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HPTLC method for estimation of reserpine, gallic acid, quercetin and ascorbic acid in cardostab tablet and crude drugs

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Abstract
High-Performance Thin-Layer Chromatography (HPTLC) has emerged as a robust analytical technique for the quantification of bioactive compounds in herbal medicines. In this study, we propose an HPTLC method for the estimation of Reserpine, Gallic Acid, Quercetin, and Ascorbic Acid in Cardostab tablet and crude drugs. These compounds are of significant therapeutic importance due to their antioxidant, cardioprotective, and neuroprotective properties. The developed HPTLC method provides a rapid, cost-effective, and reliable means for the quantitative analysis of Reserpine, Gallic Acid, Quercetin, and Ascorbic Acid in Cardostab tablet and crude drugs. This method holds promise for quality control, batch-to-batch consistency, and regulatory compliance in the manufacturing and research of herbal medicines. Its application can contribute to the advancement of herbal medicine analysis and ensure the safety and efficacy of herbal products for healthcare applications.

Keywords: HPTLC, gallic, acid, manufacturing, healthcare

Introduction
High-Performance Thin-Layer Chromatography (HPTLC) has emerged as a powerful analytical technique for the qualitative and quantitative analysis of complex mixtures of bioactive compounds present in herbal medicines. In the context of the present study, HPTLC method is proposed for the estimation of Reserpine, Gallic Acid, Quercetin, and Ascorbic Acid in Cardostab tablet and crude drugs. This introduction provides an overview of the significance of these compounds, the rationale for their estimation, and the importance of employing HPTLC in herbal medicine research and quality control.

Cardostab tablet is a polyherbal formulation containing extracts of Rauwolfia serpentina, Terminalia chebula, Emblica officinalis, and Punica granatum, among other ingredients. These herbs are traditionally used for their cardioprotective, hypotensive, and antioxidant properties. Estimation of Reserpine, Gallic Acid, Quercetin, and Ascorbic Acid in Cardostab tablet and crude drugs is essential to ensure batch-to-batch consistency, quality control, and therapeutic efficacy.

HPTLC offers several advantages for the analysis of bioactive compounds in herbal medicines, including high sensitivity, reproducibility, and cost-effectiveness. It allows for the simultaneous separation and quantification of multiple analytes in a single chromatographic run, making it well-suited for the analysis of complex herbal matrices. Moreover, HPTLC methods can be easily standardized, validated, and implemented for routine quality control testing in herbal medicine manufacturing and research settings.

Instrumentation
High-Performance Thin Layer Chromatography, 60F254 precoated silica gel (HPTLC) aluminium plates from E. Merck, and a Linomat V sample applicator with a 100 µL applicator syringe. A Revolutionary New TLC UV Cabinet. Durable stainless steel In the Camag TLC scanner IV, Twin-trough Chambers that measured 20 cm × 10 cm were used, which was controlled by the WINCATS software and operated in reflectance absorbance mode. The following items are needed: a 100 µL applicator syringe from Camag in Switzerland, a semiautomatic sample applicator called Linomat V also from Camag in Switzerland, a UV-Visible spectrophotometer with a wavelength accuracy of ± 0.5 nm made by Shimadzu in Kyoto, Japan, and two adjacent quartz cells measuring 10 mm in diameter and 2 mm in spectral width.

Reagents and Materials

Youccha Enterprises of Mumbai supplied the ascorbic acid, gallic acid, quercetin, and resperpine, all of which were analytically pure (≥98%). Toluene, ethyl acetate, glacial acetic acid, AR-grade methanol, and Whatman filter paper no. 41.

Chromatographic Condition

Pre-treatment of HPTLC plates

A twin-trough glass chamber was used to store the HPTLC plates, and methanol was used as the mobile phase. The rising process included letting the methanol rise to the top of the plate. Before being used for the experiment, the plates were taken out of the oven and left to dry at 110 ºC for 5 minutes.

