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HPTLC ANALYSIS OF NATURAL AND CHEMICALLY PRESERVED ROSE WATER

Dr. Surabhi Verma

Assistant Professor, Department of Ras Shastra Evam Bhaishajya Kalpana, Vivek college of ayurvedic sciences and hospital, Bijnor (UP)

Abstract

Background: The fingerprint analysis approach using high-performance thin-layer chromatography (HPTLC) has to turn out to be the most compelling tool for quality control of herbal medicines because of its simplicity and reliability. It can be out as a device for identification, authentication, and quality control of herbal drugs. The developed technique can be used as a quality control tool for quick authentication from a wide variety of herbal samples.

Material And Methods: For authentication of the drug, HPTLC was performed at an interval of 0 and 6 months for accelerated study and 0, 12 months for real-time study to estimate the reliability of the *Arka* samples. **Results:** In all four samples wavelength, 540nm found the maximum concentration of separated materials for accelerated storage condition, and wavelength 254nm found the maximum concentration of separated materials for real-time storage condition.

Conclusion: HPTLC analysis was also used in the stability studies. The number of spots at the initial sample vs final samples shows the change that occurred in the stability chamber. Less variation in the fingerprinting indicates the good stability of the formulations.

Key words: HPTLC, accelerated study, real-time storage condition

INTRODUCTION

Across all stages of the drug research and development process, analysis of pharmaceutical chemicals and newer pharmaceuticals is routinely employed. These analytical approaches give more exact and reliable data, which aids not just medication research and discovery, but also postmarket surveillance [1]. HPTLC is still one of the most versatile, dependable, and cost-effective separation techniques for botanicals and herbal pharmaceuticals investigation. It ensures reliable findings when used with established processes, which is critical in the routine identification of complex fingerprints of plant extracts and medicinal compounds [2]. HPTLC is a chromatographic method that is used for the identification of components, the identification and detection of impurities, and the quantitative determination of active compounds, among other things. HPTLC is an important alternative method to HPLC and gas chromatography due to the use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, as well as more effective elution techniques, high-resolution sorbents with selected particle size or chemically modified surface, the ability to combine with other instrumental methods, and the development of computer programmes for method optimization. When compared to regular TLC, HPTLC is one of the best TLC techniques for analytical applications because of its higher accuracy, repeatability, and ability to document the results [3].

MATERIAL AND METHODS

Procedure- It is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent was a relatively thin, uniform layer of dry fine powdered Silica Gel applied to the glass plate. Separation possibly will be achieved based on the partition or a combination of partition and adsorption, depending on the particular kind of support, it was prepared and used with a different solvent. Recognition can be exaggerated by observing spots of identical R_f Value and about equal magnitude obtained correspondingly, with an unknown and a reference sample chromatographed on the same plate. A visual association of the size and intensity of the spots generally serves for semi-quantitative estimation [4].

The present study was performed at Vasu Research Centre A2/624-625/2, G.I.D.C., Makarpura, Vadodara (Gujarat)

