

Development And Validation Of UV Spectrophotometric Method For Simultaneous Estimation Of Rutin And Gallic Acid In Hydroalcoholic Extract Of Triphala churna.

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Abstract: A simple, rapid, accurate, precise, and economic spectrophotometric method for simultaneous estimation of Rutin and Gallic acid in *Triphala churna* have been developed. Method is based on solving simultaneous equation. rutin and gallic acid show absorbance maximum at 359 and 273 nm respectively, so absorbance was measured at the same wave lengths for the estimation of rutin and gallic acid. Both drugs obey the Beer Lambert's law in the concentration range of 5-30 µg/mL. Methods are validated according to ICH guidelines and can be adopted for the routine analysis of rutin and gallic acid in hydroalcoholic extract of *Triphala churna*.

Key words: Rutin, Gallic acid, Simultaneous equation, *Triphala churna*, Validation.

INTRODUCTION

Triphala churna is [1:1:1] combination of three compound viz. *Terminalia chebula*, *Terminalia bellerica*, *Terminalia officinalis* each of which have its own therapeutic value but in combination it enhances overall potential these are used mostly used as antioxidant, antiaging, anti-inflammatory, mental and memory enhancing effect. The extract of *Triphala* was obtained by continuous heat extraction by using Soxhlet extraction process and ethanol water in ratio (70:30). Marker which have been found in *Triphala* by various method are quercetin, rutin, gallic acid, ellagic acid, ascorbic acid these are found in *Triphala* and known for its effect [4,5,6,8,10]. Gallic acid is phenyl propanoid, chemically it is 3, 4, 5,-Trihydroxybenzoic acid, and possess astringent activity [4]. Rutin is 5, 7, 3, 4, tetrahydroxy flavonol -3-rhamanoglucoside and widely used in medicine for maintenance of capillary integrity [5]. Both possess antioxidant activity and reduce low density lipoproteins [LDL] oxidation [3].

Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques. The literature revealed that no UV-spectrophotometric method is not yet reported for the estimation of rutin and gallic acid in *Triphala churna*. The method is developed in solvent Methanol. Method validation is done as per ICH guidelines [10].

Thus, this method is more accurate and cost effective. This paper describes simple, rapid, accurate, precise and economical method for simultaneous determination of rutin and gallic acid in *Triphala extract* form.

MATERIALS AND METHODS

Instruments

Absorbance measurements was made on Shimadzu 1800 UV/Visible spectrophotometer with a pair of matched quartz cells of 1 cm width, Elder digital balance used for weighing, and Ultra sonicator of Prama instruments was used sonicating the drug and sample solution.

Materials

Flavanoids standards (Rutin and Gallic acid) purchased from Natural remedies, Bangalore, India (purity >97%). *Triphala churna* was purchased from local market. All the chemicals and reagents were of analytical grade and were purchased from S.D fine, Mumbai.

Selection of common solvent

After assessing the solubility of drugs in different solvents Methanol has been selected as common solvent for developing spectral characteristics.

Selection of wavelength

A representative spectrum of rutin and gallic acid in Methanol has been obtained. The dilution was obtained to the concentration of 10 µg/ml for Rutin and 10 µg/ml for Gallic acid solutions. Both the solutions were scanned in UV range (200-400nm) in 10 mm cell against solvent blank. The study of spectrum revealed that rutin show a well defined max at 359 nm where as gallic acid shows at 273 nm. These two wavelengths were selected for development of simultaneous equation.

Preparation of standard stock solution and Study of Beer-Lambert's Law

The standard stock solutions of rutin and gallic acid were prepared by dissolving 50 mg of each drug in Methanol and final volume was adjusted with same solvent in 50 mL of volumetric flask to get a solution containing 1000 µg/mL of each drug. Aliquots of working stock solutions of rutin and gallic acid were prepared with Methanol solution to get concentration in range of 5-30 µg/ml for rutin and 5-30 µg/ml for gallic acid. The absorbance's of resulting solutions were measured at their respective max. A calibration curve as concentration vs. absorbance (**Fig-1, 2**) was constructed to study the Beer-Lambert's Law and regression equation.

