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Quantitative estimation of quercetin in *Nelumbo nucifera* leaves by HPTLC

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Abstract

A sensitive and reliable HPTLC method was developed for the quantitative estimation of Quercetin in Nelumbo nucifera leaves. Chromatographic analysis was performed by n-Butanol: Glacial Acetic Acid:Water:0.1% Formic Acid (7:1:1:0.25v/v/v/v) as mobile phase, TLC Silica gel 60 F_{254} as stationary phase with a dosage speed of 20µL/sec and detection was carried out at 254nm. R_f value of Quercetin was found to be 0.907. The developed method was validated for system suitability, linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) and robustness according to ICH guidelines. Linearity of Quercetin was 2000-10000ng/spot with a correlation coefficient of 0.999. The % recovery ranged between 98-102% and % RSD was <2. Hence, the proposed method can be applied for routine analysis.

Keywords: *Quercetin, Nelumbo nucifera, HPTLC*

Introduction

Flavonoids form one of the most numerous and widespread groups of natural substances accumulated in plants in significant amounts. These polyphenolic compounds are strong antioxidants [1]. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) (Figure 1) belongs to an extensive class of polyphenolic flavonoid compounds almost ubiquitous in plants and plant food sources. Frequently quercetin occurs as glycosides (sugar derivatives); e.g., rutin in which the hydrogen of the R-4 hydroxyl group is replaced by a disaccharide. Quercetin is termed the aglycone, or sugarless form of rutin [2].

Materials

Chemicals

1. Quercetin, n-Butanol, Glacial Acetic Acid, HPLC Water, Formic Acid and Methanol procured from merck and fisher scientific.

Instruments

HPTLC instrument manufactured by Aetron comprising of Hamilton syringe with sample applicator using Spraylin software, Photo Documentation was done by Aetron IDS and Quantification was done by using Just TLC software.

Preparation of standard Quercetin solution

About 10mg of standard quercetin was accurately weighed and transferred into 10mL volumetric flask and make up to 10mL with methanol (i.e., 1000µg/mL).

Preparation of working stock solution

From the above standard solution 1mL was pipette out into 10mL volumetric flak and make up to 10mL with methanol.

Preparation of sample solution

Weigh accurately about 10mg of dried extract of *Nelumbo nucifera* leaves into 10mL volumetric flask and make up to 10ml with methanol. The sample solution was filtered with 0.45µ Millipore Nylon filter. From this solution pipette out 1mL into 10mL volumetric flak and make up to 10mL with methanol.

Preparation of 0.1% Formic Acid

0.1mL of formic acid was pipette out into 100mL volumetric flask and make up to 100mL with HPLC water.

Method Development

Chromatographic Conditions

Mobile Phase: n-Butanol:Glacial Acetic Acid:Water:0.1% Formic Acid (7:1:1:0.25v/v/v/v)
Stationary Phase: TLC Silica gel 60 F_{254}
Dosage Speed: 20µL/sec
Injection Volume: 30µL
Band Width: 9mm
Detection Wavelength: 254nm
R_f: 0.907
Chromatogram of Quercetin was represented in Figure 2

Method Validation

System Suitability

Procedure

From the above working stock solution 0.6mL was pipette out into 10mL volumetric flask and make up to 10mL with methanol.
The data of system suitability was enlisted in Table 1.

Linearity

Procedure

From the above working stock solution 0.2mL, 0.4mL, 0.6mL, 0.8mL and 1.0mL (2000-10000ng/spot) were pipette out into series of five 10mL volumetric flasks and make up to 10mL with methanol.

The data of linearity and calibration curve of quercetin was represented in Table 2 and Figure 3.

Accuracy

Preparation of 50% solution

5mg of dried extract of *Nelumbo nucifera* was weighed and transferred into 10mL volumetric flask, some amount of methanol was added and sonicated for some time by intermediate shaking and the volume was made up to 10mL with methanol. The sample solution was filtered with 0.45µ Millipore Nylon filter. 1mL of above solution was pipette out into 10mL volumetric flask and make up to 10mL with methanol.

Preparation of 100% solution

10mg of dried extract of *Nelumbo nucifera* was weighed and transferred into 10mL volumetric flask, some amount of methanol was added and sonicated for some time by intermediate shaking and the volume was made up to 10mL with methanol. The sample solution was filtered with 0.45µ Millipore Nylon filter. 1mL of above solution was pipette out into 10mL volumetric flask and make up to 10mL with methanol.

Preparation of 150% solution

15mg of dried extract of *Nelumbo nucifera* was weighed and transferred into 10mL volumetric flask, some amount of methanol was added and sonicated for some time by intermediate shaking and the volume was made up to 10mL with methanol. The sample solution was filtered with 0.45µ Millipore Nylon filter. 1mL of above solution was pipette out into 10mL volumetric flask and make up to 10mL with methanol.

The data of accuracy was tabulated in Table 3.

Precision

Procedure

From the above working stock solution 0.6mL was pipette out into 10mL volumetric flask and the volume was made up to 10mL with methanol. Six replicate injections were performed. % RSD was determined for peak areas of Quercetin.

