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RESEARCH ARTICLE

Urolithiasis: HPTLC Method for Quantitative Detection of Rutin and Quercetin in an Herbal Plant

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Abstract

Anti-inflammatory, Hypoglycemic, Hepatoprotective, Antihyperlipidemic, Anti-Ulcerative, Cardioprotective Stimulant, Sedative, Hypnotic, Anticonvulsant Activity, Memory Retention, Stroke Preventive Activity, Antimicrobial Activity, Antimycobacterial, Antiviral, Larvicidal, Antiparasitic, Chemopreventative, Chemo-modulatory, Anticancer, Cytoprotective activities were found in a variety of herbal plants, including *Ocimum basilicum.* The purpose of this study was to design and develop a new HPTLC method that was accurate, precise and cost-effective for simultaneous measurement of rutin and quercetin in a hydroalcoholic extract of *Ocimum basilicum* seeds. The mobile phase was Toluene: Ethyl Acetate: Methanol: formic acid (6:4:3:1, v/v/v/v/v) and densitometric scanning was performed at 254 nm. Merck TLC aluminium sheets of silica gel 60 F254, (10 x 10 cm) with a thickness of 250 mm was used as stationary phase. At a wavelength of 254 nm, rutin and quercetin were detected. The constituents were resolved satisfactorily, with Rf values of 0.25 ± 2.01 for rutin and 0.80 ± 0.64 for quercetin, respectively. Linearity (300-1300 ng/spot for rutin and quercetin) was used to test the method's accuracy and reproducibility. For both analytes, intra- and inter-day precision, as evaluated by coefficient of variation, was less than 3%. For rutin, the detection and quantification limits were 46.52 and 140.96 ng/spot, respectively, and for quercetin, they were 81.79 and 247.84 ng/spot. The proposed approach was found to be precise, accurate, repeatable, and specific, and it could be used to measure quercetin and rutin in samples at the same time.

Keywords: Densitometry, *Ocimum basilicum,* Quercetin, High-performance Thin-layer Chromatography (HPTLC), Rutin, Validation

1. Introduction

Herbal therapies have stood the test of time due to their safety, efficacy, cultural acceptability, and absence of adverse effects. Because of their herbal character, it is thought that they will be more compatible with the biological system. A variety of herbal flowers have been employed in formulations as antidiabetic and antioxidant ingredients since ancient times[1]. Flavonoids are a type of polyphenolic chemical that can be found in abundance in plants. Flavonoids have been discovered in over 300 different types so far[2]. Flavonoids are a wide group of polyphenolic chemicals found in plants that have a benzopyrone structure and perform a variety of important functions,

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including antioxidant and chelating properties[3-5]. Human defensive enzyme systems can be induced by flavonoids. In a variety of studies, flavonoids have been found to protect against infectious (bacterial and viral infections) and cardiovascular disease, cancer, and other age-related disorders are examples of degenerative diseases[6-8]. Flavonoids also serve as a secondary antioxidant defence structure in plant tissues under a variety of abiotic and biotic challenges. They're discovered in mesophyll cell nuclei as well as *Reactive oxygen species* (ROS) generation centres. They also control boom components, as well as flora auxin[9]. For more efficient flavonoid production, biosynthetic genes have been coupled in a range of bacteria and fungi[10]. Flavonoids play an important role in a variety of biological and pharmacological processes[11,12]. Citrus fruits contain rutin, a flavonoid glycoside. Its chemical name is 5,7,3,4-tetrahydroxy flavonol-three-rhamanoglucoside, and it's used in medicine to maintain capillary integrity. Liquiritin (LIQ) is the flavanone liquiritigenin's 4'-O-glucoside. Liquiritin (LIQ) is the 4'-O-glucoside of the flavanone liquiritigenin, which is a 3,5,7,3,4′-pentahydroxyflavone. *Ocimum basilicum* is a valuable medicinal herb that has a wide range of healing effects[13]. There is currently no HPTLC method for quantifying rutin and quercetin in Ocimum *Basilicum* seeds, to the author's knowledge. The proposed method for evaluating rutin and quercetin simultaneously in *Ocimum basilicum* was confirmed according to the ICH rule[14] (International Conference on Harmonization, 1996), which is similar to the other laboratory-developed processes for natural drug standardization[15-17, 21-22].

