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Simultaneous Estimation of Gallic acid and Rutin in Marketed Polyherbal Formulations by HPTLC

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Abstract: A simple, rapid and reliable HPTLC method has been developed for simultaneous estimation of gallic acid and rutin in polyherbal formulations. Identification and quantification were performed on 20 cm x 10 cm, layer thickness 0.2 mm, aluminum- backed silica gel 60 F_{254} HPTLC plates previously washed with methanol. Toluene: Acetone: Ethyl Acetate: Formic Acid: Water (2: 3: 2: 1: 2, v / v), was used as a mobile phase. Calibration plot were established showing the dependence of response on the amount chromatographed. The validated calibration range was 400-800 ng per spot (R^2 = 0.998; 0.996). The spots were scanned at λ = 312 nm. The suitability of this HPTLC method for simultaneous estimation of the marker constituents was proved by validation in accordance with ICH Guidelines. Determination of method accuracy by the standard addition method at three concentration levels returned a mean recovery of 98.92 ± 0.16 - 101.61 ± 0.24. The developed method has the advantage of being rapid and easy. Hence it can be applied for routine quality control analysis of gallic acid and rutin in polyherbal formulations.
Key Words: gallic acid and rutin, HPTLC, Validation, Polyherbal formulations.

Introduction:

Bahera (*Terminalia belerica*, Family-Combretaceae) [1] is a proven anti-atherogenic agent that reduces cholesterol and good for eyes, hair and voice. The fruits of *Terminalia bellerica* are commonly used in the treatment of dyspepsia and diarrhea. The fruits contain about 20-30% of tannins [2] of which Gallic acid, ellagic acid, phyllemblin, ethyl gallate and galloglucose are major hydrolysable tannins and a flavanoid Rutin. Therefore, estimation of Gallic acid and Rutin would be an important parameter for quality control of polyherbal formulations. Gallic acid [3] is an organic acid also known as 3,4,5 trihydroxybenzoicacid. It is commonly used in pharmaceutical industry. It is used as a standard for determining the phenol content of various analytes by the Folin-ciocalteau assay results are reported in Gallic acid equivalents. Gallic acid seems to have antifungal and antiviral properties. Gallic acid acts as antioxidant [4] and helps to protect our cells against oxidative damage, Gallic acid was found to show cytotoxic against cancer cells, without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal hemorrhage. Gallic acid is also used to treat albuminuria and diabetes. Some ointments to treat psoriasis and external hemorrhoids contain gallic acid. Rutin[5] is a citrus flavonoid glycoside found in buckwheat the leaves and petioles of rheum species and asparagus. Rutin inhibits platelet aggregation as well as decreasing capillary permeability, making the blood thinner and improves circulation.

Extensive Literature survey reveals that, HPTLC and HPLC [6] methods are reported for the determination of Gallic acid and Rutin. But, no method is reported for the simultaneous estimation of Gallic acid and Rutin in polyherbal formulations. The aim of the work is to develop a simple, precise, rapid and cost effective HPTLC method for the simultaneous estimation of Gallic acid and Rutin in polyherbal formulations.

Materials and Methods:

Instrumentation

A LINOMAT 5-HPTLC with CAMAG- TLC Scanner 3 equipped with Win-CAT software, version 1.44 was used.

Reagents, Marker Constituents and Polyherbal formulations used

- All chemicals and reagents including Ethyl Acetate, Formic Acid, Methanol, Toluene and Alcoholic $FeCl_3$ were of analytical grade and were used throughout the experiment.
- Analytically pure samples of gallic acid and rutin were procured as gift sample from M/s Natural Remedies Pvt. Ltd., (Bangalore, India).
- The polyherbal formulations used for present study were purchased from Gururaja Pharmacy, Bangalore.

