254 nm.

- Classical Silica Plates
- HPTLC Premium Purity Plate
- LiChrospher HPTLC Plates with Spherical Particles
- RP-modified Silica Plates
- CN-, Diol-, NH2-modified Silica Plates (TLC and HPTLC)
- Cellulose Plates
- HPTLC-MS grade Plates

Mobile Phase

Volatile oil :

Toluene : Ethyl acetate : Formic acid, 93:7:0.5

Reserpine and Ajmalicine :

Toluene: Ethyl acetate: Formic acid (7:2:1)

Atropine, and Piperine :

Toluene-Ethyl acetate-Diethyl amine (7:2:1, v/v)

Development chambers

This is a classical developing tank for Thin-Layer Chromatography. Compared to a Flat Bottom Chamber less solvent is needed. A Twin-Trough-Chamber reduces disposal problems and allows preconditioning of the plate with any solvent and for any duration. For preconditioning or saturation the trough opposite to the plate is filled with developing solvent. The use of a saturation pad is recommended for a fast, homogeneous and reproducible chamber saturation process.

What is HPTLC Fingerprinting?

- HPTLC Fingerprinting is the representation of the phytochemical composition of a plant extract or formulation, in the form of an image at 254nm, 366nm, and white light. It is a sequence of peaks or zones of a chromatogram specific to a sample.
- HPTLC fingerprint is evaluated based on Rf, color, and relative intensity of bands in the electronic image.
- **Phytochemical reference standards (PRS)** are also applied along with samples. Respective plant samples/ raw materials collected from different geographical locations/ climatic conditions can be applied to check the variation in species.

- While developing an HPTLC fingerprint for certain plant materials aim should be to develop a fingerprint that shows the maximum no. of phytoconstituents with good resolution.

HPTLC for Herbal Chromatographic Fingerprinting

- The HPTLC technique can be used for herbal fingerprinting. Each plant has a different fingerprint pattern. When the plant extract is run in a suitable mobile phase, the separation pattern observed is the herbal fingerprint of the said plant.
- HPTLC is a preliminary step to **identify** phytochemical compounds and secondary metabolites of a plant.
- Fingerprints play an important role in the **quality control** of herbal medicine. It can be used for the **authentication** of raw material or the **detection** of adulterant material/ substitutes.
- Herbal Fingerprinting is the technique used for the **qualitative and quantitative** analysis of herbal components in drugs.
- ***Chromatographic fingerprinting is used for both establishing the identity and quality of the herbs being added into the formulation.***

HPTLC Fingerprinting of Volatile oils

Mobile phase: 93:7 Toluene and Ethyl Acetate

After spraying with 0.5% Anisaldehyde sulphuric acid agent

DAY LIGHT

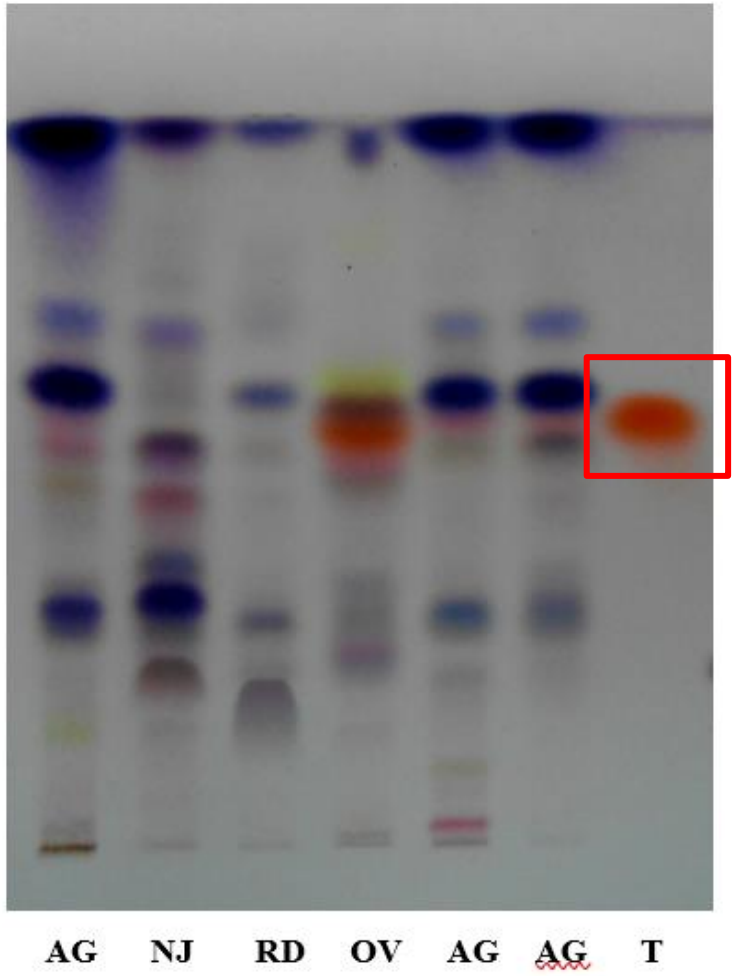
NJ: *Nardostachys jatamansi*;

