HPTLC METHOD FOR QUANTITATIVE DETERMINATION OF QUERCETIN IN *PSIDIUM GUAJAVA* LEAVES

SEEMAKURTHI SM[1*], KUPPA GK[2], PINNAMENINI P[3], GOLLAPALLI N[4] AND NADENDLA RR[5]

1: PG Scholar, Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

2: PG Scholar, Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

3: Assistant Professor, Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

4: Associate Professor, HOD, Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

5: Principal, Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

*****Corresponding Author: Satya Manohar Seemakurthi: E Mail: manoharvasantha30@gmail.com**

ABSTRACT

A simple, sensitive and specific HPTLC technique for the quantitative determination of Quercetin in *Psidium guajava* leaves was developed. TLC Silica gel 60 F_{254} as stationary phase, Toluene: Ethyl acetate: Acetic acid (5:3:2v/v/v) as mobile phase, dosage speed of 16µL/sec, and detection at 254nm. The R_f value of Quercetin was found to be 0.660. According to ICH criteria, the developed technique was validated for system suitability, linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ), and robustness. Quercetin linearity was 2000-2800ng/spot with a correlation coefficient of 0.9994. The % recovery ranged between 98-102%, and the % RSD was <2. As a result, the developed methodology can be used for routine analysis.

Keywords: Quercetin, *Psidium guajava*, HPTLC.

INTRODUCTION:

Quercetin **(Figure 1)** (2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one) is a plant pigment that is a powerful antioxidant flavonoid and, more particularly, a flavanol. It is found mostly in onions, grapes, berries, cherries, broccoli, and citrus fruits. It is a versatile antioxidant that has been shown to protect against tissue harm caused by numerous drug toxins [1].
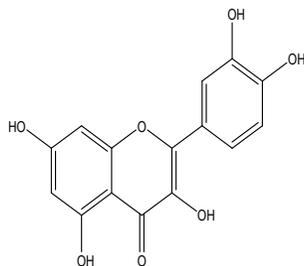
Figure 1: Structure of Quercetin

Materials:

Chemicals:

Quercetin, Toluene, Ethyl Acetate, Acetic acid, Dimethyl formamide 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, some amount of dimethyl formamide was added and dissolved, then the volume was made up to 10mL with dimethyl formamide (i.e., 1000µg/mL) [2, 3].

Preparation of working stock solution:

From the above standard solution 1mL was pipetted out into 10mL volumetric flask and made up to 10mL with dimethyl formamide. From this solution 0.2mL, 0.22mL, 0.24mL, 0.26mL and 0.28mL was pipetted out and transferred into series of five volumetric flasks and the volume was made up to 10mL with dimethyl formamide (2000ng/spot, 2200ng/spot, 2400ng/spot, 2600ng/spot and 2800ng/spot).

Preparation of sample solution:

Weigh accurately about 10mg of dried extract of *Psidium guajava* leaves into 10mL volumetric flask and made up to 10ml with dimethyl formamide. The sample solution was filtered with 0.45µ Millipore Nylon filter. From this solution 1mL was pipetted out into 10mL volumetric flask and made up to 10mL with dimethyl formamide [4, 5].

Method Development:

Chromatographic Conditions:

Mobile Phase: Toluene: Ethyl acetate: Acetic acid (5:3:2v/v/v)

Stationary Phase: TLC Silica gel 60 F_{254}

Dosage Speed: 16µL/sec

Injection Volume: 50µL

Band Width: 8mm

Detection Wavelength: 254nm

R_f: 0.660

Figure 2: Chromatogram of Quercetin

RESULTS:

System Suitability:

Procedure:

From the above working stock solution 0.26mL was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide.

The data of system suitability was enlisted in **Table 1**.

Linearity:

Procedure:

From the above working stock solution 0.20mL, 0.22mL, 0.24mL, 0.26mL and 0.28mL (2000-2800ng/spot) were pipetted out into series of five 10mL volumetric flasks and make up to 10mL with dimethyl formamide.

