International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1297-1301 www.ijprajournal.com ISSN: 2249-7781

Quantitative Estimation of Gallic Acid in Triphala Churna by HPTLC Technique

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Date Of Submission: 01-03-2021 Date Of Acceptance: 16-03-2021

ABSTRACT: A simple, reliable and rapid HPTLC method was developed for quantitative estimation of Gallic acid in the Methanol extract of Triphala Churna. The method was validated as per ICH guidelines for linearity and range, limit of detection, limit of quantification and repeatability of sample application. Pre coated silica gel on aluminum sheets were used as the stationary phase. Toluene: Ethyl acetate: Methanol: Formic acid (12:6:0.4:0.8) constituted the mobile phase. The percentage of Gallic acid was estimated through densitometry scanning using a TLC Scanner III (Camag, Switzerland) with win CATS software. The amount of Gallic acid present in the sample was found to be 1.1 mg/gm. The developed method can be applied in quantifying the amount of Gallic acid in Triphala Churna and it can be used to quantify the concentration of active principles in herbal formulations.

KEY WORDS: Gallic acid, Triphala Churna, HPTLC

I. INTRODUCTION:

The use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice from time immemorial.[1] About 75-80 % of the world population, mainly in the developing countries, uses of herbal medicines for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effect.[2]

Triphala is a traditional ayurvedic herbal formulation consisting equal parts of three Medicinal plants namely Terminalia chebula, Terminalia belerica and Emblica officinalis. Triphala has been reported to possess antioxidant activity, improves mental and physical power and also assists in weight loss. [3]

Triphala is neither a harsh purgative nor a lubricating laxative. A nutritive and cleansing property of Triphala makes it special. Triphala gently stimulates the cleansing of accumulated toxins from all the tissues of the body, reduces cholesterol and high blood pressure, and improves circulation.

Terminalaia chebula contains 30% tannins and possess anthelmentic, aphrodisiac and astringent activity. Terminalia belerica contains 17% tannins, Gallic acid and ellagic acid and possess hepatoprotective activity. Embellica officinalis is the highest known source of vitamin C in nature, cures bleeding diseases and used in treatment of scurvy. [4]

Triphala also claimed to have various biological activities like heart protective, cardio tonic, improves digestion, liver function and hepatoprotective. In this study Gallic acid is used as a marker constituent for Triphala Churna.

The aim of present research study is to carryout quantitative estimation of Gallic acid in Triphala Churna by using HPTLC technique.

Literature search revealed that very few methods have been reported for analyzing Triphala such as TLC, HPLC and HPTLC methods using Gallic acid, Chebulinic acid and Chebulagic acid as marker.[5]

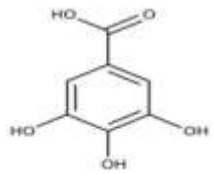

FIG: 1 Structure of Gallic acid

II. MATERIALS AND METHODS:[6]

INSTRUMENTATION:

The method was developed on CAMAG HPTLC system consisting of Linomat V applicator (Camag, Muttenz, Switzerland) CAMAG twin trough chamber, CAMAG TLC scanner, equipped with Wincats software (version 1.4.2) , CAMAG syringe of 100 µL capacity. Separation and identification of gallic acid were performed separately on pre-coated silica gel on aluminum

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plates.

REAGENTS AND CHEMICALS:

The mobile phase, composed of toluene: ethyl acetate: methanol: formic acid (12.6:0.4:0.8) separated the gallic acid with good resolution.

PREPARATION OF WORKING STANDARD SOLUTION OF GALLIC ACID:

Standard stock solution containing 2mg of gallic acid was prepared in 10 ml of water, to get stock solution containing 200 µg/ml of gallic acid. Further dilutions were made as 400-2000 ng/µl to perform linearity study. (Fig: 1)

PREPARATION OF SAMPLE SOLUTION OF GALLIC ACID:

1g of Triphala churna was weighed accurately and transferred to 10ml volumetric flask. The active principle was extracted by the addition of methanol and made up to 10ml. The solution was filtered, from which aliquots were applied.