RD: *Rosa damascena*;

AP: *Apium graveolens*;

TA: *Trachyspermum ammi*,

T: Tymol **(Standard)**

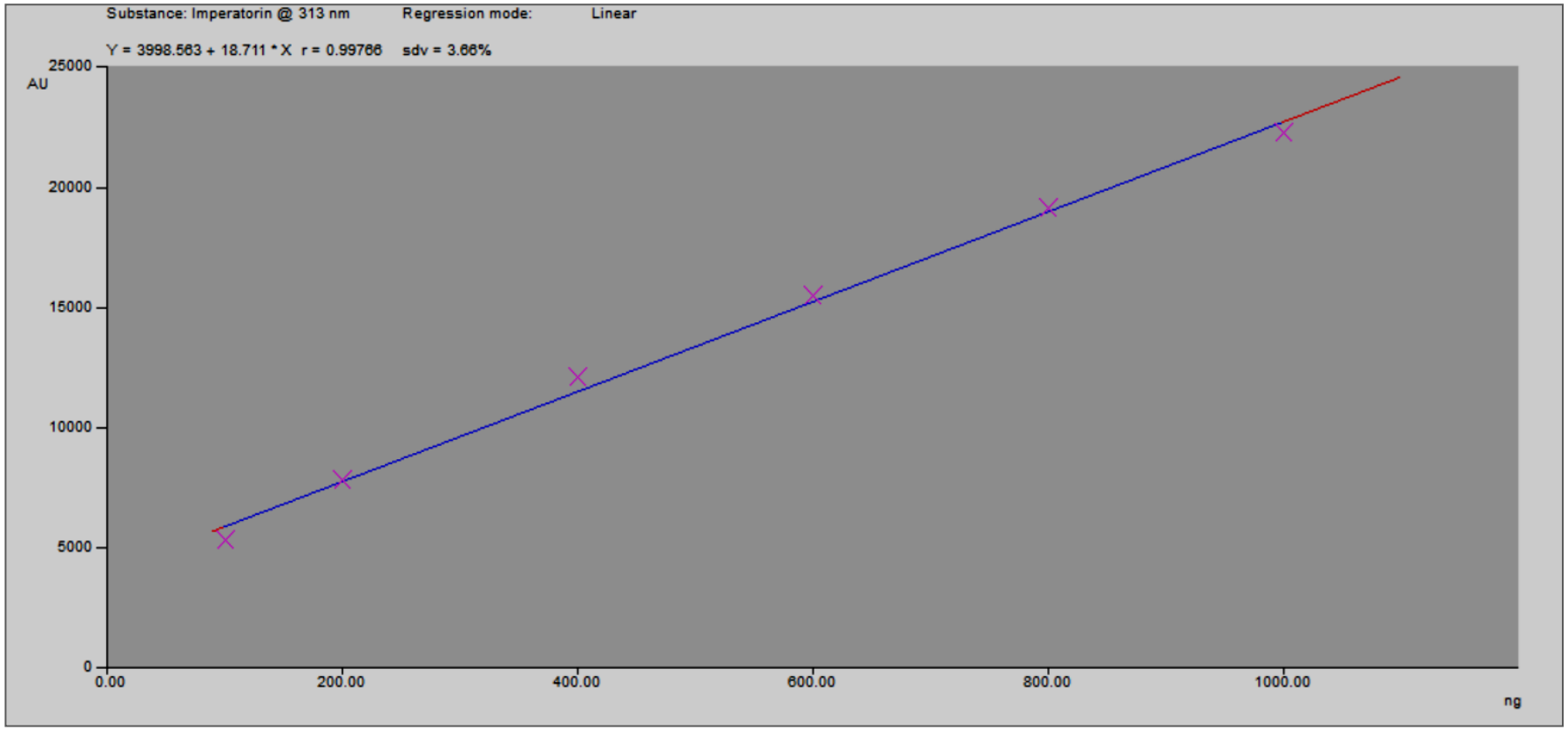


HPTLC fingerprinting analysis of volatile oils

HPTLC Quantification

- It is widely used to quantify the phytoconstituents present in a herbal drug.
- The main phytoconstituents of the plant are known as the marker compound of that plant.
- It is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability.

Calibration curve