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

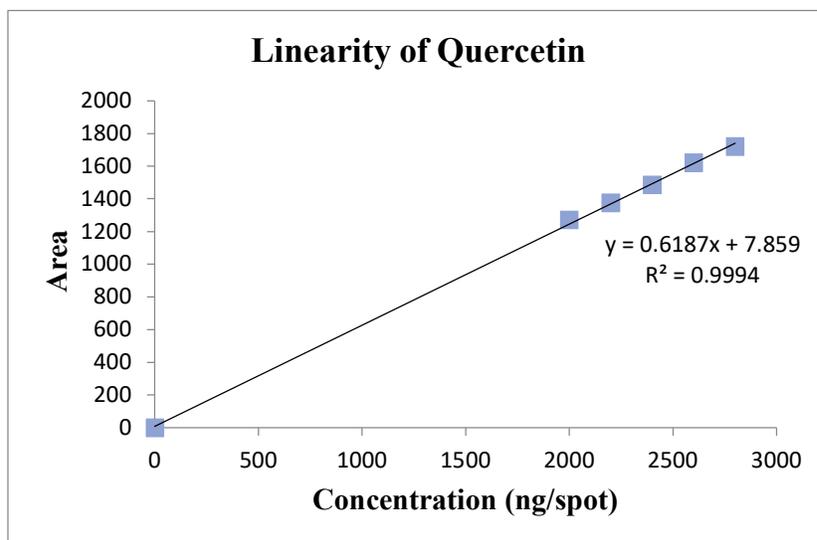**Figure 3: Calibration Curve of Quercetin**

Accuracy:

Preparation of 50% solution:

About 2mg of dried extract of *Psidium guajava* was weighed and transferred into 100mL volumetric flask, some amount of dimethyl formamide was added and sonicated for some time by intermediate shaking and the volume was made up to 100mL with dimethyl formamide. The sample solution was filtered with 0.45µ

Millipore Nylon filter. 1mL of above solution was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide.

Preparation of 100% solution:

About 6mg of dried extract of *Psidium guajava* was weighed and transferred into 100mL volumetric flask, some amount of dimethyl formamide was added and sonicated for some time by intermediate shaking and the volume was made up to 100mL with dimethyl formamide. The sample solution was filtered with 0.45μ Millipore Nylon filter. 1mL of above solution was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide.

Preparation of 150% solution:

About 10mg of dried extract of *Psidium guajava* was weighed and transferred into 100mL volumetric flask, some amount of dimethyl formamide was added and sonicated for some time by intermediate shaking and the volume was made up to 100mL with dimethyl formamide. The sample solution was filtered with 0.45μ Millipore Nylon filter. 1mL of above solution was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide.

The data of accuracy was tabulated in **Table 3**.

Precision:

Procedure:

From the above working stock solution 0.26mL was pipetted out into 10mL volumetric flask and the volume was made up to 10mL with dimethyl formamide. 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 0.20mL was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide. From this solution 1mL was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide.

Limit of Quantification (LOQ):

Procedure:

From the above working stock solution 0.20mL was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide. From this solution 3mL was pipetted out into 10mL volumetric flask and make up to 10mL with dimethyl formamide.

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

Robustness:

Effect of Mobile Phase Ratio:

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 (Toluene: Ethyl acetate: Acetic acid 4.5:3.5:2v/v/v) and increasing to (Toluene: Ethyl acetate: Acetic acid 6:2:2v/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 12µL/sec and increasing to 20µL/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 10mm.

Procedure:

From the above working stock solution 0.26mL of quercetin stock solution was pipetted out into 10mL volumetric flask and made up to 10mL with dimethyl formamide.

The data of robustness was represented in **Table 7**.