CHROMATOGRAPHIC CONDITIONS:

The experiment was performed on a silica gel 60F254 (0.2 mm thickness) HPTLC plates (20×10cm) without pre-washing. Samples were applied to the plates as 8 mm bands, 8 mm apart and 10 mm from the edges of the plate, with a Camag Linomat V automatic sample applicator.

With the fixed conditions, 1, 2,3,4,5 micro litre from the solution containing (200 µg/ml) of drug was applied on pre-coated TLC plates. The plates was analysed photometrically and the chromatogram was recorded. This was followed by application of sample solution obtained from Triphala churna formulation (Fig: 2) 3D chromatogram of Triphala churna was shown in (Fig: 3).

Calibration graph was plotted using peak area of standard vs concentration of standard solution. Peak area of sample chromatogram and amount of active constituent in Triphala churna was calculated and shown in table: 3.

VALIDATION: [7]

The validation of the developed method was carried out in terms of linearity, Limit of Detection (LOD), Limit of Quantification (LOQ) and repeatability of sample application

Linearity and range:

Calibration graphs were plotted using peak area of standard Vs concentration of standard solution. The slope, intercept and correlation coefficient value for Gallic acid were shown in the graph (Fig: 4)

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ of the development method are determined by applying decreasing amounts of the drug in triplicate on the plate. The lowest concentration at which the peak is detected is called "Limit of Detection ".The lowest concentration at which peak is quantified is called "Limit of Quantification The results were shown in the table.1

Table 1: LOD and LOQ values

PARAMETERS	VALUES
Limit of Detection(ng/ml)	100
Limit of Quantification(ng)	200-1000

Repeatability of sample application:

Repeatability of sample application was assessed by spotting of drug solution six times on a TLC plate followed by development of plate and %RSD was calculated and the results are shown in table.2

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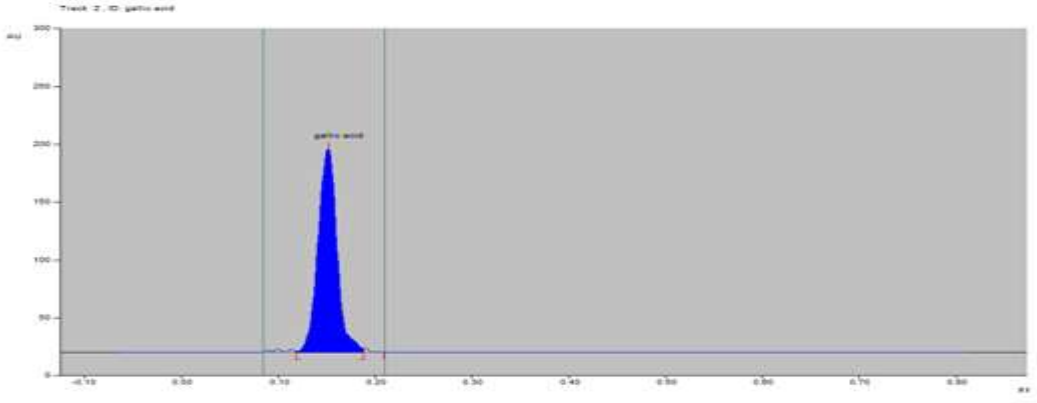