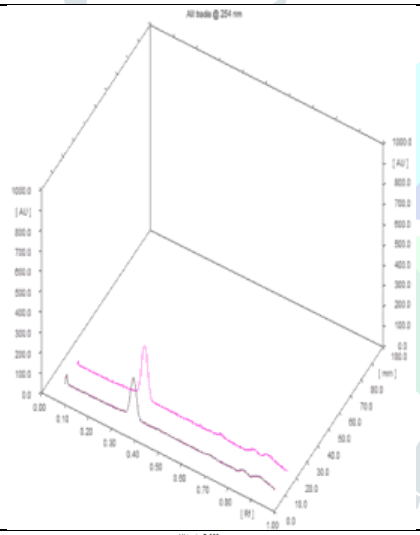
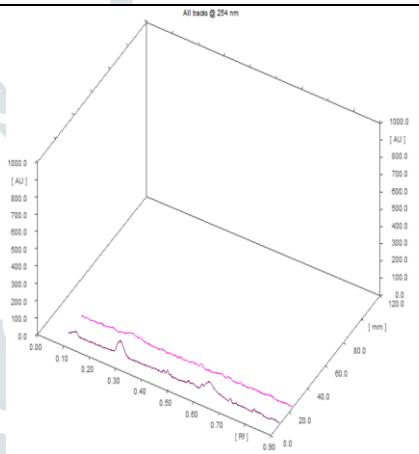
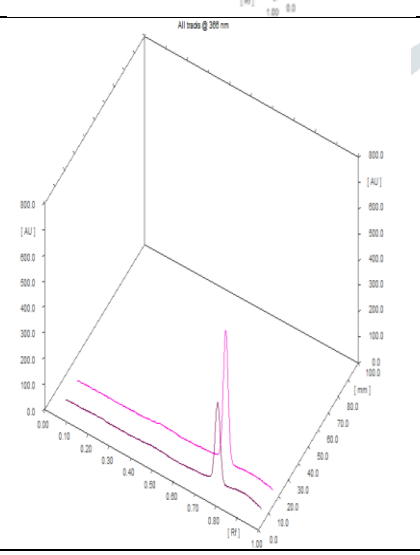
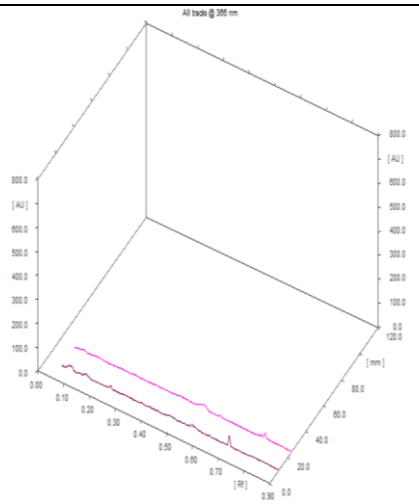
Chromatographic conditions- CAMAG Linomat 5 – Applicator, whatman filter paper No. 1 filtering system. Stationary phase and mobile Phase was MERC TLC/HPTLC Silica gel 60 F_{254} on Aluminium sheets and Toluene : Ethyl Acetate (9:1 v/v) respectively. Anisaldehyde – sulphuric acid Reagent and derivatization with CAMA (dip tank for about 1 minute). TLC Plate Heater Preheated at 100± 5°C for 3 minutes and visualization at 254 nm, 366 nm and 540 nm.

OBSERVATION AND RESULTS

Table 1.1- Sample I

Wavelength	Accelerated		Real-time	
	0 month	6 month	0 month	12 month
@254nm	3 0.34, 0.81, 0.88	4 0.34, 0.62, 0.81, 0.88	5 0.34, 0.55, 0.71, 0.81, 0.88	6 0.34, 0.55, 0.62, 0.71, 0.81, 0.88
@366nm	2 0.71, 0.88	2 0.71, 0.88	3 0.56, 0.71, 0.88	3 0.56, 0.71, 0.88
@540nm	6 0.20, 0.39, 0.47, 0.62, 0.71, 0.88	6 0.27, 0.39, 0.47, 0.62, 0.71, 0.88	2 0.47, 0.62	2 0.47, 0.62