The calibration curve area versus concentration (ng/spot) was found to be linear in the range of 100-1000 ng/spot. The linear regression data for the calibration curve (Figure) showed a good linear relationship over the concentration ranges of 100-1000 ng/spot..

HPTLC Quantification

HPTLC chromatogram of imperatorin in standard, formulation and different drugs.

Track 1-6: Imperatorin (Standard);

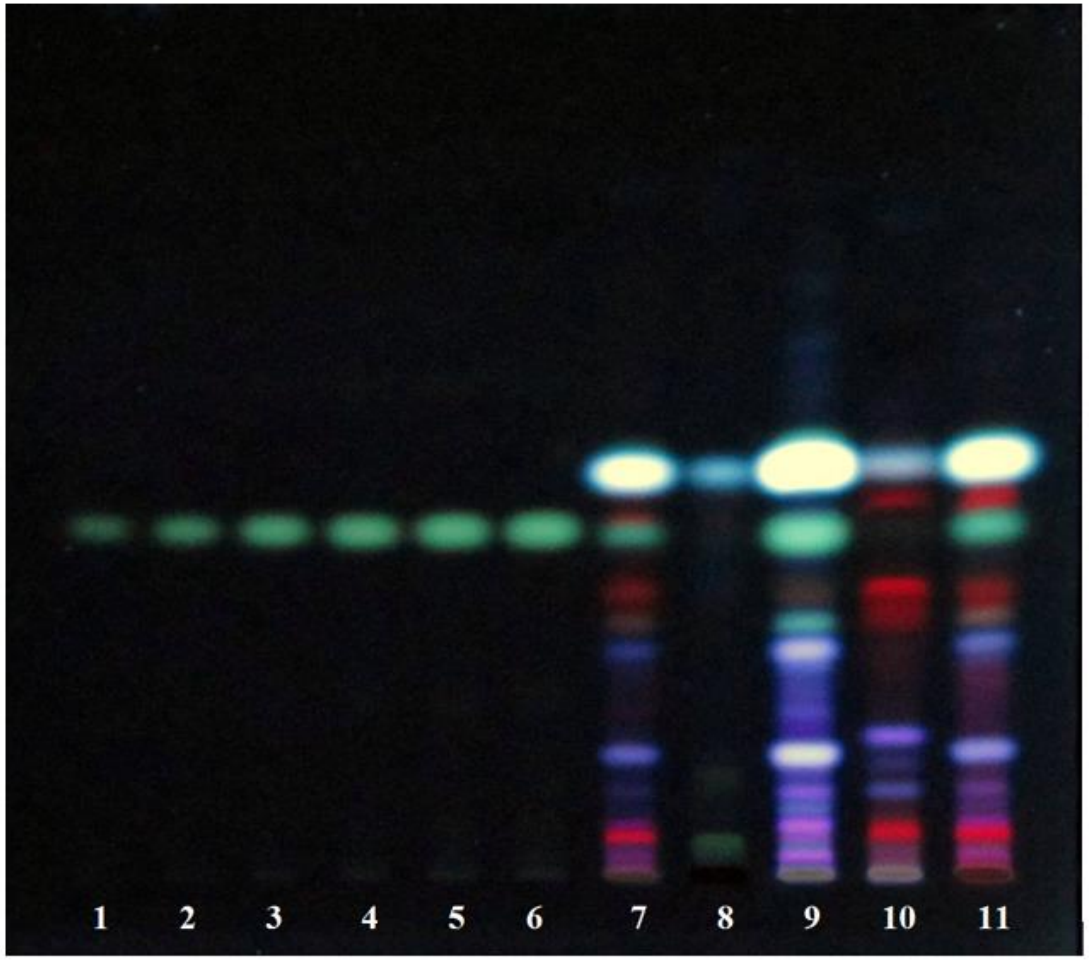
Track 7: *Nardostachys jatamansi*;

