GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

A novel stability indicating HPTLC method for comparative quantitative estimation of quercetin in herbal preparations

Aditi Gajanan Asole [1,*] and Madhuri D. Game [2]

[1] *Department of Quality Assurance, Vidyabharati College of Pharmacy, Amravati, Maharashtra, India. 444602*
[2] *Department of Pharmaceutical Chemistry, Vidyabharati College of Pharmacy, Amravati, Maharashtra, India. 444602*

GSC Biological and Pharmaceutical Sciences, 2024, 27(02), 262–275

Publication history: Received on 05 April 2024 revised on 22 May 2024; accepted on 24 May 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.27.2.0178

Abstract

This research introduces a novel stability-indicating HPTLC (High-Performance Thin-Layer Chromatography) method for the comparative quantitative estimation of Quercetin in herbal preparations. Through extensive stability studies, the method is validated, providing a robust framework for evaluating Quercetin's stability in diverse herbal formulations. The developed method ensures accurate and reliable quantification of Quercetin, facilitating comparative analysis across various herbal preparations. Additionally, results reveal that Quercetin degraded under most tested conditions, such as acidic, alkaline, oxidative and neutral conditions underscoring the necessity for intervention by experts to eliminate such challenges and ensure product efficacy and quality in the herbal industry.

Keywords: HPTLC; Quercetin; Stability study; Herbal preparations; Method development and Validation; Quantitative evaluation; Qualitative evaluation; Comparative study

1. Introduction

Herbal medicine, also known as botanical medicine or phytotherapy, refers to the use of plants or plant extracts for medicinal purposes. This traditional form of medicine has been practiced for centuries across various cultures worldwide and involves utilizing the therapeutic properties of herbs to promote health and treat illnesses. The resurgence of Ayurveda, an ancient system of medicine originating from India, as a prominent force further underscores the popularity of herbal medicine. Ayurveda emphasizes the use of herbs, diet, lifestyle modifications, and spiritual practices to promote health and well-being. As interest in holistic health approaches grows, Ayurveda's principles of personalized medicine and holistic healing are gaining recognition and acceptance worldwide.

Overall, the popularity of herbal medicine is driven by a combination of factors, including a desire for natural remedies, cultural heritage, a focus on wellness and prevention, accessibility, and increasing scientific validation. As people seek alternatives to conventional medicine and embrace holistic approaches to health, herbal medicine continues to gain attraction as a valuable and effective healing modality.[1-2]

1.1. Quercetin

Quercetin, derived from the Latin word "Quercetum," meaning Oak Forest, is a flavonol compound that is not synthesized within the human body. It possesses a yellow hue and exhibits poor solubility in hot water, moderate solubility in alcohol and lipids, and insolubility in cold water. Widely recognized as one of the most utilized bioflavonoids for addressing metabolic and inflammatory ailments, quercetin is abundantly present in various dietary sources including fruits (particularly citrus fruits), leafy greens, seeds, nuts, flowers, barks, broccoli, olive oil, apples, onions, green tea, red grapes, red wine, dark cherries, blueberries, and cranberries. Vegetables such as onions and broccoli,

* Corresponding author: Aditi G. Asole

along with fruits like apples, cherries, and berries, as well as beverages such as tea and red wine, contain the highest concentrations of flavonols.

Quercetin is purported to offer numerous health benefits, including protection against ailments like osteoporosis, lung cancer, and cardiovascular diseases. Studies have indicated a correlation between a high intake of flavonoids and a reduced risk of cardiovascular diseases. Chronic obstructive pulmonary disease (COPD), the third-leading cause of death in the USA, currently lacks fully effective therapies and may present side effects. However, emerging evidence suggests that quercetin supplementation could offer therapeutic benefits for COPD. Preclinical studies have shown that elevated plasma quercetin levels resulted in a significant reduction in lung inflammation and halted disease progression. Furthermore, quercetin has demonstrated potent anti-inflammatory properties in various animal models and human trials, with reports of reduced expression of inflammatory genes in response to a quercetin-enriched diet. Clinical trials have shown that quercetin supplementation, at a dosage of 1000 mg/day, reduced the incidence of upper respiratory tract infections in middle-aged and older individuals over a 12-week period.

Additionally, quercetin has exhibited neuroprotective properties in rat brains, particularly when combined with fish oil, and has shown promise in combating neurodegenerative diseases. Its ability to scavenge radicals suggests a potential role in preventing cancer induced by oxidative stress.[3-12]

2. Materials and methods

Equipment: A CAMAG HPTLC system comprising of a Linomat V applicator and CAMAG HPTLC scanner and single pan balance of Shimadzu model was used, for the present study.

Chemicals: Analytical grade Toluene, Ethyl acetate,Methanol, Formic acid, formic acid was obtained from Loba and CDH Chemical Pvt Ltd. Stationary phase used was silica gel G60F254, 20x10 cm TLC plate were obtained from E. Merk Ltd (Mumbai, India).