Table 1.2- Sample I

Wavelength	Accelerated	Real-time
@254nm		
@366nm		

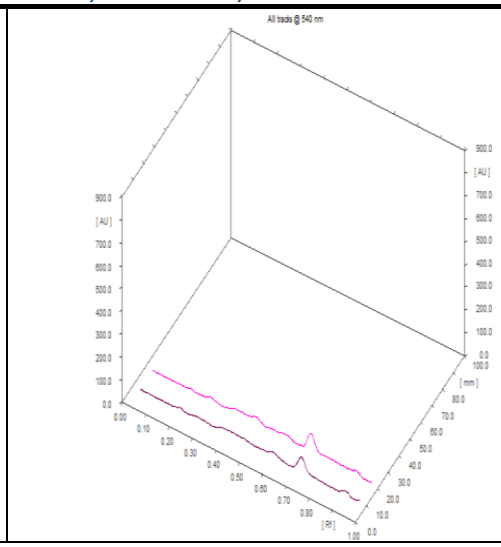
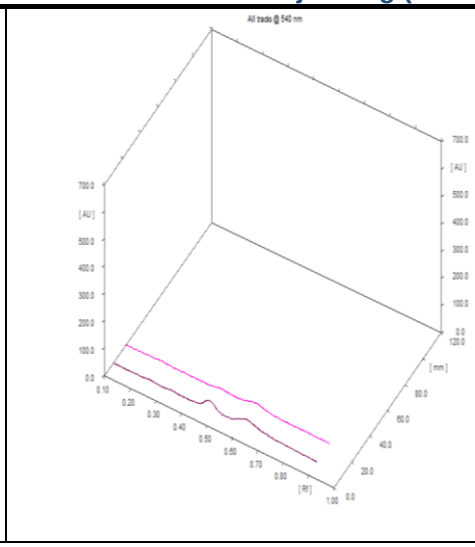
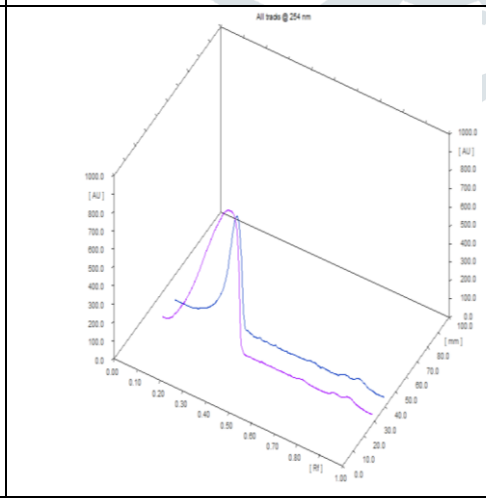
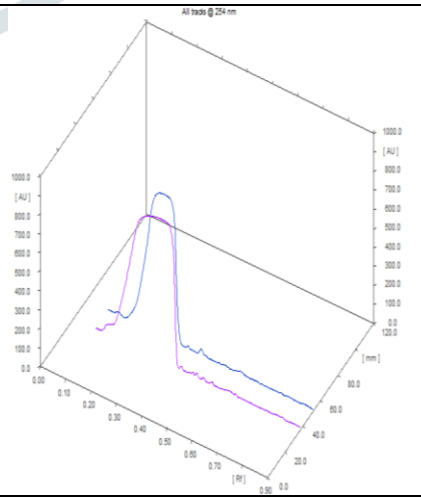
@540nm		

Table 2.1- Sample II

Wavelength	**Accelerated**		**Real-time**	
	0 month	**6 month**	**0 month**	**12 month**
@254nm	3 0.34, 0.81, 0.88	6 0.34, 0.43, 0.68, 0.71, 0.81, 0.88	5 0.34, 0.43, 0.47, 0.62, 0.68	4 0.34, 0.43, 0.47, 0.62
@366nm	3 0.43, 0.71, 0.88	2 0.71, 0.88	3 0.26, 0.43, 0.88	2 0.43, 0.88
@540nm	7 0.24, 0.27, 0.39, 0.58, 0.71, 0.81, 0.88	6 0.20, 0.39, 0.58, 0.62, 0.71, 0.88	2 0.39, 0.58	2 0.39, 0.58