Marketed Polyherbal Formulations:

Formulation 1: Sarvadi Vati

Formulation 2: Lawangadi Vati

Preparation of Standard solution

Accurately weighed 10 mg of gallic acid standard was dissolved in 10 ml of methanol in a volumetric flask (A). 2 ml of this solution was diluted to 10 ml with methanol (B). Accurately weighed 10 mg of rutin standard was dissolved in 10 ml of methanol in a volumetric flask (C). 2 ml of this solution was diluted to 10 ml with methanol (D). Working standard solution (E) was prepared by mixing 5ml of B and 5 ml of D. Solution E was used for the HPTLC analysis.

A stock solution containing 200 mcg / ml gallic acid (B) and rutin (D) were prepared in methanol. Calibration solutions were prepared by diluting the stock solution so that application of 4-8 µl volumes gave a series of spots covering the range 400 to 800 ng of gallic acid and rutin respectively (**Figure 1**).

Preparation of Sample solution

Amount equivalent to the contents of the formulation was extracted twice with 10 ml of methanol by boiling for 10 minutes. Extract obtained was filtered using Whatman filter paper, concentrated top less than 10 ml and transferred to a 10 ml volumetric flask and volume was made up with methanol. Sarvadi Vati and Lawangadi Vati were weighed in amounts of 0.35 gm and 0.34 gm respectively.

Validation of the method

After the development of HPTLC method for the simultaneous estimation of the poly herbal formulations, Validation of the method was carried out according to the ICH guidelines with respect to Linearity, Accuracy, Precision, Limit of Detection and Limit of Quantification. [7-9]

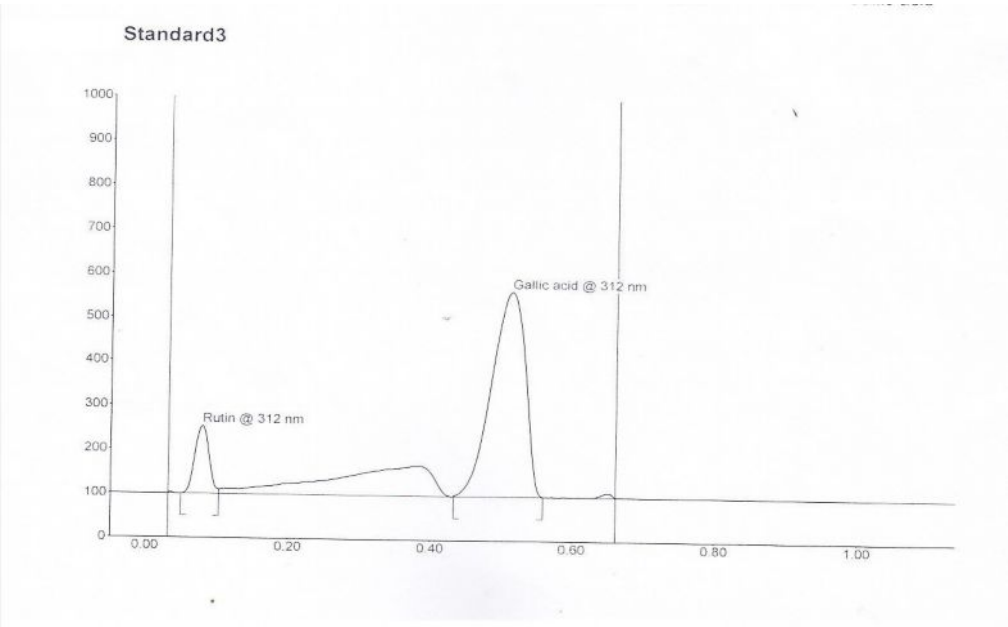

Figure 1: Typical HPTLC Chromatogram of Gallic acid and Rutin by HPTLC method

Table 1: Calibration data of Gallic acid by HPTLC method

S.NO	Amount in ng/Spot	Rf values	Peak area
1	400	0.45	1463.3
2	500	0.44	1979.8
3	600	0.43	2380.8
4	700	0.43	2856.9
5	800	0.42	3265.8