Track 8: *Rosa damascena*;

Track 9: *Apium graveolens*;

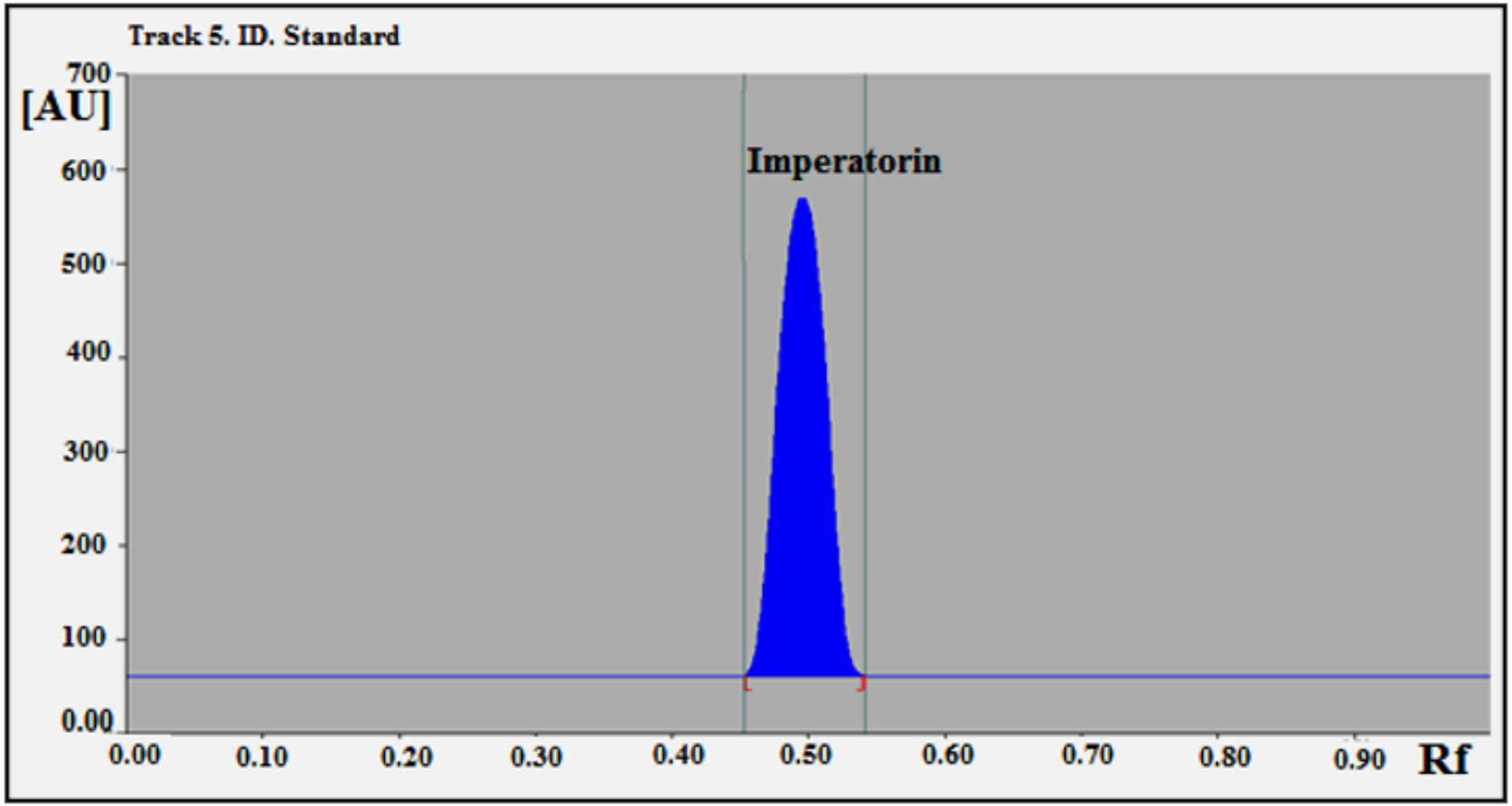
Track 10: *Trachyspermum ammi*,

Track 11: *Safoof-e-Muhazzil*.



Kamran J. Naquvi, ***Asian