Table 2.2- Sample II

Wavelength	**Accelerated**	**Real-time**
@254nm		

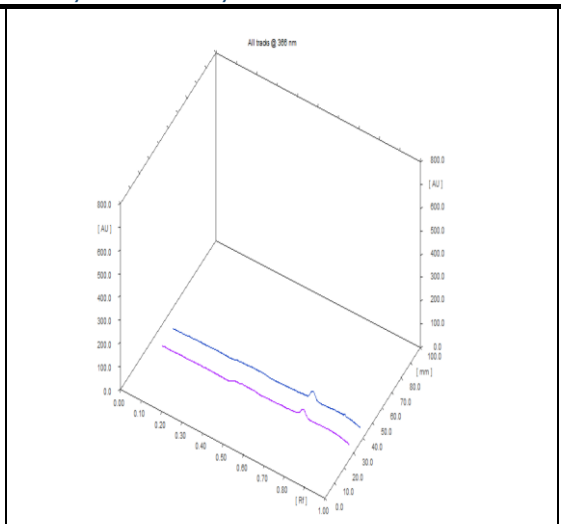
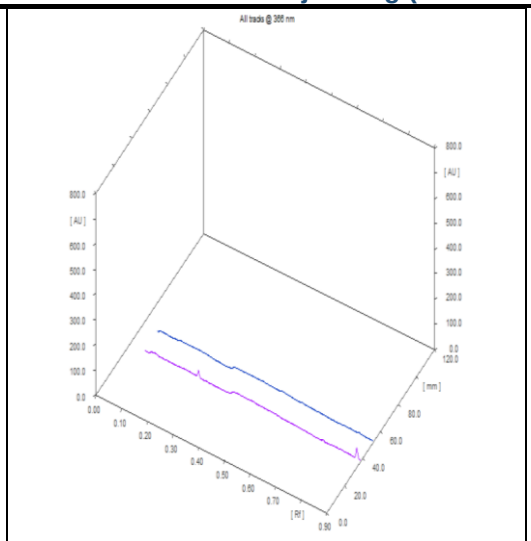
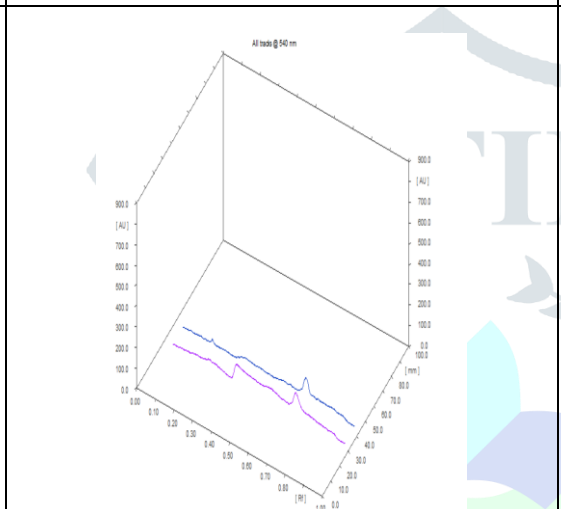
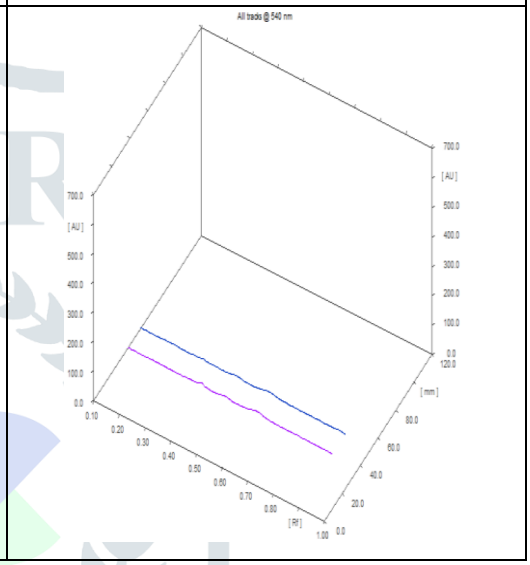
@366nm		
@540nm		

Table 3.1- Sample III

Wavelength	**Accelerated**		**Real-time**	
	0 month	**6 month**	**0 month**	**12 month**
@254nm	4 0.24, 0.34, 0.81, 0.88	8 0.08, 0.10, 0.18, 0.27, 0.34, 0.43, 0.81, 0.88	3 0.10, 0.34, 0.81	2 0.10, 0.34
@366nm	2 0.43, 0.71	4 0.08, 0.62, 0.71, 0.94	3 0.10, 0.34, 0.81	2 0.08, 0.62
@540nm	7 0.20, 0.24, 0.39, 0.58, 0.62, 0.71, 0.88	7 0.08, 0.27, 0.39, 0.58, 0.71, 0.81, 0.88	2 0.39, 0.62	2 0.08, 0.62