Table 1 Chemicals and Suppliers

Chemicals	**Supplier**
Quercetin	Sihauli Chemical Pvt Ltd
Methanol	Loba Chemical Pvt Ltd
Hydrochloric acid	Loba Chemical Pvt Ltd
Ethanol	CDH Chemical Pvt Ltd
Toluene	Loba Chemical Pvt Ltd
Ethyle-acetate	CDH Chemical Pvt Ltd
Formic acid	CDH Chemical Pvt Ltd
Water	Fusion Pharma Pvt Ltd

Table 2 Instruments

Sr.no	**Particulars**	**Details**
1.	System	CAMAG
2.	Model no	CAMAG Linomat V Sample Applicator
3.	Detector	CAMAG UV Cabinet
4.	Pump	TLC Silica gel 60 F 254
5.	Column	CAMAG TLC Scanner 3
6.	Software	Wincat
7.	Chamber	CAMAG Twin Plate Development Chamber

3. Experimental work

3.1. Qualitative Evaluation [13]

Standardization parameters were established for Quercetin :

3.1.1. Organoleptic Evaluation

Organoleptic evaluation involves assessing the drug based on its color, odor, taste, etc., utilizing our sensory organs.

- Appearance: Visual examination was conducted to assess the appearance.
- Color: The dried sample was placed in a test tube, and its color was observed in sunlight.
- Odor: Evaluation of odor was performed using a freshly prepared sample.
- Taste: The taste was evaluated using a freshly prepared sample.

3.1.2. Phytochemical Evaluation :[13]

Table 3 Organoleptic Properties of Quercetin Drug

Sr no.	Sample	Appearance	Colour	Taste	Odour
1	Quercetin Extract	Powder	Yellow	Bitter	Odourless

- Test for Sugars - Molisch's Test

To conduct the Molisch's test, a solution of Molisch's reagent is prepared by dissolving 10 g of alpha naphthol in 100 ml of 95% alcohol. A small amount of the test residue is mixed with 0.5 ml of water in a test tube, followed by the addition of 2 drops of Molisch's reagent. Then, 1 ml of concentrated sulfuric acid is carefully added from the side of the inclined test tube, forming a layer beneath the aqueous solution. The appearance of a red-brown ring at the interface of the two liquids indicates the presence of sugars.

- Test for Proteins - Xanthoproteic Test

In the Xanthoproteic test, a sample is mixed with 2 ml of water and 0.5 ml of concentrated nitric acid. The development of a yellow color indicates the presence of proteins.

- Test for Alkaloids - Dragendorff's Reagent

For the Dragendorff's reagent test, a solution is prepared by mixing solutions A and B in a 1:1 volume ratio. Solution A contains bismuth subnitrate and tartaric acid, while solution B consists of potassium iodide and water. From this solution, a working standard is prepared. The test filtrate, obtained by filtering a sample dissolved in 1.5% hydrochloric acid, is basified with dilute ammonia. Chloroform extract of this solution is applied to filter paper impregnated with Dragendorff's reagent. The appearance of an orange color indicates the presence of alkaloids.

- Test for Tannins - Ferric Chloride Test

To perform the Ferric chloride test, the test residue is dissolved in water, warmed, and filtered. A few drops of a 5% solution of ferric chloride in 90% alcohol are added to the filtrate. The formation of a dark green or deep blue color indicates the presence of tannins.

- Test for Flavonoids - Shinoda Test

In the Shinoda test, a small amount of the test extract is dissolved in 5 ml of 95% ethanol. To this solution, a few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal are added. The appearance of a pink, crimson, or magenta color within a minute or two indicates the presence of flavonoids.

- Test for Saponins - Foam Test

For the Foam test, a small amount of the test extract is vigorously shaken with a mixture of sodium bicarbonate and water in a test tube. The formation of a stable, characteristic honeycomb-like froth indicates the presence of saponins.

Table 4 Phytochemical Evaluation

Sr no.	Test	Observation	Result
1.	Sugar	-	Absent
2.	Protein	-	Absent
3.	Alkaloid	-	Absent
4.	Tannin	-	Absent
5	Flavonoids	+	Present
6.	Saponin	-	Absent

3.1.3. Physical Evaluation

Physical Standards are determined for drug. These are rarely constant for crude drug but may help in evaluation, specifically with reference to moisture content, drug gravity, density optical rotation, refractive index, melting point and solubility in different solvents.

- Solubility

The presence of adulterant in a drug was indicated by solubility studies- Quercetin is soluble in organic solvents such as Ethanol, Methanol and DMSO.

- Melting Point

Melting point determination involves heating a dry, impurity-free sample in a melting point apparatus, observing the temperature range at which it transitions from solid to liquid phase, and comparing it to known values to confirm identity and purity.

The melting point of Quercetin is 314°C-316 °C

3.2. Quantitative evaluation

HPTLC Method Development, Validation and Stability Study [14-24]

3.2.1. Preparation of Standard Solutions

Solution A: Approximately 25 mg of Quercetin was accurately weighed and dissolved in 25.0 mL volumetric flask using methanol, and then the volume was adjusted to mark with the same solvent (Concentration: 1 mg/mL).

Solution B: 5.0 mL of Solution A was accurately measured and further diluted in a 50.0 mL volumetric flask with methanol (Concentration: 100 µg/mL).