Table 2: Calibration data of Rutin by HPTLC method

S.NO	Amount in ng/Spot	Rf values	Peak area
1	400	0.05	13989.8
2	500	0.05	15214.1
3	600	0.05	16112.1
4	700	0.05	17121.2
5	800	0.05	18412.3

Table 3: Characteristic parameters for the proposed HPTLC method

Parameters	HPTLC	
	GALLIC ACID	RUTIN
Calibration range (ng / spot)	400-800	400-800
Detection wavelength	312nm	312nm
Mobile phase (Toluene : Acetone : Ethylacetate : Formic acid : Water)	2 : 3 : 2 : 1 : 2	2 : 3 : 2 : 1 : 2
Rf value	0.43	0.05
Regression equation (y*)	Y = 4.482x – 299.9	Y = 10.75x + 9718.6
Slope (b)	4.4821	10.7521
Intercept (a)	-299.94	9718.6
Correlation coefficient(R^2)	0.998	0.996
Limit of detection (ng/spot)	9.504	0.306
Limit of quantitation (ng/spot)	28.802	0.930

*y = b x + a, where x is the concentration of Gallic acid and Rutin in ng/spot and y is the peak area at respective wavelength.
Mean** = Average of three linearity curves

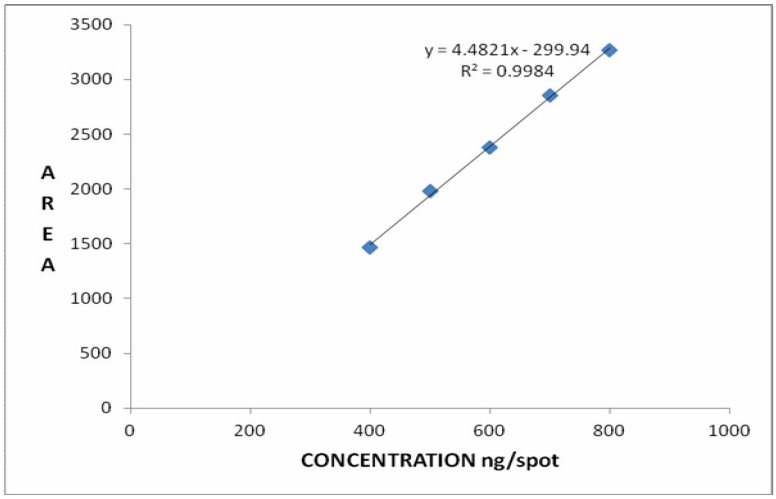

Figure 2: Calibration curve of Gallic acid by HPTLC method

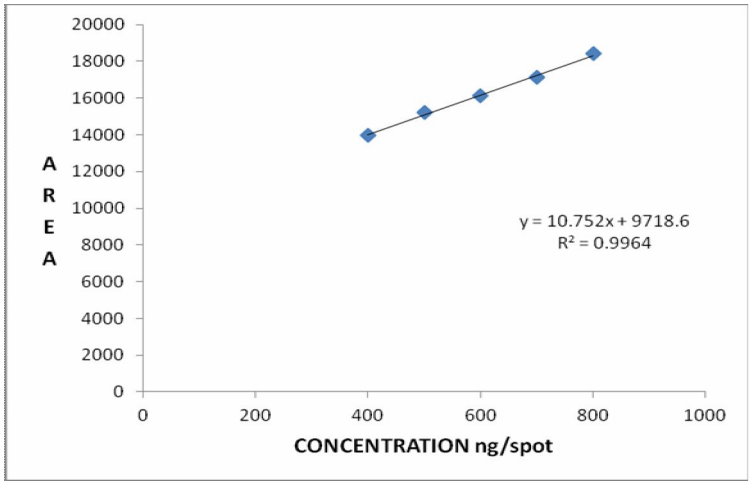

Figure 3: Calibration curve of Rutin by HPTLC method

Results and Discussion:

A wavelength of 312 nm was chosen for quantification. The Rf value of gallic acid and rutin after development with the mobile phase Toluene: Acetone: Ethyl Acetate: Formic Acid: Water (2: 3: 2: 1: 2, v / v) was 0.43 and 0.05 respectively. When the concentrations of gallic acid and rutin and their respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (R^2 = 0.998; 0.996) was observed between the concentrations of gallic acid and rutin and the respective peak areas in the range 400 - 800 ng / spot. The regression of gallic acid and rutin was found to be Y = 4.482 X – 299.9 and Y= 10.75 X + 9718 respectively, where 'Y' is the peak area and 'X' is the concentration of gallic acid and rutin respectively are shown in **Table: 3.** The regression equations were used to estimate the amounts of gallic acid and rutin, in tablet Polyherbal formulations or in validation study (precision and accuracy). The content of Gallic acid and Rutin present in polyherbal formulations were shown in **Table: 8.** The chromatograms containing peaks of Gallic acid and Rutin in polyherbal formulations are shown in **Figure: 4 and 5** respectively.

Precision

The precision of the method in terms of intra - day precision (% RSD) was determined by analyzing gallic acid and rutin standard solutions in the range (400 - 800 ng / spot) three times on the same day. Inter - day precision (% RSD) was assessed by analyzing these solutions (400 - 800 ng / spot) on three different days over a period of one week. The results of the precision studies are shown in **Table: 4 and 5.**

Accuracy

Determination of method accuracy by the standard addition method at three concentration levels returned a mean recovery of 98.92 ± 0.16 - 101.61 ± 0.24 is given in **Table: 6 and 7.**

Table 4: Precision of Gallic acid by HPTLC method

S. No.	Concentration (ng/spot)	Intraday precision (Area)	Interday precision (Area)
1	600	2380.7	2452.8
2	600	2390.8	2491.3
3	600	2415.7	2501.8
4	600	2438.5	2520.4
5	600	2445.9	2544.8
6	600	2456.8	2589.7
Mean		2421.4	2516.8
Std.Dev		30.890	47.079
%RSD		1.275	1.870