Table3.2- Sample III

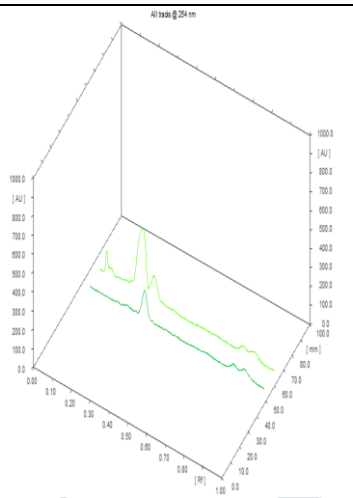
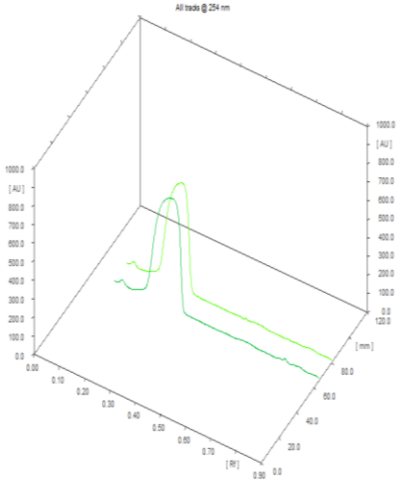
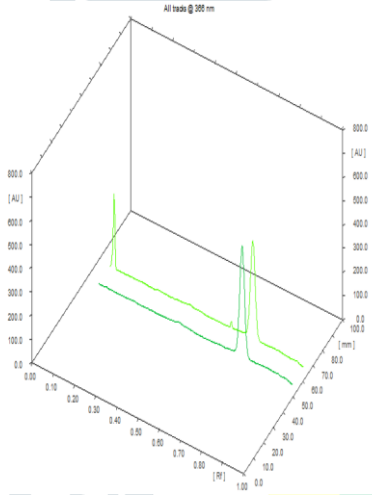
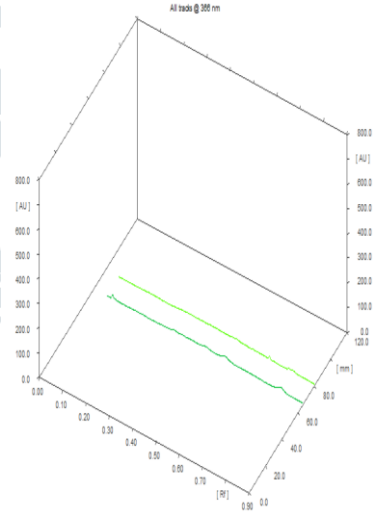
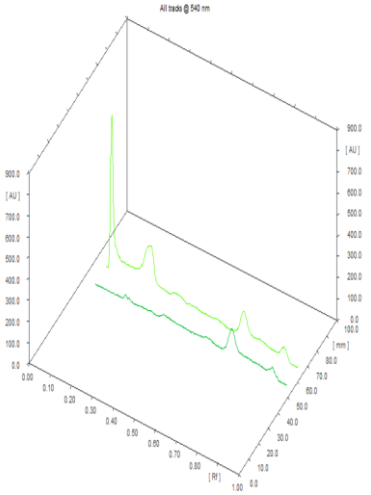
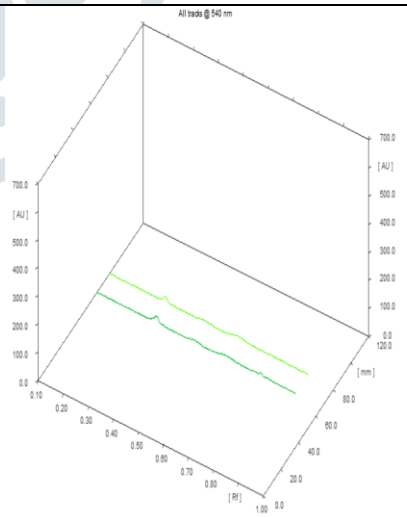