Chromatographic separation

Chromographic separation was accomplished on HPTLC plates using a mobile phase that consisted of toluene, ethyl acetate, methanol, and glacial acetic acid in a proportion of 7:2:1:0.3 v/v. The samples were applied to the plates in 6 mm bands using the Linomat V applicator, which is equipped with a 100 µL syringe. There was a 14 mm gap between each band, an 8 mm distance from the plate's edge, and a 15 mm distance from the plate's base. A flow rate of five microliters per second was used for all samples. The plates were polished using a 20 cm x 10 cm Camag twin-trough chamber that had been paper-lined and soaked with mobile phase vapour for 30 minutes at room temperature (25±2 ºC). The spacing between the plates for development was eight centimetres. After removing the plates from the chamber, they were dried using hot air. A combination of the TLC Scanner III and the winCATS programme was used to scan the plates at four different wavelengths: 254 nm, 261 nm, 240 nm, and 268 nm. The slit size was 6.00 x 0.45 mm, and the scanning speed was 100 nm/s. The radiation came from a deuterium lamp that continually released ultraviolet light between 190 and 360 nanometers in wavelength.

Solution preparation

Preparation of the Mobile Phase

An equal volume of toluene, ethyl acetate, methanol, and glacial acetic acid (0.3 mL) was combined to make the mobile phase. The mobile phase was immersed in a covered twin-trough chamber for 30 minutes before use.

Preparation of Standard Solution

The usual components A stock solution with a concentration of around 1000 micrograms per millilitre was prepared by dissolving ascorbic acid, gallic acid, quercetin, and resperpine in 10 millilitres of methanol in separate 10 millilitre volumetric flasks. A concentration of 10 µg/mL may be achieved, the original solution of each phytoconstituent was pipetted out and 10 mL of methanol was added.

Preparation of Sample Solution

Emblica officinalis, Terminalia bellerica, Rauwolfia serpentina, and Terminalia chebula all have 62.5 milligrams each in a single pill. In separate 10 ml volumetric flasks, A stock solution with a concentration of about 1000 µg/mL was prepared by dissolving 10 mg of standard Ascorbic acid, Gallic acid, Quercetin, and Reserpine in 10 ml of methanol. The concentration of each phytoconstituent was brought down to 10 µg/mL by combining 10 mL of methanol with 1 mL of the original solution, which was pipetted out. The mixture was subjected to a water bath heating at 50 °C for 20 minutes after the dry residue and 30 cc of methanol were added. A 0.45µ filter was used to filter the solution in order to get the sample of the working solution.

Data Analysis

The formulation's methanolic extract was made, and 10µL of the extract was added to the TLC plate. The ascorbic acid, gallic acid, quercetin, and reserpine concentrations of the applied plate were determined using the provided technique. In order to determine if Ascorbic acid, Gallic acid, Quercetin, or Reserpine are present, a methanolic extract of the crude extract was made for the powders of Amala, Vibhitak, and Rauwolfia serpentina root. The quantity of ascorbic acid, gallic acid, quercetin, and reserpine was determined by running each analysis five times using the regression equation.

Table 1: Chromatographic condition

Stationary phase	**Pre-coated Silica gel 60F $_{254}$ plate: E. Merck**
Mobile phase	Toluene: Ethyl acetate: Methanol: glacial acetic acid (7: 2: 1: 0.3v/v/v/v)
Saturation time	20 min
Migration Distance	8 em
Temperature	Room Temperature
Band length	6 mm
Spotting volume	lOpl
Scanning wavelength	254 mm
Scanning speed	200 mm/sec
Slit size	6 X 0.45 nun
Scanner	Camas scanner IV

Mobile Phase optimization

Table 2: Experiments to improve the mobile phase

Sr. No.	Mobile Phase Composition	Inference	Conclusion
1.	Toluene: Ethyl acetate:Methanol: Formic acid (4: 3: 2:1 v/v/v)	Spots run withsolvent front	Not satisfactory
2.	Toluene: Ethyl acetate:Methanol: Formic acid(42, 4 • 1 • 1 vlv)	Spot of Gallic acid, Quercetin and Reserpine was observed but with poor resolution	Not satisfactory
3.	Toluene: Ethyl acetate: Methanol: Formic acid (5: 1: 1 v/v)	Spots run withsolvent front	Not satisfactory
4.	Toluene: Ethyl acetate: Methanol: Glacial acetic acid (42, 5: 1: 1 v/v)	Spot run with solvent front	Not satisfactory
5.	Ethyl acetate: Methanol: Glacial acetic acid (5._:. 6: 1 v/v/v)	Spots of Reserpine was not observed	Not satisfactory
6.	Toluene: Ethyl acetate: Methanol: Glacial acetic acid(72. 2: 1: 0.3 v/v)	Spot of Ascorbic acid, Reserpine, Gallic acid andQuercetinwas observed with good resolution and reproducibility at RF= 0.2, 0.52, 0.75 and 0.47, respectively	Satisfactory