Solution C: 10.0 mL of Solution A was accurately measured and further diluted in a 50.0 mL volumetric flask with methanol (Concentration: 200 µg/mL).

3.2.2. Selection of Mobile Phase

Solution B of quercetin was applied to TLC plates, and various solvents of different polarities were tested individually and in combination to optimize plate development for sharp, stable, and distinct peaks. The mobile phase consisting of toluene: ethyl acetate :methanol (4:4:2 v/v) was found to be the most satisfactory and suitable for further experimentation.

3.2.3. Selection of Detection Wavelength for Densitometric Evaluation of Sample Spots

After chromatographic development and plate drying, bands were scanned over a wavelength range of 200-400 nm. The wavelength selected for detection was 254.0 nm.

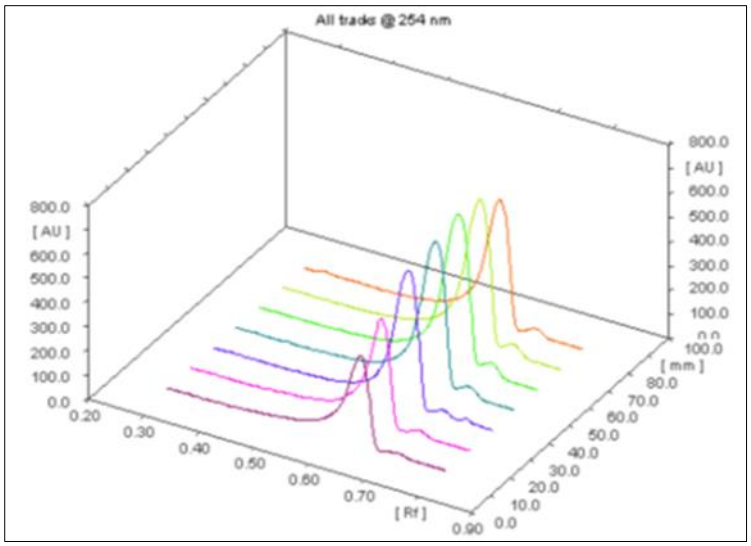

Figure 1 UV Spectra 254.0 nm**.**

3.2.4. Finalized Chromatographic Conditions

The chromatographic conditions were optimized by evaluating various parameters and were kept constant during further experimentation:

- Samples were applied as 6 mm wide bands with a spacing of 6 mm using a micro syringe (Hamilton, Switzerland) on pre-coated silica gel aluminum TLC plates 60F_{24} (20 x 10 cm, Merck) with a thickness of 250 µm using a CAMAG LINOMAT V automatic sample applicator.
- Slit dimensions of 3.0 x 45 mm and a scanning speed of 20 mm/s were employed in the analysis.
- Linear ascending development was conducted in a twin-trough glass chamber (10 x 10 cm, Camag, Switzerland) using toluene: ethyl acetate:methanol (4:4:2 v/v) as the mobile phase.
- Chamber saturation time was 30 minutes, migration time was 15 minutes, and migrating and the distance was 75 mm. After application, TLC plates were dried using a dryer with a current of air. Densitometric scanning was conducted using the CAMAG TLC SCANNER 3 at 254 nm, employing WINCATS software. The scanner utilized a deuterium lamp as a radiation source emitting a continuous UV spectrum ranging from 200 to 400 nm.

3.2.5. Construction and Study of Calibration Curve

Following the general procedure, calibration curves were constructed by spotting 5-35µL of solution B, achieving a concentration range of 50 to 350 ng/band on TLC plates. The plates were developed and scanned under the optimized chromatographic conditions. This process was repeated three times, and the mean peak height and peak area were recorded for different drug concentrations. Calibration curves were then constructed plotting concentration versus peak height and peak area. The resulting calibration curves are depicted in Figure 2.

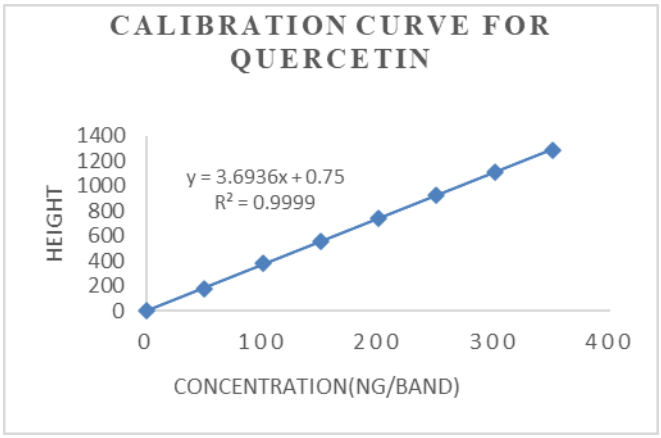

Figure 2 Calibration curve for Quercetin Conc. Vs Height and Conc. Vs AUC

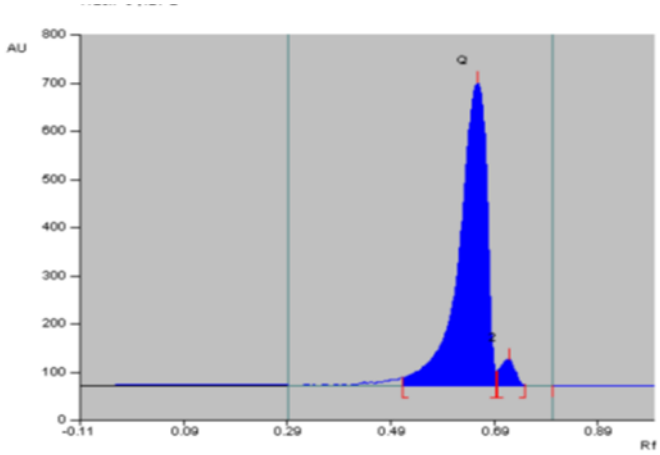

Figure 3 Typical densitograms of standard is shown (Rf = 0.65)