2. Methods and Materials

2.1 Plant Identification and Collecting

In October 2018, Dr. A. S. Reddy of Sardar Patel University's Department of Biosciences, Vallabh Vidyanagar-388120 Gujarat, India, taxonomically identified and certified the plant material as seeds of *Ocimum basilicum*. The vouched specimens with the number NO. SMSHAH-01 was stored in the herbarium for future reference.

2.2 Chemical and Reagents

Rutin and quercetin were procured from Yucca Enterprise, Mumbai, India. All of the chemical ingredients and reagents were of AR grade. TLC plates with 2 mm layer of silica gel 60 F254 (10cm x 10cm) was used as stationary phase which is obtained from E. Merck (Germany).

2.3 Preparation of Standard Stock Solution

By dissolving 10 mg of correctly weighed rutin and quercetin in methanol and diluting to ten ml methanol in a Standard volumetric flask (1 mg/ml). Before being evaluated on a TLC plate, the stock solutions were filtered with a whatman filter paper.

2.4 Preparation of Samples

The plant material was thoroughly cleaned, then dried in the shade before being coarsely pulverized and extracted. In a nutshell, 20 g of powder is placed in the cellulose cartridge before being placed in the soxhlet assembly. 150 mL Hydro-alcoholic [ethanol: water (70:30, v/v)] was added to the flask, which was then brought to a boil for 4 hours at roughly 65°C. The dried extract was then kept at 4°C in an airtight container. The suitably weighed samples (30 mg) were diluted in 10 mL of extraction solvent (70:30, v/v) and sonicated in an ultra sonicator water bath for 20 minutes at 27±3^{0}C. With the help of whatman filter paper, the sample solutions were filtered and applied on TLC plates.

2.5 TLC Instrumentation and Operating Conditions

The samples were applied using a Camag Linomat V sample applicator with a Hamilton syringe on a precoated silica gel aluminium plate 60 F254 (10 cm 10 cm with 0.2 mm thickness, E. Merck, Germany) and a Camag HPTLC machine with a Hamilton syringe on a precoated silica gel aluminium plate 60 F254 (10 cm 10 cm with 0.2 mm thickness (Switzerland). The samples were applied at a constant rate of 150 nL/s, with a gap of 11.6 mm between the two bands. The scanning speed was set to 20 mm/s and the slit measurement

was kept at 6.0 x 0.45 mm. Twin trough glass container saturated with the mobile phase Toluene : Ethyl acetate: Methanol: formic acid (6:4:3:1, v/v/v/v/v) was used for linear ascending development. At room temperature, the mobile phase compartment was saturated for 30 minutes, and the densitogram was obtained up to an 80-mm length. Using an air-dryer, the TLC plates were dried in current of air. Densitometric scanning in the absorbance mode at 254 nm was performed with the Camag TLC scanner III. Deuterium lamp was used as radiation source.

2.6. Linearity (Calibration Curve)

Rutin and quercetin standard solutions of 1 mg/mL were prepared in methanol. For rutin and quercetin, different amounts of standard solution were spotted three times on the TLC plate, yielding a final concentration of 300-1300 ng/spot. Following that, the data of peak area vs. concentration was used to generate a regression equation using linear least squares regression analysis.

2.7 Method Validation

According to ICH criteria, the proposed technique was validated for accuracy, precision, linearity, detection and quantification limits.