Chromatographic separation of Ascorbic acid, Reserpine, Gallic acid and Quercetin in standard, formulation and crude extract

Optimized Mobile phase – Toluene: Ethyl acetate: Methanol: Glacial acetic acid (7:2:1:0.3 v/v)

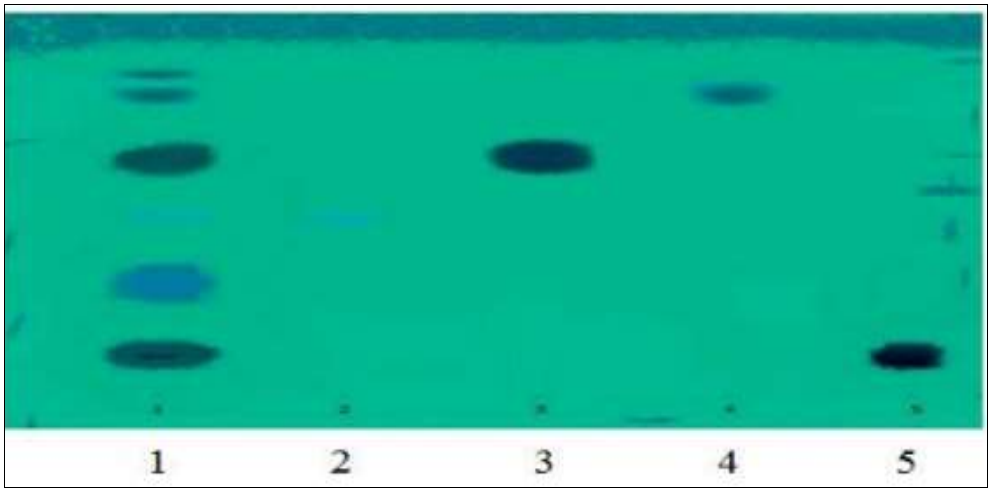

Fig 1: HPTLC plate demonstrating formulation and standard concentrations of Reserpine, Ascorbic acid, Quercetin, and Gallic acid

Methanolic extract of formulation.
Reserpine.
Ascorbic acid.
Quercetin.
Gallic acid.