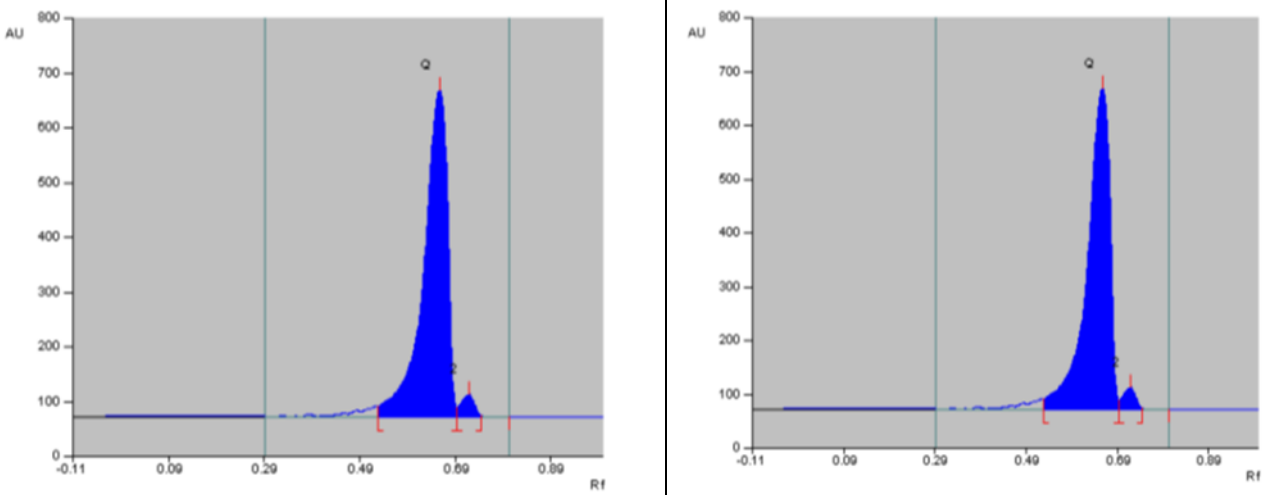

Figure 4 and 5 Typical densitograms of sample 1 and 2 (Rf = 0.65,0.65)

3.2.6. Application of proposed method in marketed formulation

Table 5 Marketed sample 1

Trade Name: Inlife				Average weight: 845 mg			
Sr.No	**Wt of Capsule taken (mg)**	**CA Estimated in (µg)**		**Amount estimated in average wt of capsule**		**% Labeled Claim***	
		Height	Area	Height	Area	Height	Area
1	8.450	596.76	25007.9	595.76	25005	100.16	100.01
2	8.470	597.80	25234	596.70	25200	100.34	100.13
3	8.457	595.26	25192.0	596.10	25115	99.35	100.30
4	8.475	596.26	25205.3	595.62	25100	100.10	100.41
5	8.520	598.42	25347.2	594.72	25292	100.62	100.21
					Mean	100.414	100.21
					S.D	0.2832	0.1375
					C.V	0.2821%	0.1372%
					R.S.D	0.315	0.153
					S.E	0.1416	0.0687

Table 6 Marketed sample 2

Trade Name: Pure Nutrition				**Average weight: 569 mg**			
Sr.No	**Wt of Capsule taken (mg)**	**CA Estimated in (µg)**		**Amount estimated in average wt of capsule**		**% Labeled Claim***	
		Height	Area	Height	Area	Height	Area
1	5.650	629.95	27814	628.92	27805	100.1637	100.0323
2	5.670	630.29	27947	629.28	27807	100.217	100.50
3	5.657	629.98	27842	629.98	27834	100	100.028
4	5.675	630.39	27954	629.39	27849	100.15	100.37
5	5.720	631.10	28102	628.18	28204	100.464	99.63
					Mean	100.196	100.112
					S.D	0.1494479	0.304249920
					C.V	0.0015	0.030
					R.S.D	0.167	0.153
					S.E	0.07474	0.15219

3.2.7. Validation parameters

Validation of the proposed method was carried out as per the USP guidelines.

- Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies. Recovery studies were performed by standard addition method.