Method (Simultaneous equation method) [4]:

If a sample contains two absorbing drug each of which absorbs at the max of the other, it may be possible to determine both drugs by the technique of simultaneous equation. Two wavelengths selected for the development of the simultaneous equations are 359 nm and 273 nm. The absorptivity values determined for Rutin are 0.0246 (ax1), 0.0224 (ax2) and for Gallic acid are 0.002 (ay1), 0.0186 (ay2) at 359 nm and 273 nm respectively. These values are means of six estimations.

Analysis of the *Triphala churna*

A quantity of *Triphala extract* 50mg was transferred to 50 mL volumetric flask and dissolved in methanol and final volume was made up with methanol. The sample solution was then filtered through Whatman filter paper No.41. From the above solution 0.373ml of solution was taken and diluted to 10 mL with methanol to get final concentration containing 37.31µg of solution containing 5µg/mL of rutin and 5.59 µg/mL of gallic acid. Analysis procedure was repeated six times with *Triphala extract*. The results *Triphala* extract analysis are reported in Table 4.

VALIDATION OF THE DEVELOPED METHODS [7, 8]:

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method the Beer- Lambert's concentration range was found to be 5-30 µg/mL for rutin and 5-30 µg/mL for gallic acid. The linearity data for method is presented in Table 1.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The result of the recovery studies are reported in **Table 3**.

Precision:

Interday and Intraday precision

The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively (six replicates). **The results of the same are presented in Table 2.**

Ruggedness study:

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by spiking the standard 3 times with different analyst by using same equipment. The results of the same are presented in **Table 5**.

Limit of detection

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$DL = \frac{3.3 \sigma}{S}$$

Where σ = the standard deviation of the response
S = the slope of the calibration curve

Limit of quantitation

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

$$QL = \frac{10 \sigma}{S}$$

Where σ = the standard deviation of the response
S = the slope of the calibration curve

RESULT AND DISCUSSION

Linearity range for Rutin and Gallic acid are 5-30 μ g/mL and 5-30 μ g/mL at respective selected wavelengths. The coefficient of correlation for Rutin at 359 nm and for Gallic acid at 273 nm is 0.999 and 0.9941 respectively. Both drugs shows good regression values at their respective wavelengths and the results of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation of Rutin and Gallic acid in *Triphala extract* was found by method is 99.78 \pm 0.326 and 101.35 \pm 0.947 \pm standard deviation with standard deviation <2.