FIG: 1.Chromatogram of Gallic acid

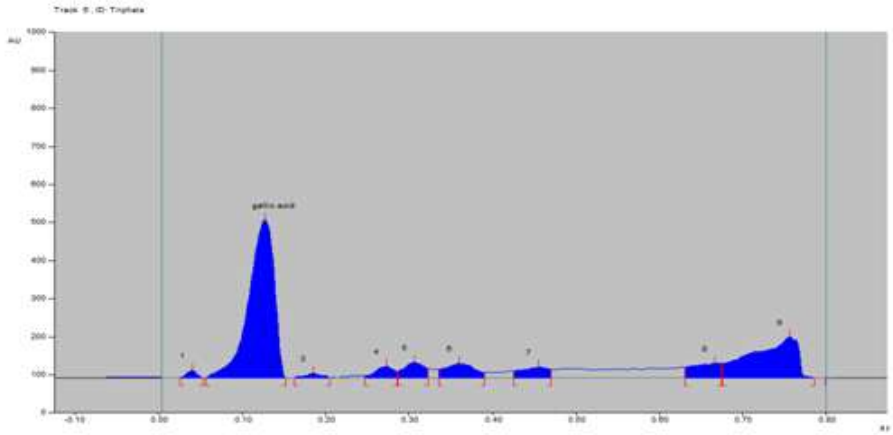

FIG: 2. Chromatogram of Triphala Churna

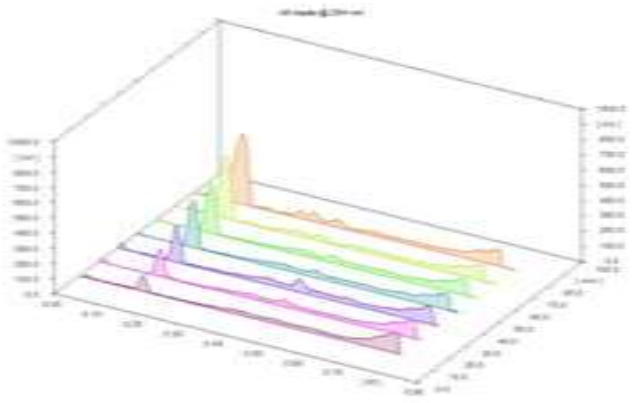

FIG:3. 3D Chromatogram of Gallic acid

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Concentration(ng/ml)	Peak area
200	1723.3
400	3200.6
600	4180.8
800	5991.9
1000	6415.9
Unknown	7325.8

Table: 3 Calibration of Gallic acid

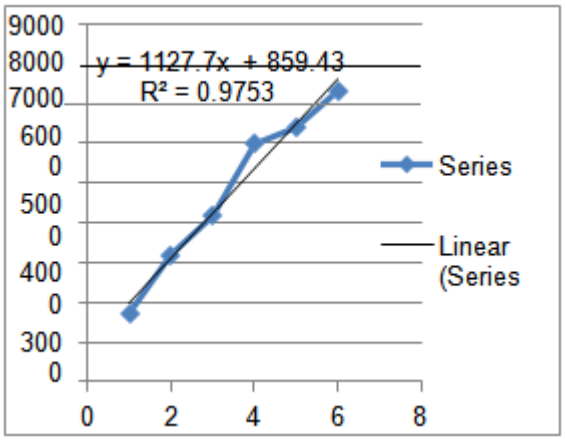

FIG: 4 Calibration graph of Gallic acid

Concentration (µg/ml)	Peak area	%RSD
600	4180.8	0.7961
	4160.5	
	4120.3	
	4117.5	
	4102.2	

Table: 2 Repeatability of sample

III. RESULTS AND DISCUSSION:

A rapid, simple, precise method was developed for the estimation of active principle in Triphala Churna formulation by HPTLC method.

In HPTLC method, a wavelength of 254 NM was selected and the mobile phase which consists of Toluene: Ethyl acetate: Formic acid : Ammonia in the ratio of 12:6:0.4:0.8 were found to be optimum condition for the analysis. The Gallic acid is the main marker compound of this formulation. Hence the assay results at these compounds can be kept as standard. The Effective value for Gallic acid was found to be 0.16.The linearity range was found to be 200-1000ng/spot.

Slope, intercept and correlation values were found to be 1127.7, 859.43 and 0.98.

Precision of this method was studied by making repeated sample application and standard deviation was determined.

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IV. CONCLUSION:

The prepared HPTLC method was found to be rapid, simple and accurate for quantitative estimation of Gallic acid in Triphala Churna formulation extracts.

The method quantify the Gallic acid effectively, it can be used to quantify the concentration of the active principles in the herbal formulations.

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