Table 7 Accuracy

Label claim(mg in capsule)	**Initial amount in µg**	**Amount of standard drud added (µg)**	**Total amount recoverd**	**% Recovery**
250	200	0	199	99.5
	200	200	401	100.25
	200	250	451	100.22
	200	300	499	99.81
			Mean	99.945
			S.D	0.3102
			C.V	0.003104
			R.S.D	0.36%
			S.E	0.179

3.3. Precision

Precision of an analytical method is expressed as S.D. or R.S.D. of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method. The results of estimation are shown in Table 5

3.4. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot of Quercetin in sample was confirmed by comparing the Re and spectra of spot with that of standard. The peak purity was assessed by comparing the spectra at three different levels peak start (S), peak apex (M) and peak end (E).

3.5. Linearity

The study was performed by applying different volumes of standard solution on TLC plates, developing and scanning the plates as per the optimized chromatographic conditions.The test results that are directly proportional to the concentration of the analyte within a given range. The response (e.g., peak area or peak height) obtained from each standard solution is then plotted against its corresponding concentration.That is shown in fig. 2

3.6. Robustness and ruggednes

Robustness is the measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions and is an indication of the reliability of the method. To study the robustness of the method, small but deliberate variations in mobile phase composition (±2%) chamber saturation period (±10%), development distance (±10%), time from application to development (0, 10, 15 20 min), time from development to scanning (0, 30, 60, 90 min) were carried out. Results of robustness studies are given in Table 8 and 9

Table 8 Results of robustness studies

i)Chromatographic changes (% toluene in mobile phase)

% in mobile phase	**Rf ***
-2%	0.63
0%	0.65
2%	0.66
Mean+-S.D	0.64667 +- 0.015275

ii) Chromatographic changes (chamber saturation)

Chamber saturation (time in min)	Rf *
33	0.64
30	0.65
27	0.66
Mean+-S.D	0.65 +- 0.01

iii) Chromatographic changes (development distance)

Development distance (mm)	Rf *
75	0.63
70	0.65
65	0.66
Mean+-S.D	0.6466 +- 0.015275

iv) Chromatographic changes (time from application to development)

Time from application to development	Rf *
10	0.63
20	0.64
30	0.65
Mean+-S.D	0.64 +- 0.01

v) Chromatographic changes (time from development to scanning)

Time from development to scanning	Rf *
10 min	0.62
20 min	0.64
30 min	0.65
Mean+-S.D	0.6366 +- 0.015275

4. Results of ruggedness studies

The ruggedness of the method was studied under three different parameters.

4.1. Intraday variation

The samples were analysed on different times on same day by proposed method. The percent labeled claim was calculated and results of estimation proposed method.

Table 9 Results and statistical data for intraday study

Time	**%Labeled claim***	
	Height	Area
Time - 1	100.16	100.01
Time - 2	100.34	100.19
Time - 3	99.85	100.1
Mean	100.1166	100.1466
S.D	0.2478	0.1457
C.V	0.002021	0.001188
R.S.D	0.25%	0.15%
S.E	0.1431	0.08413

4.2. Interday variation

The samples were analysed by proposed method on three different days (1 st, 3rd and 5th day). The percent labeled claim was calculated and results of estimation are shown in Table 10

Table 10 Results and statistical data for interday study

Days	**%Labeled claim***	
	Height	Area
Day - 1	99.85	100.30
Day- 2	100.10	100.41
Day – 3	100.62	100.21
Mean	100.19	100.30
S.D	0.39281	0.1007
C.V	0.003201	0.000815
R.S.D	0.392%	0.1%
S.E	0.227	0.057831

4.3. Different analysts

The samples were analysed by three different analysts as per the proposed method. The percent labeled claim was calculated and results of estimation are shown in 11.

Table 11 Results and statistical data for different analysts

Analysts	**%Labeled claim***	
	Height	Area
Analyst - 1	99.85	100.30
Analyst - 2	99.63	100.01
Analyst – 3	99.73	99.98
Mean	99.736	100.10

S.D	0.1102	0.17673
C.V	0.000902	0.00144
R.S.D	0.11%	0.177%
S.E	0.0636	0.1020

4.4. Forced degradation of Quercetin [25-34]

In order to ensure the stability indicating property and specificity of the proposed method stress studies were performed. In all following degradation studies, the average peak area Quercetin of was determined by applying 200 ng/band .

4.4.1. Acid induced degradation

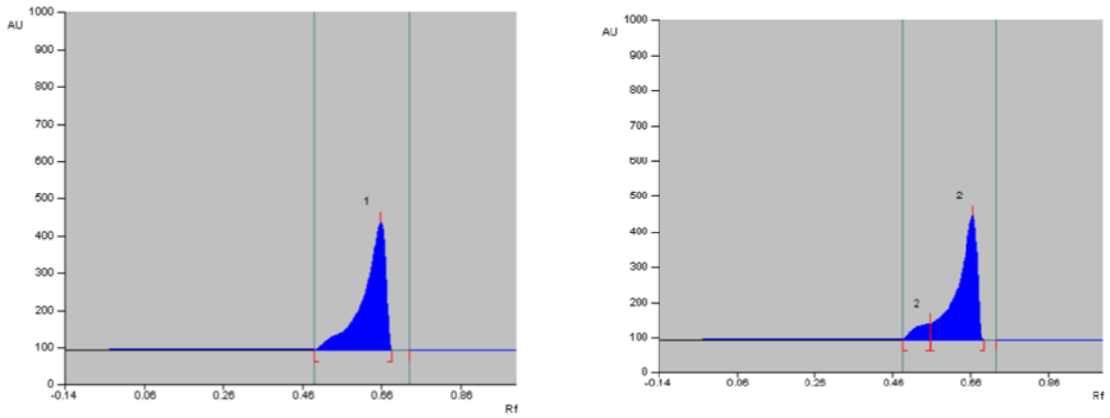

Figure 6 Densitogram of Quercetin stress condition acidic medium marketed 1 and 2