2.7.1 Accuracy (% Recovery)

Calculating rutin and quercetin recoveries using the traditional addition method was used to determine the accuracy of the method. To a pre-quantified rutin and quercetin sample solution, known amounts of standard rutin and quercetin solutions were added at 80, 100, and 120 percent levels. By plotting the acquired values into the relevant regression line equations, the amount of rutin and quercetin was calculated.

2.7.2 Precision

The reproducibility of sample application and measurement of peak regions for six repetitions of the same band established the precision of the system. Six samples were produced and tested at three different concentrations on three different days (300, 900, and 1300 ng per spot for quercetin and rutin, respectively). Intra-day precision was investigated by comparing trials performed on the same day (intermediate precision). The relative standard deviation (RSD percent) and the standard deviation (SD) of peak area were used to express the precision of the system and method.

2.7.3 Robustness

The results were computed with slight changes in the chamber saturation time and analysis wavelength. The method's robustness was tested three times at 900ng/band Rutin and Quercetin concentrations. The area average, the Rf average, and the percent RSD of the peak Rf were calculated.

2.7.4 Limit of Detection and Limit of Quantification

Signal to noise ratio was used to determine the limit of detection (LOD) and limit of quantification (LOQ). The signal-to-noise ratio was calculated as 3:1 for LOD and 10:1 for LOQ.

2.8 Estimation of Rutin and Quercetin in a Hydro-alcoholic Extract of *Ocimum basilicum* Seeds

On TLC plates, two mL of each sample (as discussed in sample preparation) were applied in triplicates. It was created and scanned according to the instructions. The regression equation was used to quantify samples using peak area data for rutin and quercetin. The average amount of quercetin and rutin in percent w/w was calculated.

3. Result

3.1 Optimization and Selection of Mobile Phase

The ideal mobile phase for the detection and quantification of rutin and quercetin in an *Ocimum basiicum* seed hydro-alcoholic extract were selected based on trials taken on different mobile phase. By altering the mobile phase, the TLC process was improved. Toluene: Ethyl acetate: Methanol: Formic acid: in a ratio of 6:4:3:1, v/v/v/v/v produced high-resolution rutin and quercetin spots. The bands were compact and well resolved at Rf value of 0.25 ± 2.01 and 0.80 ± 0.64 for rutin and quercetin respectively

(Figures 1A and 1B). Other chromatographic parameters include sample application rate and volume, run length, chamber saturation time, and sample application adjusted for exact and repeatable Rf values, increased resolution, and symmetrical peak shape for the substances. For exact and repeatable Rf values, greater resolution and symmetrical peak shape for the compounds, the positions, detecting wavelength, and distance between tracks were tuned.

3.2 Calibration of Curves

As shown in Tables 1 and 2 and Figures 2 and 3, the linear regression data gathered for the calibration curves (n=6) revealed a good linear correlation for rutin and quercetin with reference to the calibration curve throughout a wide concentration range of 300-1300 ng per spot.

3.3 Validation

Precision, robustness, accuracy, LOD, LOQ, and specificity were all tested as part of the validation process.

3.3.1 Precision

As indicated in Table 3, the examination of peak area three times inter-day and intra-day revealed a percent R.S.D (3%) showing that the method was precise.