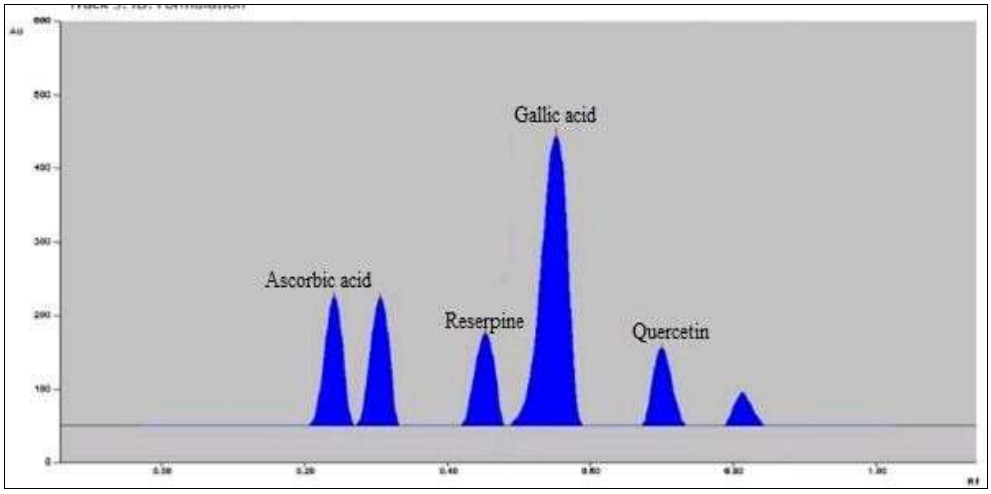

Fig 2: HPTLC Chromatogram of formulation

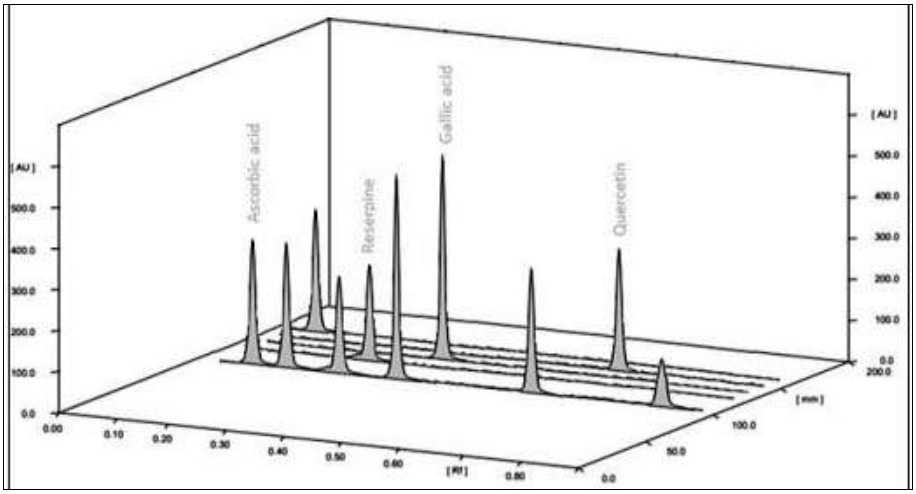

Fig 3: 3D overlay HPTLC Chromatogram of standard and formulation

Ascorbic acid

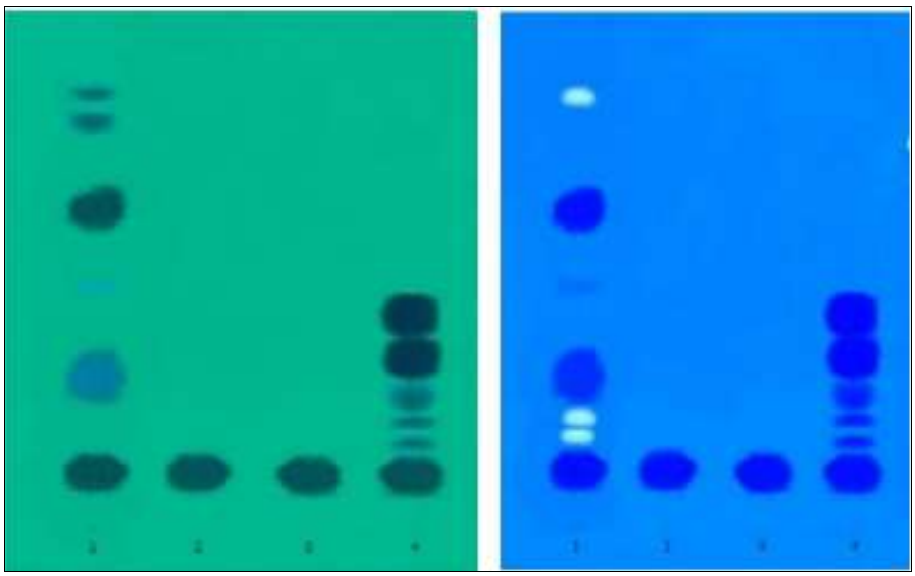

Fig 4: HPTLC plate showing estimation of Ascorbic acid in formulation, standard and crude drugs

Methanolic extract of formulation.
Ascorbic acid standard spot.
Methanolic extract of *Emblica officinalis.*
Methanolic extract of Triphala churna Visualization: 254 nm and long UV.

