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Simultaneous Estimation Of Ascorbic Acid And Gallic Acid In Triphala Ghrita Formulation By Hptlc

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ABSTRACT

A sensitive, selective and precise thin-layer chromatographic method has been developed and validated for the analysis of Ascorbic acid and Gallic acid in Triphala Ghrita laboratory prepared and Market formulation. Separation and quantification was achieved by TLC using mobile phase of Toluene: ethyl acetate : methanol : Gl. acetic acid v/v/v/v (4:4:5:0.5) (Rf 0.41 & 0.81 for Ascorbic acid and Gallic acid respectively) on precoated silica gel 60F254 aluminum plates and determination was carried out at 254 nm. The calibration curve was linear in the concentration range of 2–10 µg spot-1. The method was validated for precision, repeatability and accuracy. The proposed method was found to be simple, precise, specific, sensitive and accurate for the quantification of Ascorbic acid and Gallic acid.

This is the first TLC report for the simultaneous estimation of Ascorbic acid and Gallic acid in Triphala Ghrita formulation and may be useful for the routine quality control.

KEYWORDS: Triphala Ghrita, Ascorbic Acid, Gallic Acid

INTRODUCTION

Triphala ghrita is the most favourite formulation used by Ayurvedic scholars and practitioners in ancient time as either whole or separately with other drugs; locally as well as systematically . Triphala ghrita contains Triphala (which consist of Emblica officinalis, Terminalia chebula and Terminalia belerica) and ghrita (cow ghee) having the various classical usefulness that includes Timira (cataract), Visarpa (erysipelas), Netra ruja (pain in eyes), Netra srava (lacrimation), Kandu (itching), Svayathu (oedaema), Visama jvara (intermittent fever), Sukla netra roga (eye disorders related to sclera), Vartma roga (disorders of eyelids). Tillotson et al 2001 and Varma et al have reported that Oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the development of cataract. Sabu et al and Naik et al have proved Triphala to be an antioxidant. All its constituents are also proved to posses antioxidant activity like gallic acid. Even ghrita (cow's ghee) consists of vitamin A and E which are the well established antioxidant.

Famous yogas containing Triphala as main ingredient, which are utilized in day to day ophthalmic practice are Triphala Churna, Sadanga kwatha guggulu, Triphaladi kwatha ,Triphaladi anjana,Tripahala varti, Triphaladi rasakriya, Nagarjuna varti, Kokilavarti, Drustiprada varti, Candhanadi varti, Lohadi guggulu, Timirahara lauha, Saptamruta loha, Triphaladi ghrita, Mahatriphaladi ghrita and Triphala Guggulu etc.

Considering wide therapeutic applications of Triphala ghrita, and presence of the marker constituents, to ensure identity and quality of Triphala ghrita a simple, sensitive, specific and reproducible HPTLC method is developed for the quantification of Ascorbic acid and Gallic acid markers in the Triphala ghrita formulation.

MATERIALS AND METHODS:

The selected formulation of Triphala ghritawas procured from the Ayurvedic Pharmacy in the local Market from Pune, the crude drugs required for the preparation of lab formulation were procured from Soham Ayurved Rasashala, Solapur, Maharashtra.

Solvents and chemicals

Standard Ascorbicacid and Gallic acid were procured from Yucca enterprises. All chemicals and reagents used were of analytical grade and purchased from Rankem and S. D. Fine Chemicals, India. Silica gel 60F HPTLC pre-coated plates were purchased from Merck.

Preparation of standard solution

A stock solution of Ascorbic acid and Gallic acid (1 mg/ml) were prepared by dissolving 10 mg of accurately weighed Ascorbic acid and Gallic acid in methanol and making up the volume to 10 ml with methanol. This concentration was used as the working standard for the HPTLC method.

Sample preparation

The test samples were prepared by 100mg extracted with 100 ml of acetone for 1 hr on sonicator with heat. the extract was filtered and allowed to dry completely the residue was weighed to 10 mg and dissolved in 10 ml of acetone.

HPTLC Instrumentation and Method development

The stationary phase used was precoated silica gel aluminium plate $60F_{254}$ (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland)on to which the test solutions were spotted in the form of bands of width 6 mm with a Camag microlitre syringe. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 5mm × 0.45 mm, bandwidth was set at 20 nm, each track and 10 mm/s scanning speed was employed.