4.4.2. Base Induced Degradation

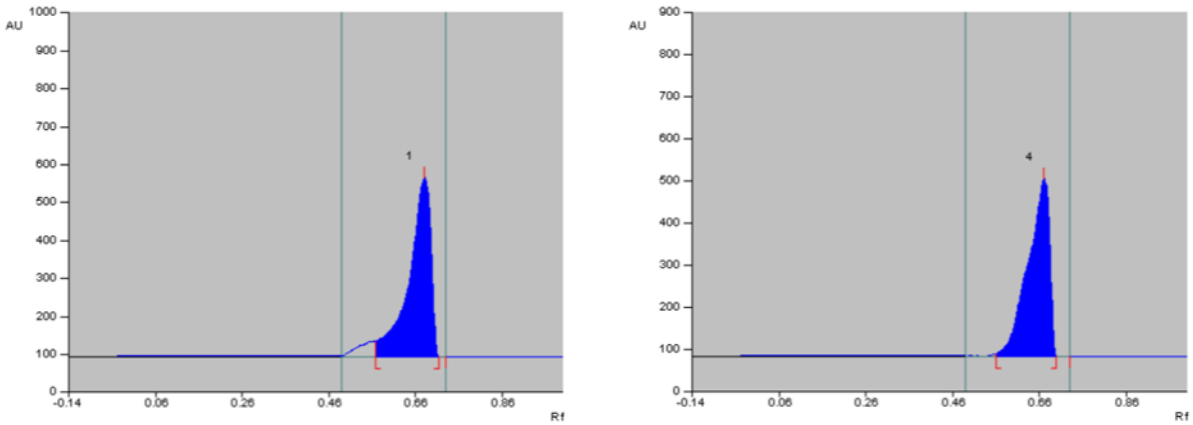

Figure 7 Densitogram of Quercetin stress condition alkaline medium marketed 1 and 2

4.4.3. Hydrogen peroxide-induced degradation

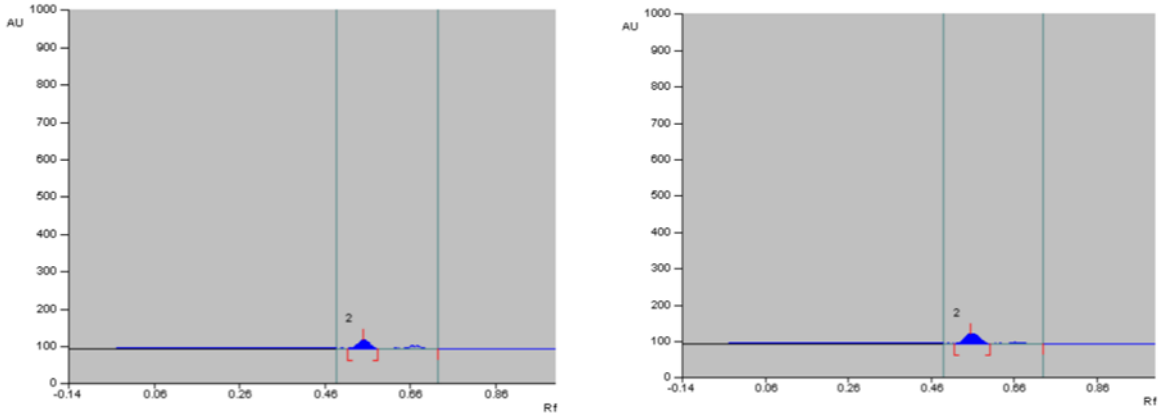

Figure 8 Densitogram of Quercetin stress condition oxidative medium marketed 1 and 2

4.4.4. Neutral degradation

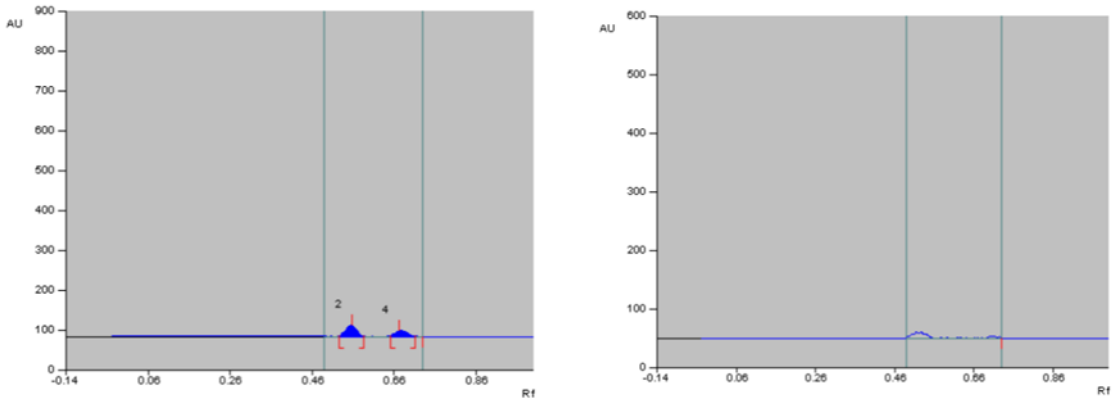

Figure 9 Densitogram of Quercetin stress condition neutral medium marketed 1 and **2**