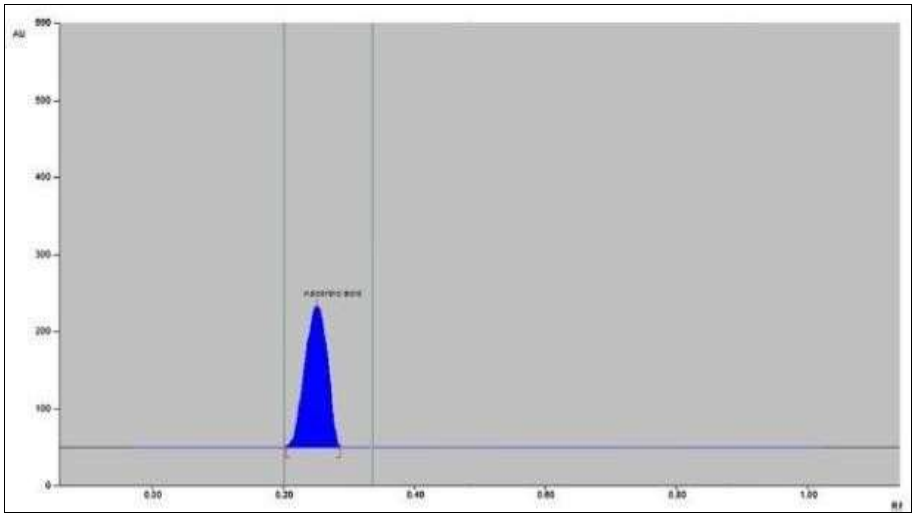

Fig 5: Chromatogram of Standard Ascorbic acid

Table 3: Retention factor (R_f) and Area of standard, formulation and Crude drug

Name		Standard	Methanolic extract of formulation	Methanolic extract of Triphala powder
Ascorbic acid Acid	R_f	0.27	0.26	0.24
	Area	11363	6279	3788

Gallic acid

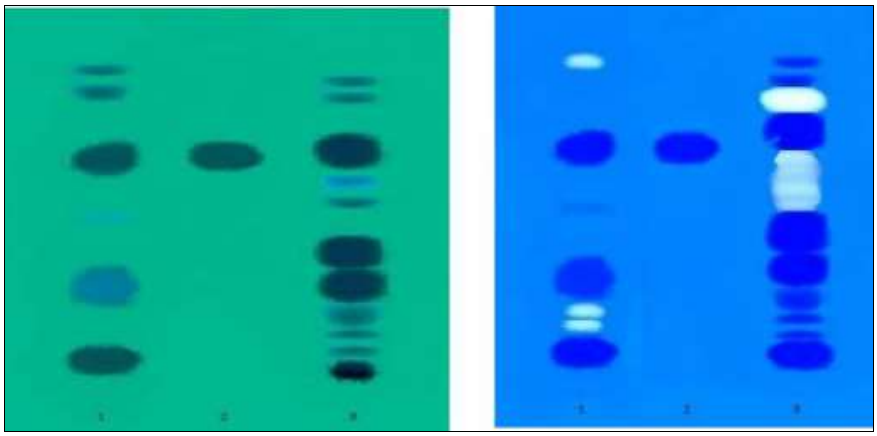

Fig 6: HPTLC plate showing estimation of Gallic acid in formulation, standard and crude drugs