**Average of six determinations

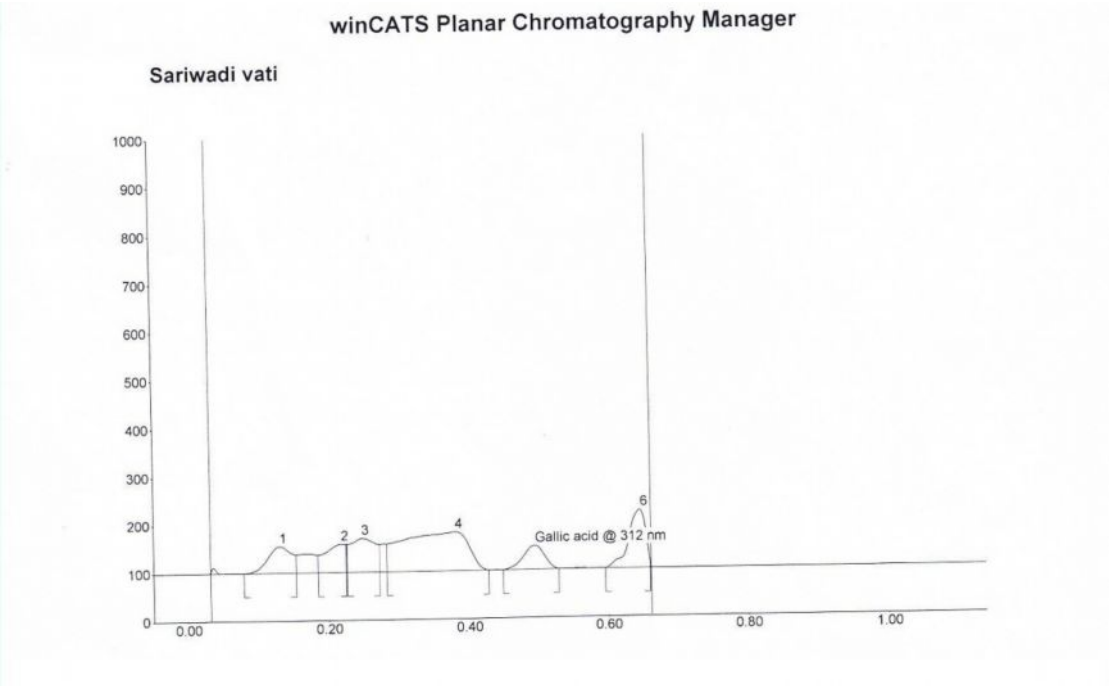

Figure 4: Typical HPTLC Chromatogram of Formulation I by HPTLC method

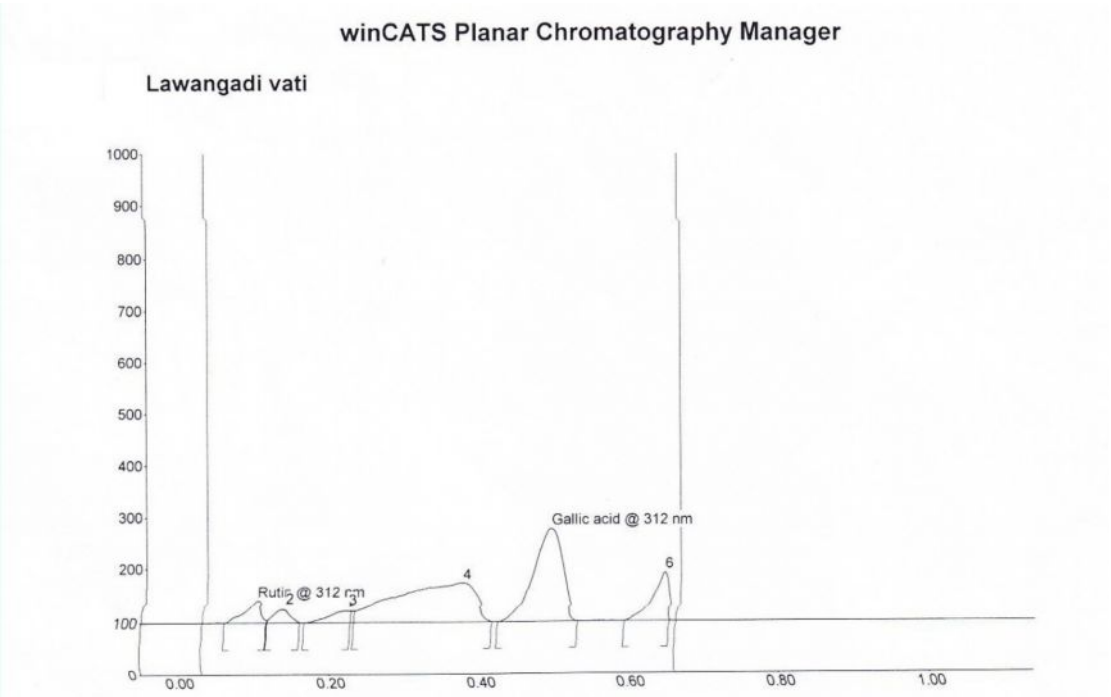

Figure 5: Typical HPTLC Chromatogram of Formulation II by HPTLC method

Table 5: Precision of Rutin by HPTLC method

S. No.	Concentration (ng/spot)	Intraday precision (Area)	Interday precision (Area)
1	600	16112.1	15914.1
2	600	16114.1	15863.1
3	600	16113.1	15962.1
4	600	15962.1	16063.1
5	600	15863.1	15564.1
6	600	15914.1	15712.1
Mean		16013.1	15846.4
Std.Dev		113.93	180.52
%RSD		0.71	1.13