The composition of the mobile phase was Toluene: ethyl acetate: methanol: Gl. acetic acid v/v/v/v (4:4:5:0.5) for Ascorbic acid and Gallic acid was employed. The linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 minutes at room temperature (25 ± 2°C). The length of the chromatogram run was 80 mm. Subsequently, the plate was allowed to dry at room temperature. The separated bands on the HPTLC plates were scanned over the wavelength of 254 nm. The source of radiation utilized was the tungsten lamp and the detection and quantification was carried at 254nm.

Method validation

The validation of the developed HPTLC method was carried out according to ICH guidelines.

Linearity

The linearity was analyzed for different concentration ranging from 0.5-2.5 µg/ spot and 2-10 µg/ spot for Ascorbic acid and Gallic acid were spotted. The data of the peak areas plotted against the corresponding concentrations were treated by least-square regression analysis.

LOD and LOQ were determined by using standard deviation method. Detection limit =3.3σ /S and quantification limit=10 σ /S where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve.

Precision studies

Precision of the method was evaluated by repeatability (intra-day) and reproducibility (inter-day). The triplicates of three different concentrations of standard mixture solution were spotted and analyzed on same day for intra-day study and two different days for inter-day study with respective chromatographic conditions.

Accuracy studies

Recovery study method was employed to evaluate accuracy of the method. The samples were spiked with 80, 100 and 120 % of median concentrations of standards. Accuracy was calculated from the following equation:

[(spiked concentration − mean concentration)/spiked concentration] × 100.

Robustness

For the determination of the robustness of method, chromatographic parameters, such as mobile phase composition and detection wavelength and saturation time were intentionally varied to determine their influence on the retention factor and quantitative analysis.

The mobile phase composition was altered by ± 2 % changes in the composition of methanol. The chamber saturation time was altered from 15 min to 30 min. The method was analyzed using two altered wavelengths ; 252nm and 256 nm.

RESULTS & DISCUSSION

Method optimization

The proposed method gave very good separation and resolution of the standard Ascorbic acid and Gallic acid.

Method validation

Linearity, limit of detection and quantitation

Under the above described experimental conditions, linear correlation between the peak area and applied concentration was found to occur in the concentration range for Ascorbic acid 0.5-2.5 µg/ spot and Gallic acid2-10 µg/ spot

Precision

Precision data on repeatability (intra-day) and instrumental variation was obtained for Ascorbic acid and Gallic acidat three different concentration levels. Precision studies showed R.S.D. less than 2%, indicating a good precision.

Accuracy

The sample containing 1.5µg of Ascorbic acid and 6µg of Gallic acid was spiked with the known amount of standards, and the percent ratios between the recovered and expected concentrations were calculated. Recoveries were obtained in the range of 80-120%, depicting the HPTLC proposed method for estimation is accurate for the quantification of Ascorbic acid and Gallic acid.

Robustness

No changes were observed in retention time and peak shape of both the standards with the changes made with mobile phase and chamber saturation time. The resolution and the separation of markers were also unaltered.

Analysis of TGMF and TGLF formulations

The developed method was able to be provide a well resolved chromatogram with no alterations in peaks of Ascorbic acid and Gallic acid.