Table 1: Data of System Suitability

S. No	R_f	Area
1.	0.661	1461
2.	0.67	1472
3.	0.67	1490
4.	0.67	1497
5.	0.665	1478
6.	0.67	1498
Average		1483
SD		13.536
% RSD		0.91

Table 2: Data of Linearity

S. No	Concentration (ng/spot)	Area
1.	2000	1271
2.	2200	1375
3.	2400	1485
4.	2600	1620
5.	2800	1720

Table 3: Data of Accuracy

Level (%)	Standard concentration	Sample concentration	R_f	Standard area	Sample area	Standard area + Sample area	% Recovery	Mean % Recovery	Over all % Recovery
50	2400	2000	0.660	1483	1237	2720	100.09	100.71	
	2400	2000	0.661	1483	1242	2725	100.50		
	2400	2000	0.660	1483	1255	2738	101.55		
100	2400	2400	0.664	1483	1482	2965	99.93	100.40	100.47
	2400	2400	0.663	1483	1496	2979	100.88		
	2400	2400	0.664	1483	1489	2972	100.40		
150	2400	2800	0.672	1483	1729	3212	99.93	100.28	
	2400	2800	0.670	1483	1740	3223	100.57		
	2400	2800	0.674	1483	1736	3219	100.34		

Table 4: Data of System Precision

S. No	Concentration (ng/spot)	R_f	Area
1.	2600	0.671	1486
2.	2600	0.670	1498
3.	2600	0.668	1496
4.	2600	0.670	1497
5.	2600	0.670	1463
6.	2600	0.671	1472
Average			1485.333
SD			13.462
% RSD			0.91

Table 5: Data of Method precision

S. No	Concentration (ng/spot)	R_f	Area
1.	2600	0.670	1499
2.	2600	0.672	1495
3.	2600	0.670	1497
4.	2600	0.671	1479
5.	2600	0.672	1473
6.	2600	0.671	1460
Average			1483.833
SD			14.358
% RSD			0.97

Table 6: Data of LOD and LOQ

S. No	LOD (ng/spot)	LOQ (ng/spot)
1.	940	2750

Table 7: Data of Robustness

S. No	Parameters	Concentration (ng/spot)	R_f	Area	Average	SD	% RSD
1.	Change in Mobile Phase Ratio (4.5:3.5:2v/v/v)	2600	0.558	1493	1479	14	0.95
			0.664	1465			
2.	Change in Mobile Phase Ratio (6:2:2v/v/v)	2600	0.665	1460	1476	16	1.08
			0.664	1492			
3.	Change in Dosage Speed (12μL/sec)	2600	0.660	1462	1480	18	1.22
			0.660	1498			
4.	Change in Dosage Speed (20μL/sec)	2600	0.660	1467	1481	14	0.95
			0.660	1495			
5.	Change in Band Width (6mm)	2600	0.660	1238	1251.5	13.5	1.08
			0.660	1265			
6.	Change in Band Width (10mm)	2600	0.660	1760	1742.5	17.5	1.00
			0.660	1725			

DISCUSSION:

In the optimized method, The R_f value of Quercetin was found to be 0.660. According to ICH guidelines the developed method was validated. The linearity concentration range was 2000-2800ng/spot with a correlation coefficient(R^2) of 0.9994. The % recovery was 100.47% which is within the acceptance limit. Both system precision and method precision were within the limit with a % RSD of NMT 2.0. The LOD and LOQ were found to be 940ng/spot and 2750ng/spot.

CONCLUSION:

The developed method was found to be simple, sensitive and specific for the HPTLC method for quantitative determination of Quercetin in *Psidium guajava* leaves. Method was validated with mobile phase Toluene: Acetic acid: Ethyl

acetate (5:2:3v/v/v), stationary phase TLC Silica gel 60 F_{254} and dosage speed of 16µL/sec at a detection wavelength 254nm. The R_f value of quercetin was found to be 0.660. Linearity range was 2000-2800ng/spot with a correlation coefficient of 0.9994. The % recovery was 100.47% which is within the acceptance limit. Both system precision and method precision were within the limit with a % RSD of NMT 2.0. The LOD and LOQ were found to be 940ng/spot and 2750ng/spot. Hence the chromatographic method developed can be effectively applied for routine analysis in institutes and pharmaceutical laboratories.

Acknowledgement:

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