Journal of Traditional Medicines***, 2014, 9(2): 34-44.

HPTLC Quantification



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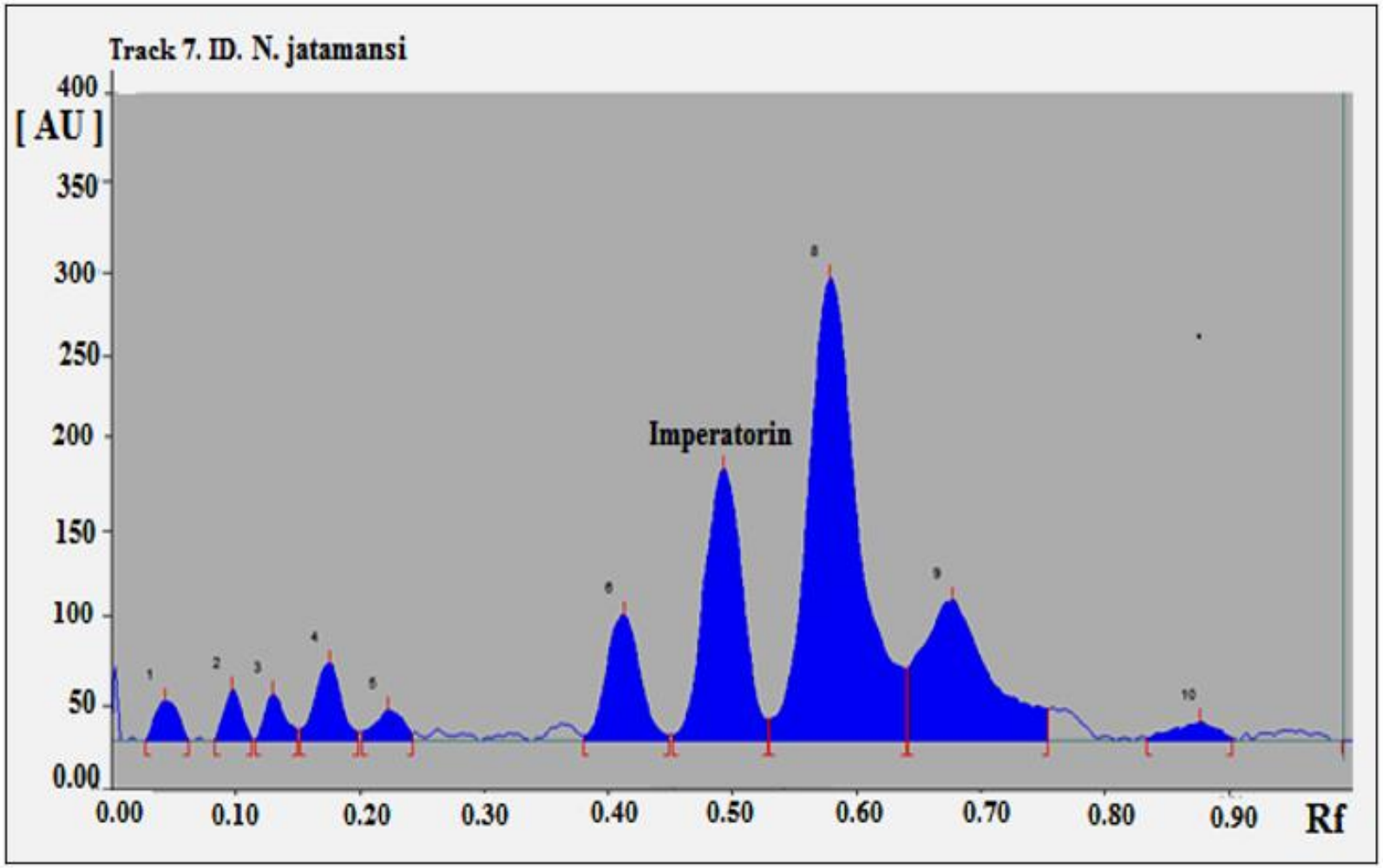
HPTLC Quantification