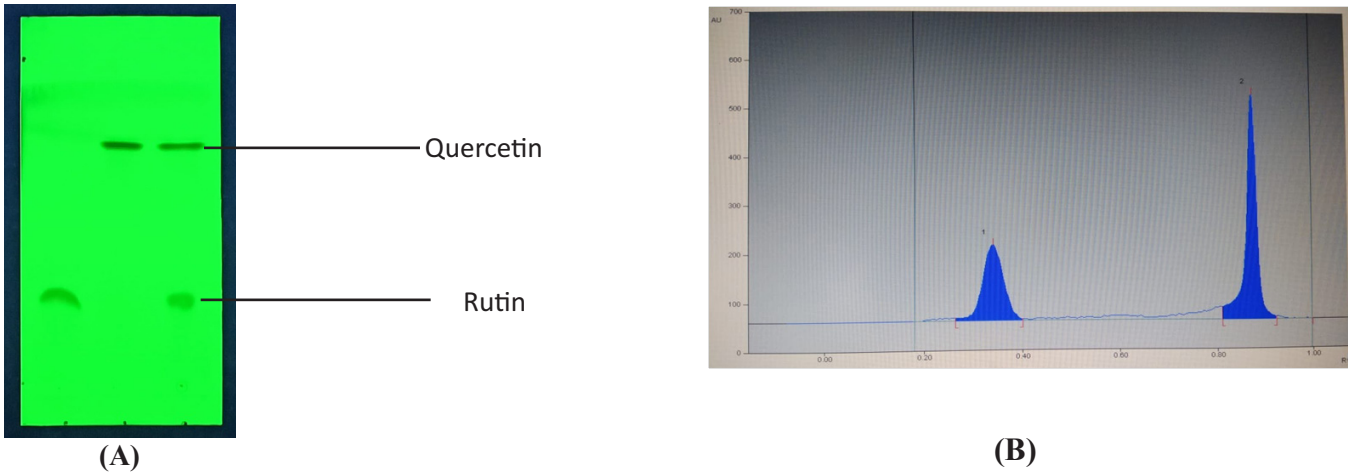

Figure 1. **(A)** TLC plate showing resolution of rutin and quercetin in Hydro-alcoholic extract of *Ocimum basiicum*. **(B)** A typical TLC chromatogram of rutin and quercetin (Rf = 0.25 ± 2.01 and 0.80 ± 0.64).

Table 1. Data from linear regression for rutin calibration (n=6)

Concentration (ng/band)	Area (n=6) ± SD	% RSD
300	2075.1 ± 43.07197	2.075658
500	3264.92 ± 45.15094	1.382911
700	4275.16 ± 52.41138	1.225951
900	5172.06 ± 152.8504	2.95531
1100	6153.56 ± 93.32825	1.516655
1300	7096 ± 104.6897	1.475333

Table 2. Data from linear regression for quercetin calibration (n=6)

Concentration (ng/band)	**Area (n=6) ± SD**	**% RSD**
300	3330.26 ± 74.90876	2.249337
500	4441.66 ± 105.1977	2.368433
700	5498 ± 71.97892	1.309184
900	6930.7 ± 112.4304	1.622209
1100	7978.84 ± 109.722	1.375163
1300	8923.88 ± 184.704	2.069772

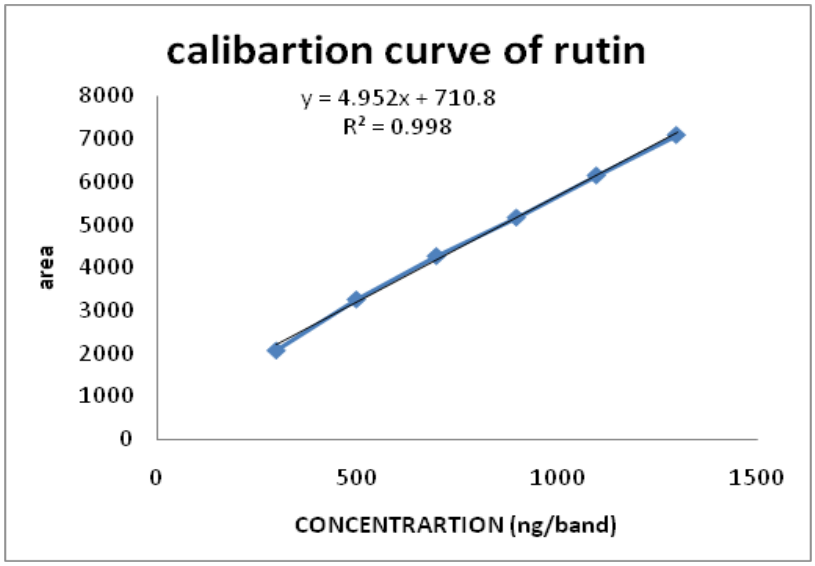

Figure 2. Calibration curve for standard rutin (n= 6).