Methanolic extract of formulation
Gallic acid standard spot

Methanolic extract of Triphala churna
Visualization: 254 nm and Long UV

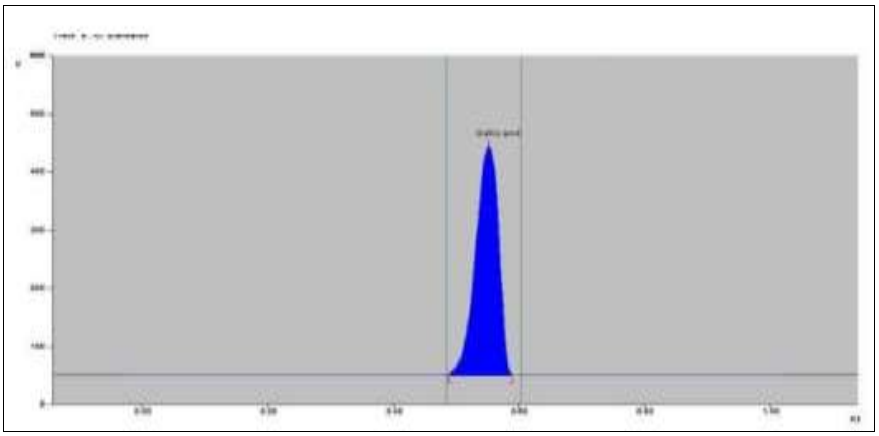

Fig 7: Chromatogram of Standard Gallic acid

Quercetin

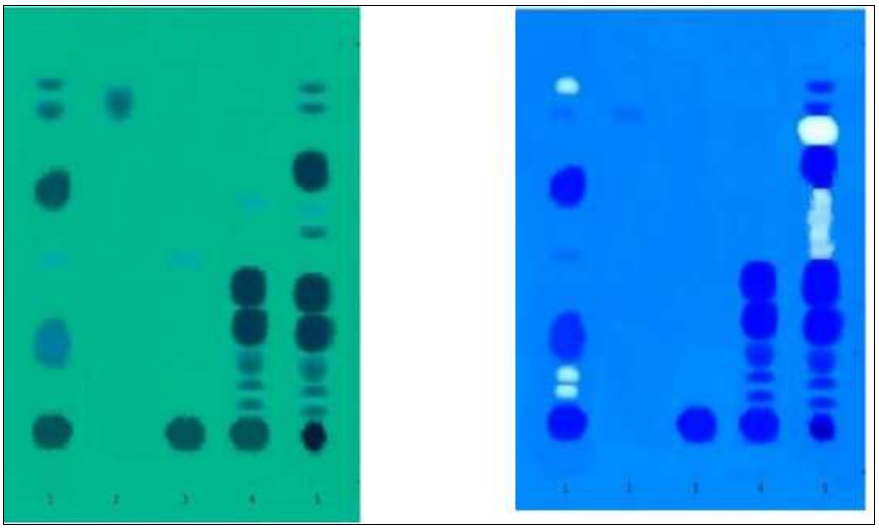

Fig 8: HPTLC plate showing estimation of Quercetin in formulation, standard and crude drugs

Table 4: Retention factor (R_f) and Area of standard, formulation and Crude drug

Name		Standard	Methanolic extract of formulation	Methanolic extract of Triphala powder
Gallic acid	R_f	0.52	0.57	0.56
	Area	19666	4356	2519

Methanolic extract of formulation.
Quercetin standard spot.
Methanolic extract of *Emblica officinalis.*
Methanolic extract of *Terminalia chebula.*
Methanolic extract of Triphala churna Visualization: 254 nm and Long UV.

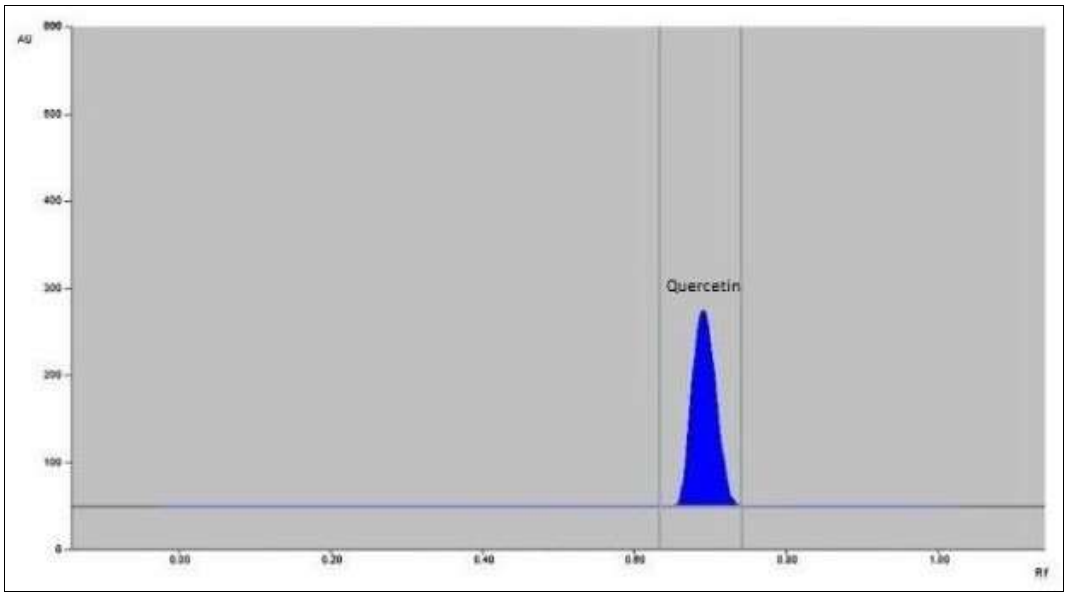

Fig 9: Chromatogram of Standard Quercetin

Table 5: Retention factor (R_f) and Area of standard, formulation and Crude drug

Name		Standard	Methanolic extract of formulation	Methanolic extract of Triphala powder
Quercetin	R_f	0.75	0.76	0.76
	Area	13306	10506	3256