**Average of six determinations

Table 6: Recovery studies of Gallic acid by HPTLC method

S.No	Sample	Initial amount (ng/spot)	Amount added (ng/spot)	Amount recovered* (ng/spot)	Recovery ± SD* (%)	% RSD
1	Sarvadi vati	600	300(50%) 600(100%) 900(150%)	99.13 100.08 99.96	99.13±1.20 100.08±1.80 99.96±0.72	1.205 1.805 0.723
2	Lawangadi vati	600	300(50%) 600(100%) 900(150%)	99.24 101.14 99.54	99.24±0.11 101.14±0.17 99.54±0.07	0.118 0.173 0.076

Mean* ± S.D. from six determinations

Table 7: Recovery studies of Rutin by HPTLC method

S.No	Sample	Initial amount (ng/spot)	Amount added (ng/spot)	Amount recovered* (ng/spot)	Recovery ± SD* (%)	% RSD
1	Sarvadi vati	600	300(50%) 600(100%) 900(150%)	99.13 101.29 99.48	99.13±0.16 101.29±0.24 99.48±0.09	0.167 0245 0.100
2	Lawangadi vati	600	300(50%) 600(100%) 900(150%)	98.92 101.61 99.64	98.92±0.16 101.61±024 99.64±0.59	0.162 0.237 0.592

Mean* ± S.D. from six determinations

Table 8: Content of Gallic acid and Rutin in polyherbal formulations

S.NO	Samples	Gallic acid (%)	Rutin (%)
1.	Sarvadi Vati	0.0432	ND
2.	Lawangadi Vati	0.8383	0.01397

Linearity

The linearity was found in the concentration range of 400 - 800 ng / spot. The correlation coefficient was found to be 0.998 and 0.996 for gallic acid and rutin respectively. The results are presented in **Table: 1 and 2** and **Figure 2 and 3** respectively.

Limit of Detection and Limit of Quantitation

The LOD and LOQ of gallic acid were found to be 9.504 and 28.802 respectively. The LOD and LOQ of rutin were found to be 0.306 and 0.930 respectively. % assay were calculated and reported in **Table: 3**

Conclusion:

In the present study, on the simultaneous estimation of Gallic acid and Rutin in marketed polyherbal formulations by HPTLC, wide variations in the content of Gallic acid and Rutin in the formulations to be administered or prescribed by the physicians were observed. This shows that Polyherbal formulations are not standardized. This leads to marked differences in the therapeutic efficacy of the formulations when administered. Hence, the newly developed method for the simultaneous estimation of Gallic acid and Rutin in Polyherbal formulations can be adapted to standardize the formulations, and the content of Gallic acid and Rutin can be altered during the formulation stage, thus ensuring desired therapeutic efficacy of the herbal product. This would also minimize or avoid the batch-to-batch variations in the therapeutic efficacy of such Polyherbal formulations.

References:

1. Marker Compounds of Ayurvedic Drugs, WHO Country Office for India, New Delhi, July 2009.
2. Kokate CK, Purohit AP and Gokhale SB. Text book of Pharmacognosy. Jan2007; p.258-59.
3. URL: http://en.wikipedia.org/wiki/File:Gallic_acid.svg.
4. Natvarbhai M Petal, Vineet C Tain, Anurag Bhargav and Dhiren Pshah. HPTLC method for Quantification of Gallic acid in root powder of *Alangium salvifolium* wang. Int J Pharm Research. April-June2010; 2(3):40-44.
5. URL: http://en.wikipedia.org/wiki/File:Rutin_structure.svg
6. Ashok kumar BS, Lakshman K, Jayaveera KN and Satish KV. Estimation of Rutin and Quercetin in *Terminalia chebula* by HPLC. The Internet J Aesthetic & Antiaging medicine. 2009.
7. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text on Validation of Analytical Procedures Q2A, 1994.
8. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Methodology Q2B, 1996.
9. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005.

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