The data of system precision and method precision was given in Table 4 and Table 5.

Limit of Detection (LOD)

Procedure

From the above working stock solution 0.2mL was pipette out into 10mL volumetric flask and make up to 10mL with methanol. From this solution 1mL was pipette out into 10mL volumetric flask and make up to 10mL with methanol.

Limit of Quantification (LOQ)

Procedure

From the above working stock solution 0.2mL was pipette out into 10mL volumetric flask and make up to 10mL with methanol. From this solution 3mL was pipette out into 10mL volumetric flask and make up to 10mL with methanol.

The data of LOD and LOQ was represented in Table 6.

Robustness

Effect of Mobile Phase

To determine the effect of mobile phase a standard solution was prepared and sprayed on to the TLC Silica gel 60 F_{254} by keeping the variation in decreasing to n-Butanol: Glacial Acetic Acid:Water:0.1%Formic Acid (6.5:0.5:1:0.25v/v/v/v) and increasing to n-Butanol: Glacial Acetic Acid: Water:0.1%Formic Acid (7.5:1.5:1:0.25v/v/v/v). The effect of mobile phase was evaluated.

Effect of Dosage Speed

To determine the effect of dosage speed a standard solution was prepared and sprayed on to the TLC Silica gel 60 F_{254} by keeping the variation in decreasing to 16µL/sec and increasing to 25µL/sec. The effect of dosage speed was evaluated.

Effect of Band Width

To determine the effect of band width a standard solution was prepared and sprayed on to the TLC Silica gel 60 F_{254} by keeping the variation in decreasing to 6mm and increasing to 12mm.

Procedure

From the above working stock solution 0.6mL of quercetin stock solution was pipette out into 10mL volumetric flask and made up to 10mL with methanol.

The data of robustness was represented in Table 7.

Tables and Figures

Table 1: Data of System Suitability

S. No	R_f	Area
1.	0.909	1141
2.	0.904	1150
3.	0.905	1159
4.	0.907	1165
5.	0.909	1160
6.	0.906	1175
Average		1158.333
SD		11.793
% RSD		1.02

Table 2: Data of Linearity

S. No	Concentration (ng/spot)	Area
1.	2000	420
2.	4000	765
3.	6000	1150
4.	8000	1525
5.	10000	1850

Table 3: Data of Accuracy

S. No	Level (%)	Concentration (ng/spot)	R_f	Area	% Recovery	% Average Recovery	Overall % Average Recovery
1.	50	2000	0.907	425	99.30	99.57	99.70
		2000	0.906	427	99.90		
		2000	0.906	423	99.50		
2.	100	6000	0.908	1150	99.92	99.71	
		6000	0.905	1153	99.54		
		6000	0.904	1156	99.67		
3.	150	10000	0.909	1853	99.99	99.83	
		10000	0.907	1855	99.60		
		10000	0.907	1849	99.90		

Table 4: Data of System Precision

S. No	Concentration (ng/spot)	R_f	Area
1.	6000	0.906	1165
2.	6000	0.905	1167
3.	6000	0.906	1150
4.	6000	0.906	1142
5.	6000	0.904	1156
6.	6000	0.905	1170
Average			1158.333
SD			10.930
% RSD			0.94

Table 5: Data of Method Precision

S. No	Concentration (ng/spot)	R_f	Area
1.	6000	0.904	1154
2.	6000	0.906	1170
3.	6000	0.907	1175
4.	6000	0.905	1155
5.	6000	0.907	1146
6.	6000	0.906	1150
Average			1158.333
SD			11.535
% RSD			1.00

Table 6: Data of LOD and LOQ

S. No	LOD (ng/spot)	LOQ (ng/spot)
1.	200	600

Table 7: Data of Robustness

S. No	Parameters	Concentration (ng/spot)	R_f	Area	Average	SD	% RSD
1.	Change in Mobile Phase Ratio (6.5:0.5:1:0.25v/v/v/v)	6000	0.907	1224	1237	18.385	1.49
			0.906	1250			
2.	Change in Mobile Phase Ratio (7.5:1.5:1:0.25v/v/v/v)	6000	0.905	1122	1133.5	16.263	1.43
			0.906	1145			
3.	Change in Dosage Speed (16µL/sec)	6000	0.906	1008	1016.5	12.021	1.18
			0.906	1025			
4.	Change in Dosage Speed (25µL/sec)	6000	0.907	1360	1347.5	17.678	1.31
			0.907	1335			
5.	Change in Band Width (6mm)	6000	0.905	900	905	7.071	0.78
			0.905	910			
6.	Change in Band Width (12mm)	6000	0.906	1460	1470	14.142	0.96
			0.906	1480			