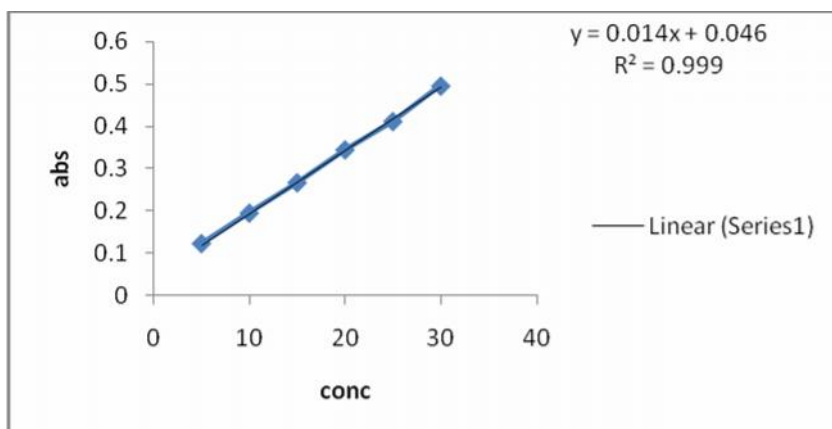


Fig.1. Calibration curve of Rutin

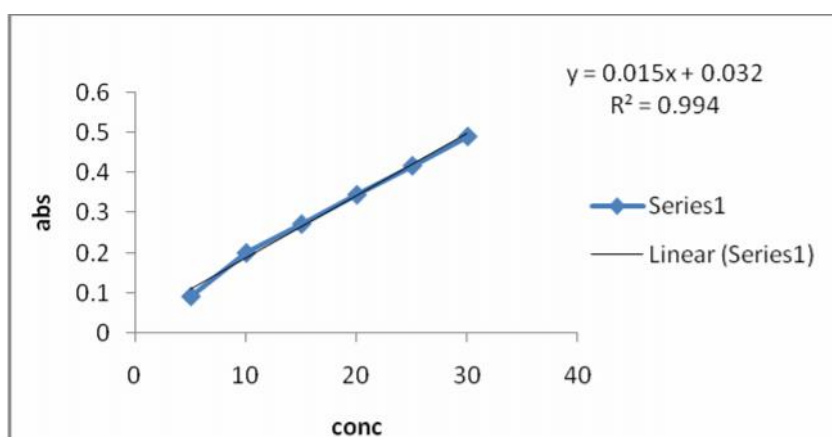


Fig.2. Calibration curve of Gallic acid

Table 1. Result of Validation parameters [7, 8]:

Parameters	Rutin	Gallic acid
Detection wavelength	359 nm	273nm
Linearity range	5-30 μ g/mL	5-30 μ g/mL
Slope	0.014	0.01550
Intercept	0.004693	0.001905
Correlation coefficient	0.999	0.994
Regression equation (y =a + bc)	Y=0.014x-0.046	Y=0.015x-0.032
Limit of detection	0.023 μ g/mL	0.014 μ g/mL
Limit of quantitation	0.068 μ g/mL	0.045 μ g/mL

The validity and reliability of proposed methods are assessed by recovery studies. Sample recovery for both the methods is in good agreement, which suggest non interference of other extracted content in estimation ss(Table 3).

Precision is determined by studying the interday and intraday precision. In both intra and inter day precision study for both the methods % RSD are not more than 2.0% indicates good repeatability and intermediate precision (Table 2).

Table 2. Interday and Intraday precision [7, 8]:

Interday precision			Intraday precision	
	%Amount found \pm SD*	% RSD	%Amount found \pm SD*	% RSD
RUTIN	99.78 \pm 0.326	0.3230	99.78 \pm 0.816	0.818
GALLIC ACID	101.35 \pm 0.947	0.9378	99.63 \pm 0.416	0.417

*Average of six determinations

Table 3. Recovery studies [7, 8]:

Concentration of the drug added to the formulation	RUTIN % Recovery \pm SD*	%RSD	Gallic acid % Recovery \pm SD*	%RSD
80%	100.73 \pm 0.450	0.442	102.36 \pm 0.172	1.17
100%	101.73 \pm 0.907	0.891	103.4 \pm 0.435	0.42
120%	100.90 \pm 0.725	0.718	103.17 \pm 0.0519	0.0503

*Average of three determinations

Table 4. Result of analysis of *Triphala Extract* :

Formulation	Drug	Amount Found μ g/ml \pm S.D*
<i>Triphala extract</i>	Rutin	0.67 \pm 0.005144
	Gallic acid	0.76 \pm 0.01171

*Average of three determinations

Table 5: Ruggedness study:

<i>Triphala churna</i>	Drug	%Amount found \pm S.D*
Analyst 1	Rutin	100.93 \pm 0.461
	Gallic acid	100.7 \pm 1.039
Analyst 2	Rutin	100.93 \pm 0.461
	Gallic acid	13.12 \pm 1.039

CONCLUSION

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for simultaneous estimation of Rutin and Gallic acid in *Triphala churna*.

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