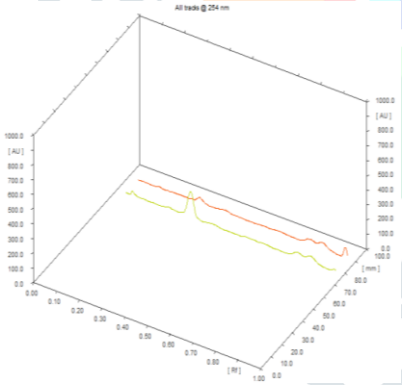
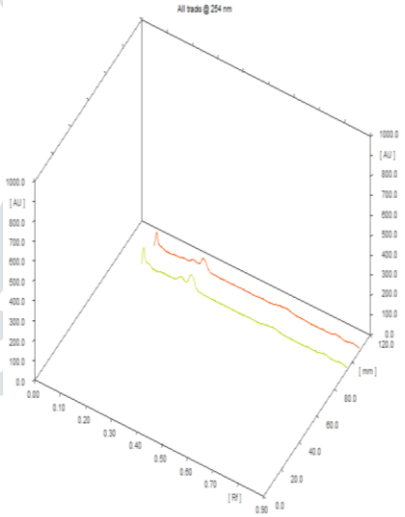
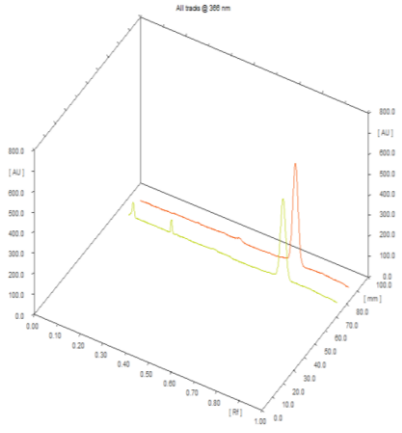
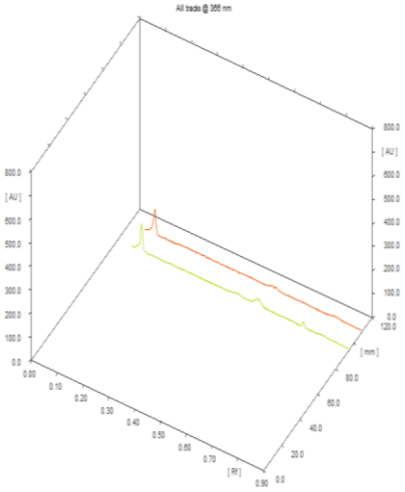
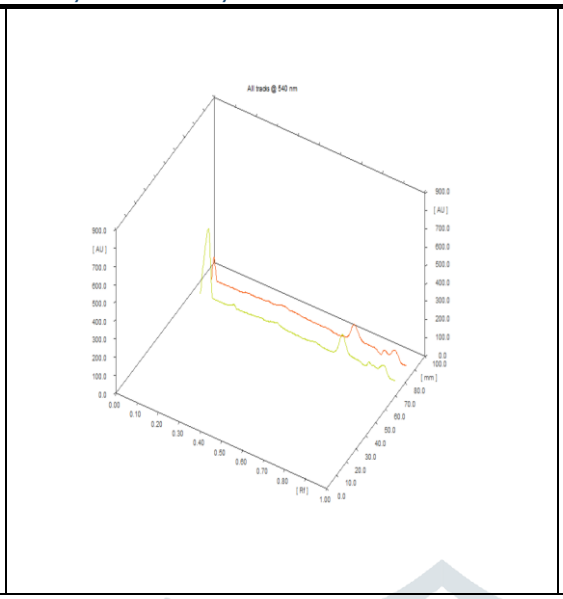
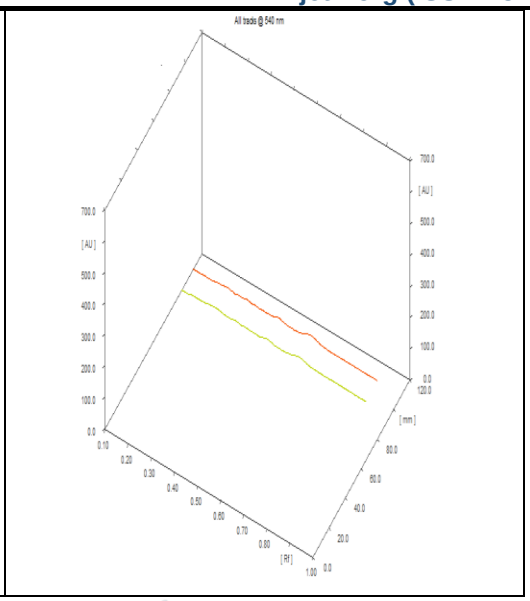
Wavelength	Accelerated	Real-time
@254nm		
@366nm		
@540nm		