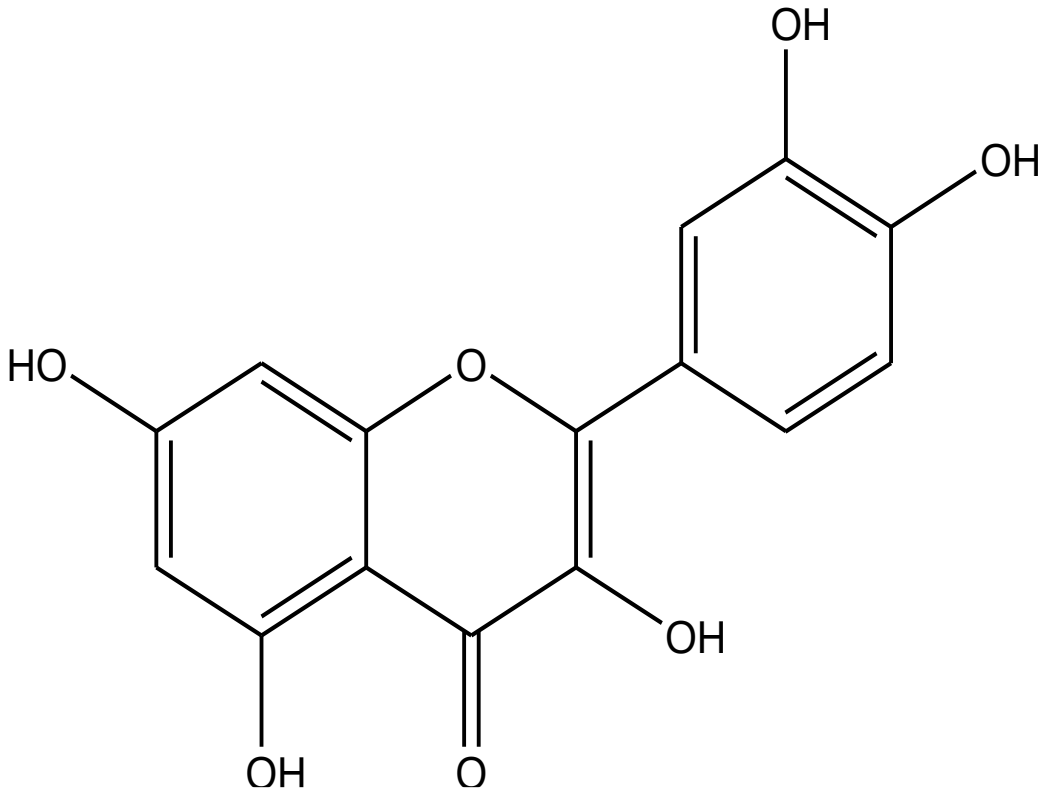

Fig 1: Structure of Quercetin

Fig 2: Chromatogram of Quercetin

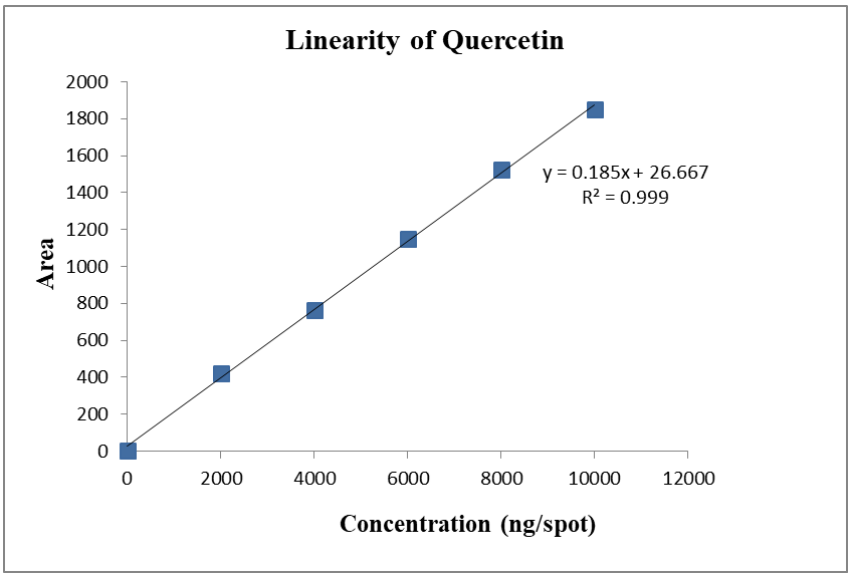

Fig 3: Calibration Curve of Quercetin

Conclusion

The proposed method was found to be sensitive and reliable for quantitative estimation of quercetin in *Nelumbo nucifera* leaves by HPTLC has been developed and validated. n-Butanol:Glacial Acetic Acid:Water:0.1% Formic Acid (7:1:1:0.25v/v/v/v) as mobile phase, TLC Silica gel 60 F_{254} as stationary phase with a dosage speed of 20µL/sec at a detection 254nm. The R_f value of quercetin was found to be 0.907. The linearity was observed in the range of 2000-10000ng/spot with a correlation coefficient of 0.999. The % recovery ranged between 98-102% which is within the acceptance limit. Precision was within the limit with a % RSD of NMT 2.0 and the method was found to be robust. Hence the chromatographic method developed can be effectively applied for routine analysis.

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