Figure 1 Spectral Overlay of Ascorbic Acid Standard and Sample Tracks

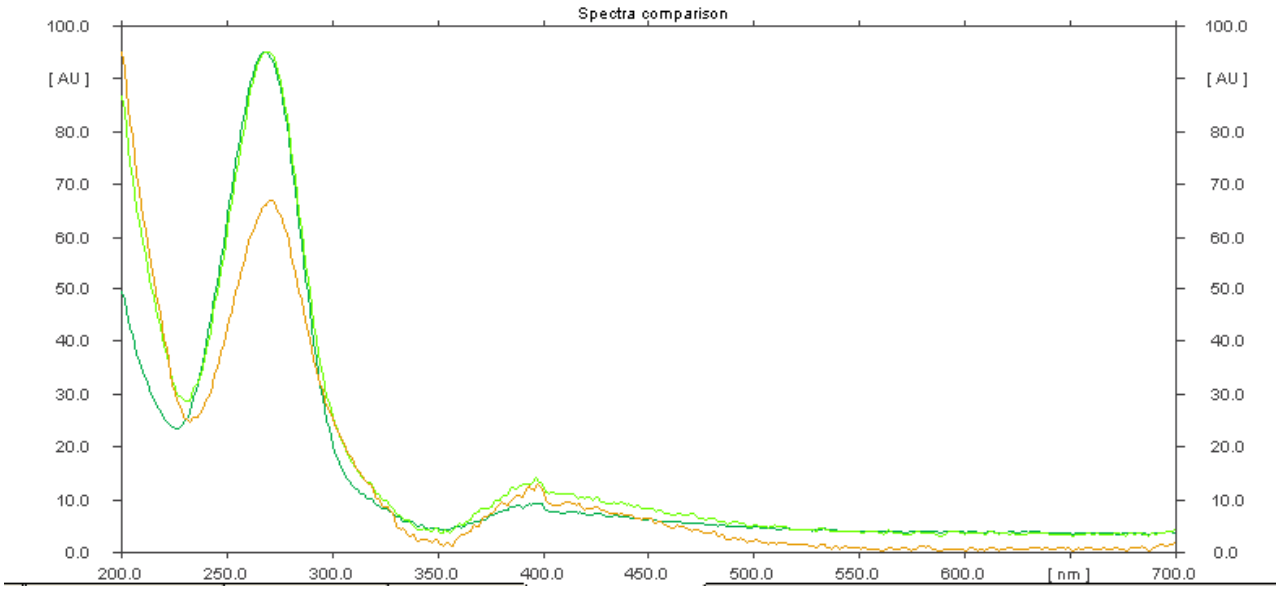


Figure 2 Spectral Overlay of Gallic Acid Standard And Sample Tracks

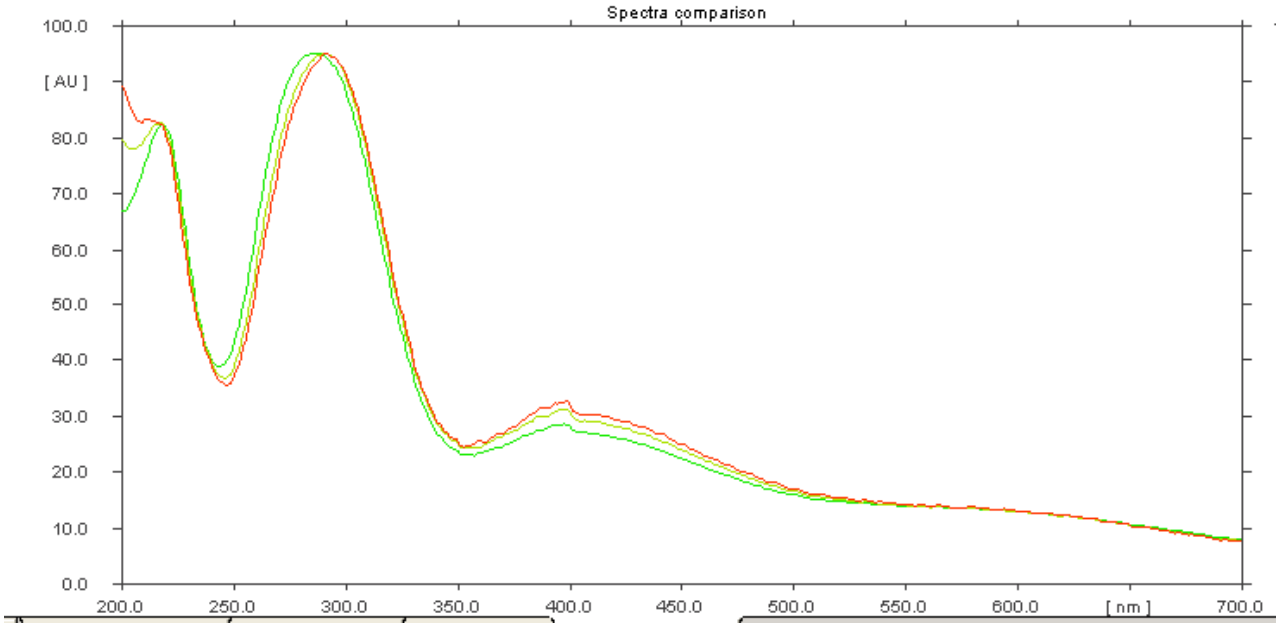


Figure 2 Calibration Plot of Ascorbic Acid

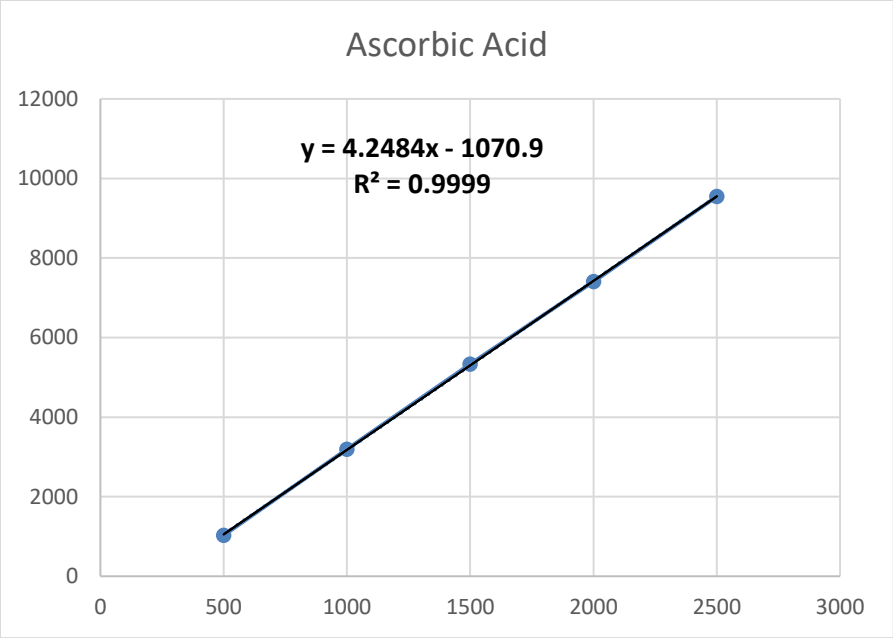


Figure 3 Calibration Plot of Gallic Acid

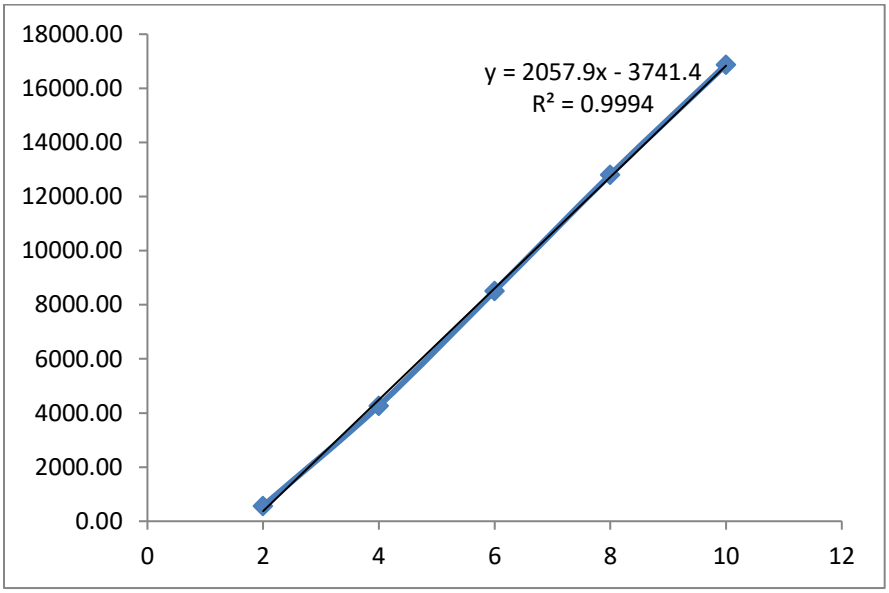


Figure 4 3 D Overlaid Chromatogram of Standard and Sample Tracks

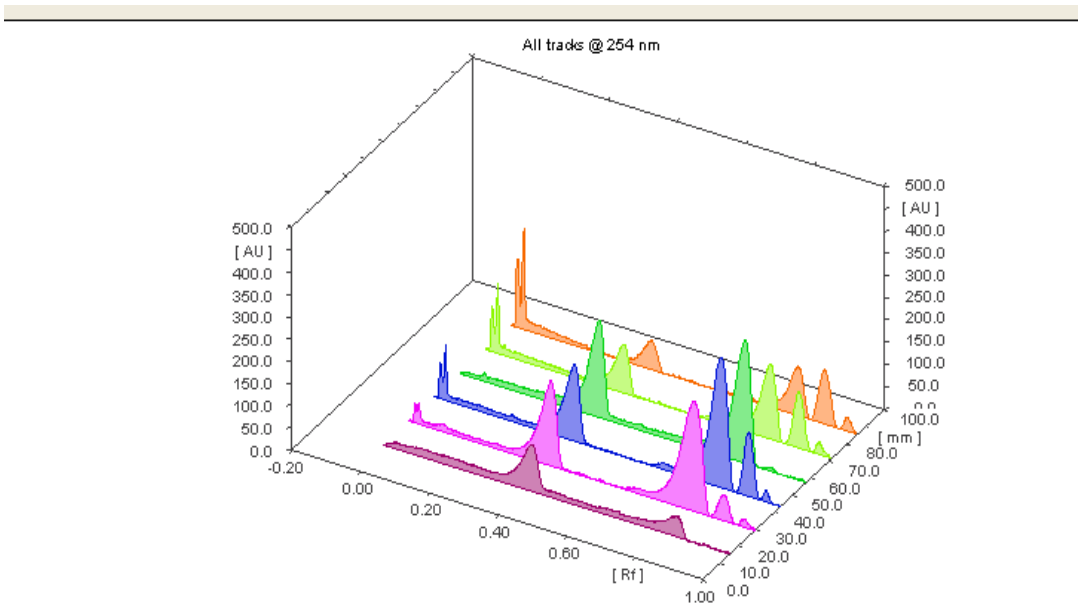
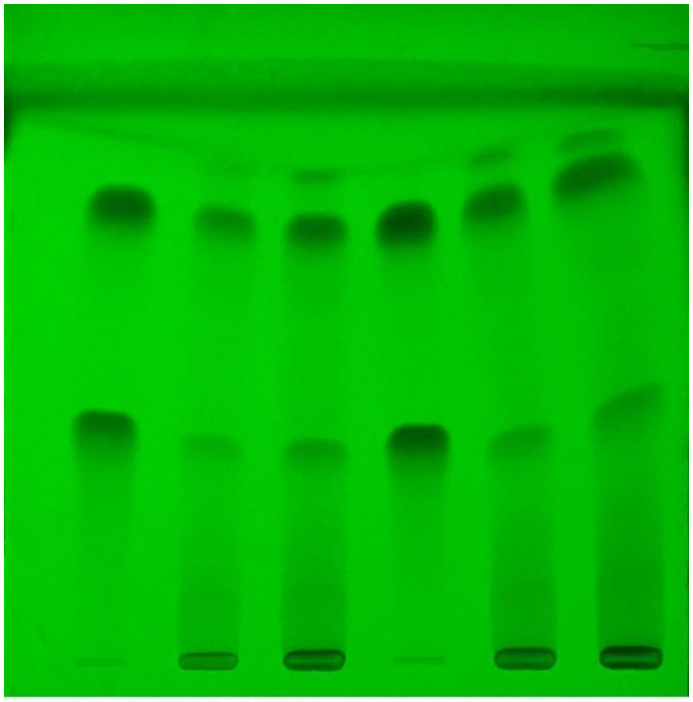


Figure 6 Chromatogram of Standard and Sample



a) Lineartity regression Data

Sl No.	Parameter	Results

		Ascorbic acid	Gallic acid