Table 12 Results of forced degradation of marketed formulation

Sr.No	Degradation condition	concentration used(µg/ml)	Sample 1(% Recovery)	Sample 2 (% Recovery)
1	Acid Hydrolysis	20	49.85	60.90
2	Alkali Hydrolysis	20	68.96	67.79
3	Oxidative Hydrolysis	20	2.004	3.25
4	Neutral Hydrolysis	20	2.421	0

5. Conclusion

In this study, we developed and applied a validated HPTLC (High-Performance Thin-Layer Chromatography) method for the quantitative estimation of Quercetin in pharmaceutical marketed formulations. The chromatographic conditions were meticulously optimized, considering various parameters such as mobile phase composition, linearity range, detection wavelength, band size of the spots applied, chamber saturation time, solvent front migration, and slit width. These optimizations were crucial to ensure the accuracy and reproducibility of the results.

The Rf value obtained for Quercetin was found to be 0.65 ± 0.02, indicating its characteristic migration behavior under the specific chromatographic conditions employed. The linearity of the method was established over a concentration range of 5 - 35 µg/mL, with high correlation coefficient values of 0.9998 and 0.9999 obtained for height and area, respectively. These high correlation coefficient values signify the excellent linear relationship between the concentration of Quercetin and its corresponding response in the chromatogram.

The method exhibited high accuracy, with the results showing an accuracy of 99.945 ± 0.321%. This high level of accuracy indicates the reliability of the method in quantifying Quercetin content in the tested formulations.

Furthermore, degradation studies were conducted to assess the stability of Quercetin under various stress conditions. It was observed that Quercetin underwent degradation in all stress conditions tested, highlighting the importance of monitoring its stability in pharmaceutical formulations.

Overall, the developed HPTLC method proved to be robust, sensitive, and suitable for the quantitative analysis of Quercetin in pharmaceutical formulations. The optimized chromatographic conditions, along with the observed accuracy and reproducibility, validate the applicability of this method for routine quality control analysis in the pharmaceutical industry.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Zakaria, Dastoom. (2023). Herbal Medicine – Origins and Major Systems of Herbal Therapy with Selected Evidence-based Interventions. doi: 10.1002/9781119823551.ch9

[2] Bishnu, Kumar, -., J., .. (2023). A Review Study of Importance of Herbal Medicine. International Journal For Multidisciplinary Research, doi: 10.36948/ijfmr.2023.v05i01.1667

[3] Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals (2023)., doi: 10.3390/ph16071020

[4] Dharmendra, Kumar., Pramod, Kumar, Sharma. (2023). Quercetin: A Comprehensive Review. Current Nutrition and Food Science, doi: 10.2174/1573401319666230428152045

[5] Exploitation of Quercetin Antioxidative Properties in Potential Alternative Therapeutic Options of Neurodegenerative Diseases (2023). doi: 10.20944/preprints202305.2174.v1

[6] Fauzia, Mahanaz, Shorobi., Fatema, Yasmin, Nisa., Srabonti, Saha., Muhammad, Abid, Hasan, Chowdhury., Mayuna, Srisuphanunt., Kazi, Helal, Hossain., Md., Atiar, Rahman. (2023). Quercetin: A Functional Food-Flavonoid Incredibly Attenuates Emerging and Re-Emerging Viral Infections through Immunomodulatory Actions. Molecules, doi: 10.3390/molecules28030938

[7] Ramos FA, Takaishi Y, Shirotori M, Kawaguchi Y, Tsuchiya K, Shibata H, et al. Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin. J Agric Food Chem. 2006;54:3551–7.

[8] Coles LS. Quercetin: A Review of Clinical Applications.

[9] Zahedi M, Ghiasvand R, Feizi A, Asgari G, Darvish L. Does quercetin improve cardiovascular risk factors and inflammatory biomarkers in women with type 2 diabetes: A Double-blind Randomized Controlled Clinical Trial. Int J Prev Med. 2013;4:777–85.

[10] Han M, Song Y, Zhang X. Quercetin suppresses the migration and invasion in human colon cancer caco-2 cells through regulating toll-like receptor 4/nuclear factor-kappa B pathway. Pharmacogn Mag. 2016;12(Suppl 2):S237–44.

[11] Cruz-Correa M, Shoskes DA, Sanchez P, Zhao R, Hylind LM, Wexner SD, et al. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. Clin Gastroenterol Hepatol. 2006;4:1035–8.