Reserpine

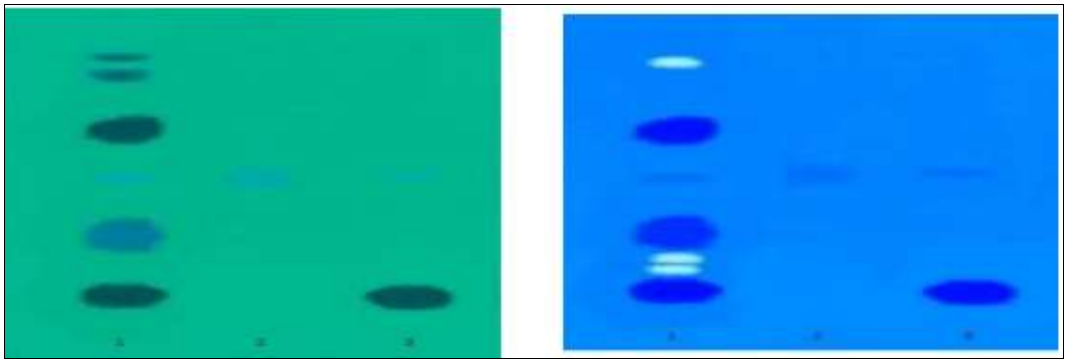

Fig 10: HPTLC plate showing estimation of Reserpine in formulation, standard and crude drugs

Methanolic extract of formulation.
Reserpine standard spot.
Methanolic extract of *Rauwolfia serpentina* root powder Visualization: 254 nm and Long UV.

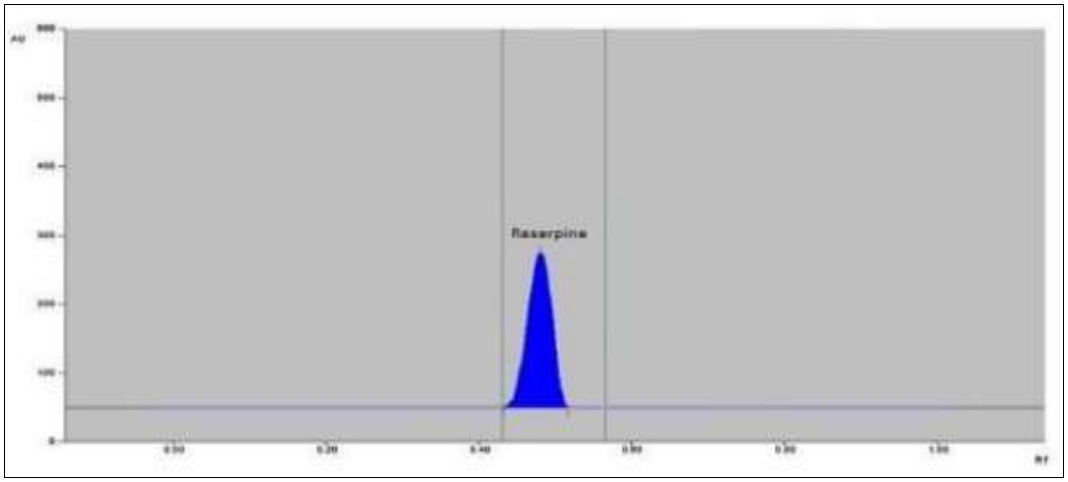

Fig 11: Chromatogram of Standard Reserpine

By superimposing their UV absorption spectra on top of the reference standards, the identification of the band for Gallic acid, Ascorbic acid, Quercetin, and Reserpine in the formulation and crude drug extract was verified.

Table 6: Retention factor (R_f) and Area of standard, formulation and Crude drug

Name		Standard	Methanolic extract of formulation	Methanolic extract of *Rauwolfia serpentina* root powder
Reserpine	R_f	0.48	0.47	0.49
	Area	20156	2650	2098

Conclusion

The proposed HPTLC method for the estimation of Reserpine, Gallic Acid, Quercetin, and Ascorbic Acid in Cardostab tablet and crude drugs holds promise for ensuring the quality, safety, and efficacy of herbal medicines. By employing HPTLC-based analytical techniques, researchers and manufacturers can accurately quantify bioactive compounds, monitor batch-to-batch consistency, and comply with regulatory standards. This study aims to contribute to the advancement of herbal medicine research and quality control practices, ultimately promoting the safe and effective use of herbal medicines for healthcare worldwide.

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