1	R_F	0.41	0.81
2	Dynamic range (µg $spot^{-1}$)	1-15µg/µl	10-100µg/µl
3	Equation	y = 4.283x - 1097	y = 2057.x - 3741
4	Slope	4.227	2057
5	Intercept	1097	-3741
6	Limit of Detection	21.79	0.114
7	Limit of Quantification	63.87	0.345
8	Linearity (Correlation coefficient)	0.999	0.999
9	Specificity	Specific	Specific

b) Precision studies data

Instrumental Precision (% RSD)	Concentration Conc (µg /spot)	Method Precision (% RSD)	Concentration Conc ((µg/spot)	Method Precision(% RSD)
	Ascorbic acid		Gallic acid	
Intra day	1.0	1.68	4	0.89
	1.5	1.77	6	1.09
	2.0	1.28	8	1.35
Inter day	1.0	0.62	4	1.04
	1.5	0.63	6	0.90
	2.0	0.48	8	0.92

c) Recovery studies of Ascorbic acid

Sl No.	Amount of Ascorbic acid present in the sample((µg)	Amount of Ascorbic acid added ((µg)	Amount of Ascorbic acid found (µg)	Recovery (%)
1	1.5	1.2	2.74	100.1
2	1.5	1.5	3.74	100.2
3	1.5	1.8	3.30	101.58

Recovery studies of Gallic acid

Sl No.	Amount of Gallic acid present in the sample(µg)	Amount of Gallic acid added (µg)	Amount of Gallic acid found (µg)	Recovery (%)
1	6	4.8	10.83	100.73

2	6	6	12.20	103.32
3	6	7.2	13.24	100.59

CONCLUSION

The TLC method developed here for the quantification of Ascorbic acid and Gallic acid in Triphala ghrita is simple, rapid, cost-effective and easily adaptable for the screening and quantitative determination.

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