Table 4.1- Sample IV

Wavelength	Accelerated		Real-time	
	0 month	6 month	0 month	12 month
@254nm	6 0.08, 0.24, 0.34, 0.81, 0.88, 0.94	5 0.34, 0.43, 0.81, 0.88, 0.94	4 0.08, 0.24, 0.34, 0.61	4 0.08, 0.24, 0.34, 0.61
@366nm	3 0.08, 0.24, 0.71	2 0.43, 0.71	3 0.08, 0.61, 0.71	2 0.08, 0.61
@540nm	7 0.08,0.20,0.39, 0.58, 0.71, 0.84, 0.94	7 0.08, 0.39, 0.58, 0.71, 0.81, 0.84, 0.94	1 0.61	1 0.61

Table 4.2- Sample IV

	Accelerated	Real-time
@254nm		
@366nm		

@540nm		

DISCUSSION

The developed technique can be used as a quality control tool for quick authentication from a wide variety of herbal samples. HPTLC analysis was also used in the stability studies. The number of spots at the initial sample vs final samples shows the change that occurred in the stability chamber. Less variation in the fingerprinting indicates the good stability of the formulations. For authentication of the drug, HPTLC was performed by using Toluene: Ethyl acetate (9:1 v/v) at an interval of 0 and 6 months for accelerated study and 0, 12 months for real-time study to estimate the reliability of the *Arka* samples. In all four samples wavelength, 540nm found the maximum concentration of separated materials for accelerated storage condition, and wavelength 254nm found the maximum concentration of separated materials for real-time storage condition [table 1.1, 1.2, 2.1, 2.2, 3.1, 3.2, 4.1, 4.2].

Table 5.1- Spots observed in HPTLC

@254nm				
Sample	**Spot no. (accelerated)**		**Spot no. (real-time)**	
	0 month	**6 month**	**0 month**	**12 month**
Sample I	3	4	5	6
Sample II	3	6	5	4
Sample III	4	8	3	2
Sample IV	6	5	4	4
@366nm				
Sample	**Spot no. (accelerated)**		**Spot no. (real-time)**	
	0 month	**6 month**	**0 month**	**12 month**
Sample I	2	2	3	3
Sample II	3	2	3	2
Sample III	2	4	3	2
Sample IV	3	2	3	2

@540nm				
Sample	**Spot no. (accelerated)**		**Spot no. (real-time)**	
	0 month	**6 month**	**0 month**	**12 month**
Sample I	6	6	2	2
Sample II	7	6	2	2
Sample III	7	7	2	2
Sample IV	7	6	1	1

Above mentioned spots [Table 5.1] indicated that the variation at an interval of 0 and 6 months for accelerated stability condition and 0, 12 months for real-time stability condition was less, so the samples were stable during both the studies.

CONCLUSION

HPTLC fingerprinting was done for qualitative assessment of *Gulab Arka* samples. The variable number of the spots were found in different samples. The chromatogram showed 03, 02, 06 spots at wavelength 254 nm, 366nm, 540nm respectively for sample I (WP), 03, 03, 07 spots at wavelength 254 nm, 366nm, 540nm respectively for sample II (MP+PP), 04, 02, 07 spots at wavelength 254 nm, 366nm, 540nm respectively for sample III (PE+TEG) and 06, 03, 07 spots at wavelength 254 nm, 366nm, 540nm respectively for sample IV (PEA+CG). In all four samples wavelength, 540nm found the maximum concentration of separated materials.

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