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HPTLC Quantification

Test samples	Imperatorin content (Mean ± SD, % *w/w*)
Nardostachys jatamansi DC.	1.58 ± 0.03
Rosa damascena Mill.	0.44 ± 0.03
Apium graveolens L.	3.05 ± 0.02
Trachyspermum ammi L.	1.23 ± 0.01
Safoof-e-Muhazzil	2.16 ± 0.02

Kamran J. Naquvi, ***Asian Journal of Traditional Medicines***, 2014, 9(2): 34-44.

Applications of HPTLC

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◦ **Pharmaceutical Industry:**

- **Quality Control:** HPTLC is extensively used for quality control and analysis of pharmaceuticals. It helps in separating and quantifying active ingredients, impurities, and degradation products in drugs.
- **Stability Studies:** HPTLC can be employed to assess the stability of pharmaceutical formulations over time, aiding in determining shelf life and storage conditions.

◦ **Food and Beverage Industry:**

- **Quality Assurance:** HPTLC is used for analyzing food additives, preservatives, pesticides, and contaminants in food and beverages, ensuring compliance with regulatory standards.
- **Authenticity Testing:** It can be applied to verify the authenticity and origin of food products, including the identification of adulterants.

Applications of HPTLC ii

- **Environmental Analysis:**

  Pollutant Detection: HPTLC is employed to analyze environmental samples for the presence of pollutants, such as pesticides, herbicides, and other organic and inorganic contaminants.

  Water Quality Monitoring: It can be used for the assessment of water quality by detecting and quantifying pollutants in water samples.

- **Botanical and Herbal Analysis:**

  Herbal Medicine Quality Control: HPTLC is utilized for the quality control of herbal medicines, helping to identify and quantify active compounds and ensure product consistency.

  Plant Extract Analysis: It can be used to analyze plant extracts for the presence of specific compounds, facilitating the study of phytochemical profiles.

Applications of HPTLC iii

- **Cosmetics and Personal Care Products:**

Ingredient Analysis: HPTLC is employed to analyze the composition of cosmetic and personal care products, ensuring that they meet regulatory requirements and do not contain harmful substances.

- **Forensic Science:**

Drug Analysis: HPTLC is used in forensic laboratories for the analysis of drugs and controlled substances, aiding in criminal investigations.

Toxicology Studies: It can be applied to analyze biological samples for the presence of toxins and drugs in forensic toxicology.

Applications of HPTLC iv

- **Research and Development:**

  Chemical Research: HPTLC is used in chemical research for the separation and identification of compounds in various samples.

  Method Development: It is employed to develop and optimize chromatographic methods for specific compounds of interest.

- **Clinical Analysis:**

  Biochemical Analysis: HPTLC can be used for the separation and quantification of biomolecules in clinical samples, supporting diagnostic and research efforts.

References

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- **Kamran Javed Naquvi***, S.H. Ansari, A.W. Siddiqui, Afrin Salma, Javed Ahamad. (2020) HPTLC quantification of thymol in different extracts and volatile oil of *Safoof-E-Muhazzil*. ***J Global Trends Pharm Sci***, 2020; 11 (2): 77481-7487.
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- https://youtu.be/VUNY0uTiXdo?si=v7K0XhNG6KGyGVgm
- https://youtu.be/95vx4RRZlmE?si=3MY47j6RQ3IhlDNY

Thank You