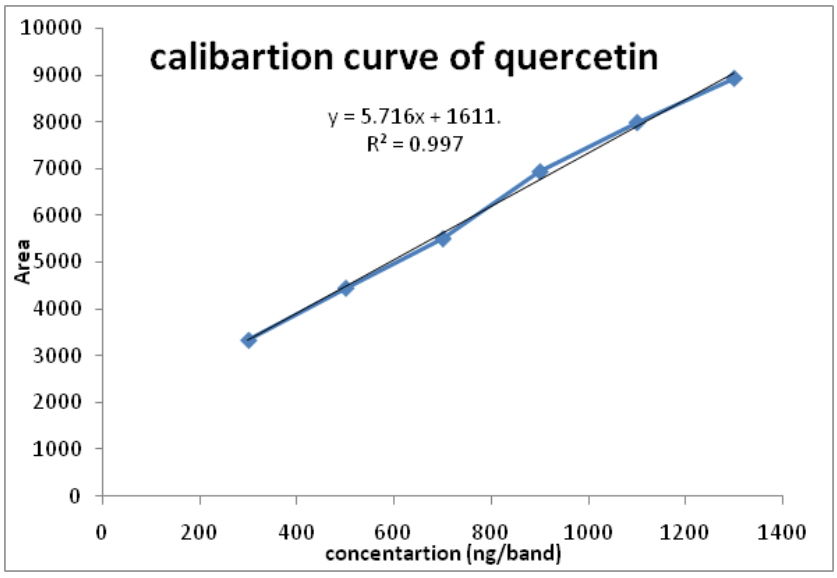

Figure 3. Calibration curve for standard quercetin (n=6).

3.3.2 Robustness

The proposed technique's robustness was proven by the lowest RSD values after making small but deliberate changes to the specification of the created HPTLC technology. The statistical data for robustness are shown in Table 4.

3.3.3 Studies on Accuracy

As shown in Table 5 and 6, the suggested approach for withdrawal and successive quantification of rutin and quercetin in specimen following spiking with 80, 100, and 120 percent more standard constituents shows good recovery of for rutin (24.34%) and quercetin (54.00%).

3.3.4 LOD and LOQ are Two Different Terms for the Same Thing

With a S/N ratio of 3:1, the LOD for rutin and quercetin was 46.52 ng/spot and 81.79 ng/spot, respectively. With a S/N ratio of 10:1, rutin and quercetin had LOQs of 140.91 ng/spot and 247.86 ng/spot, respectively.

3.3.5 Quercetin and Rutin Concentrations in Samples

The proposed approach is used to calculate rutin and Quercetin in an *Ocimum basilicum* seed hydroalcoholic extract. Table 7 shows that the percent amount of rutin and quercetin was 26.19 percent and 57.46 percent, respectively.

4. Discussion

HPTLC is a new technique that is primarily used for drug sample identification, qualitative, quantitative, and visual analysis[18]. The current study found that HPTLC techniques using these solvents are quite effective for rutin and quercetin analysis. The method is believed to solve a wide range of qualitative and quantitative analytical problems in a variety of pharmaceutical fields, including pharmaceuticals, biological sciences, food analysis, toxicology, and environmental studies[19].