[12] Yang F, Song L, Wang H, Wang J, Xu Z, Xing N. Quercetin in prostate cancer: Chemotherapeutic and chemopreventive effects, mechanisms and clinical application potential (Review) Oncol Rep. 2015;33:2659–68.

[13] Evans W. C. Evans D. and Trease G. E. (2009). Trease and evans pharmacognosy (16th ed.).

[14] Saunders/Elsevier.

[15] https://www.researchgate.net/publication/345600243_A_Review_Article_on_Analytical_Method_Validation

[16] https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/high-performance-thin-layer-chromatography

[17] https://www.researchgate.net/publication/317754072_HPTLC_method_development_and_validation_An_over view

[18] Arivukkarasu, R. "Detection and estimation of well-known free radical scavengers rutin, quercetin and gallic acid in market herbal anti-inflammatory and anti-arthritis formulations by HPTLC Methods."

[19] Jain, Pankaj Kumar, et al. "Evaluation of anti-diabetic and antihypertensive activity of Phoenix sylvestris (L.) Roxb leaves extract and quantification of biomarker Quercetin by HPTLC." *Phytomedicine Plus* 1.4 (2021): 100136.

[20] Shailajan, S., Patil, Y., Joshi, M., Menon, S., and Mhatre, M. (2019). Simultaneous Quantification of Pharmacological Markers Quercetin and Berberine Using High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) from a Polyherbal Formulation *Pushyanuga Churna*. *Journal of AOAC International*, *102*(4), 1003–1013. https://doi.org/10.5740/jaoacint.18-0380

[21] Rashmin, P., Mrunali, P., Nitin, D., Nidhi, D., and Bharat, P. (2012). HPTLC method development and validation: strategy to minimize methodological failures. *Journal of food and drug analysis*, *20*(4), 20.

[22] Aiyalu, R., and Mani, K. (2012). HPTLC method development, validation, and stress degradation studies for atorvastatin and ezetimibe in multicomponent tablet dosage form. *Medicinal Chemistry Research*, *21*, 1297-1301.

[23] Rani, R., Medhe, S., and Srivastava, M. (2015). HPTLC–MS based method development and validation for the detection of adulterants in spices. *Journal of Food Measurement and Characterization*, *9*, 186-194.

[24] Patel, G. H., Prajapati, S. T., and Patel, C. N. (2011). HPTLC Method development and validation for simultaneous determination of cinitapride and Pantoprazole in capsule dosage form. *Research Journal of Pharmacy and Technology*, *4*(9), 1428-1431.

[25] Jadhav, V. M., Kedar, U. S., Gholve, S. B., and Kadam, V. J. (2009). Development and validation of HPTLC method for determination of glycyrrhizin in herbal extract and in herbal gel. *Development*, *1*(4), 826-831

[26] Narayanan, S., Jadhav, A. P., and Kadam, V. J. (2015). Forced Degradation Studies of Aloe Emodin and Emodin by HPTLC. *Indian journal of pharmaceutical sciences*, *77*(6), 795–798. https://doi.org/10.4103/0250-474x.174997

[27] T., P., Aneesh., Rajasekaran, A. (2012). Forced degradation studies-a tool for determination of stability in pharmaceutical dosage forms.

[28] Mohamed, R., Elghobashy., Ali, M., Yehia., Aya, Helmy, Helmy., Nadia, F., Youssef. (2018). Forced degradation of gliquidone and development of validated stability-indicating HPLC and TLC methods. Brazilian Journal of Pharmaceutical Sciences, doi: 10.1590/S2175-97902018000317223

[29] Rini, Yulianita., Iyan, Sopyan., Muchtaridi, Muchtaridi. (2018). Forced degradation study of statins: a review. International Journal of Applied Pharmaceutics, doi: 10.22159/IJAP.2018V10I6.29086

[30] Makhija, S. N., and Vavia, P. R. (2001). Stability indicating HPTLC method for the simultaneous determination of pseudoephedrine and cetirizine in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis*, *25*(3-4), 663-667.

[31] Kotiyan, P. N., and Vavia, P. R. (2000). Stability indicating HPTLC method for the estimation of estradiol. *Journal of pharmaceutical and biomedical analysis*, *22*(4), 667-671.

[32] Kaul, N., Agrawal, H., Patil, B., Kakad, A., and Dhaneshwar, S. R. (2005). Application of stability-indicating HPTLC method for quantitative determination of metadoxine in pharmaceutical dosage form. *Il Farmaco*, *60*(4), 351-360.

[33] Kulkarni, S. P., and Amin, P. D. (2000). Stability indicating HPTLC determination of timolol maleate as bulk drug and in pharmaceutical preparations. *Journal of pharmaceutical and biomedical analysis*, *23*(6), 983-987.

[34] Singh, M., Kamal, Y. K., Parveen, R., and Ahmad, S. (2011). Development and validation of a stability-indicating HPTLC method for analysis of arjunolic acid in a herbal formulation. *JPC-Journal of Planar Chromatography-Modern TLC*, *24*(2), 172-175.

[35] Choudhari, V. P., and Nikalje, A. P. G. (2009). Stability-indicating HPTLC method for the determination of tamsulosin in pharmaceutical dosage forms. *Chromatographia*, *69*, 1463-1467.