Table 3. Precision studies by proposed method

	Concentration (ng/band)	Intraday		Interday	
		Mean ± SD (n=3)	%RSD	Mean± S.D (n=3)	%RSD
Rutin	300	2091.53 ± 34.51	1.65	2151.53 ± 41.05	1.90
	900	5116.80 ± 91.67	1.79	5126.80 ± 93.73	1.82
	1300	7057.06 ± 108.71	1.54	7063.73 ± 170.84	2.41
Quercetin	300	3310.86 ± 41.21	1.24	3327.53 ± 65.21	1.96
	900	6813.20 ± 93.03	1.36	6968.70 ± 146.19	2.09
	1300	8920.43 ± 176.41	1.97	8913.76 ± 193.56	2.17

Table 4. The results of robustness tests for the suggested approach

Area					
Normal condition	**Changed condition**	**Rutin 900(ng/band)**		**Quercetin 180(ng/band)**	
		mean ±S.D	**%RSD**	**mean ±S.D**	**%RSD**
Chamber saturation time 30 min	25 min	5173.46 ± 69.27	1.33	6999.23 ± 86.99	1.24
	35 min	5463.46 ± 60.97	1.11	7432.56 ± 80.11	1.07
Wavelength 254 nm	252 nm	5378.8 ± 100.34	1.86	7335.90 ± 84.38	1.15
	256 nm	5612.13 ± 70.02	1.24	7639.23 ± 88.82	1.16

Table 5. Proposed accuracy study of rutin

No	Amount of extract taken from samples (ng/band)	% level	Amount of standard drug spiked (ng/band)	Total conc.	Conc. found	% Recovery
1	500	0	0	500	114.2182	22.84364
2	500	80	400	900	521.7131	24.34263
3	500	100	500	1000	625.996	25.19919
4	500	120	600	1100	722.5953	24.51906

Table 6. Proposed accuracy study of Quercetin

No	Amount of extract taken from samples (ng/band)	% level	Amount of standard drug spiked (ng/band)	Total conc.	Conc. found	% Recovery
1	500	0	0	500	274.8892	54.97784
2	500	80	400	900	670.0315	54.0063
3	500	100	500	1000	771.4719	54.29438
4	500	120	600	1100	876.4696	55.29391

Table 7. Proposed assay study of rutin and Quercetin

Hydroalcoholic extract	Drugs	The amount of medicine that was taken (ng/band)	Average of medicine found (ng/band) (n=3)	%Amount of drug discovered (n=3)
	Rutin	1200	2267.00	26.19%
	Quercetin	1200	5552.667	57.46%

Validation parameters are summarized in Table 8

Table 8. A list of the validation parameters

Parameter	Rutin	Quercetin
Range (ng/band)	300-1300	300-1300
Regression Coefficient	0.9967	0.9964
Slope	4.95248	5.716
Intercept	710.82	1611.06
LOD (µg/ml)	46.52	81.79
LOQ (µg/ml)	140.96	247.84
Retention Factor (n=6)	0.25 ± 0.005	0.80 ± 0.005
Intraday (n=3)	1.54 -1.79	1.24 - 1.97
Interday (n=3)	1.82-2.41	1.96- 2.17
Repeatability(n=6)	0.87	0.89
Assay	26.19%	57.46%
Accuracy	22.84-25.19 %	54.00-55.29 %
Robustness	Robust	

Because of the advancement of TLC with forced flow technology, enhanced Phases: fixed and mobile selection, and novel quantification approaches, the HPTLC methodology is regarded helpful[20]. For the measurement of rutin and quercetin in *Ocimum basilicum*, HPTLC has been proven to be stable analytical approach has already been designed. The proposed method is specific, accurate, easy, exact, time-saving, and cost-effective, and it can separate pharmaceuticals from their other elements.

Because it has been adequately refined and validated, the suggested method outperforms other TLC densitometric methods. It also offers a greater linearity range and determination limits. The unique approach is repeatable and selective, according to a statistical analysis of the data acquired, and it can be employed on a regular basis, both qualitatively and quantitatively. *Ocimum basilicum* rutin and quercetin levels.

5. Conclusion

The TLC process that has been developed is precise, specific, and accurate. The approach is acceptable for estimation of rutin and quercetin in *Ocimum basilicum*, supported by statistical analysis. TLC is a less expensive, simpler, faster, and more adaptable approach than LC.

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7. References

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