Hygeia :: journal for drugs and medicines

April 2013 - September 2013
OPEN ACCESS
A half yearly scientific, international, open access journal for drugs and medicines
Research article section: Natural products/short communication
Researcher ID: J-7381-2012, Article ID- Hygeia.J.D.Med/95/13

Development and Validation of a RP- UFLC Method for Simultaneous Estimation of Quercetin and Rutin

Shanmugam. R[1], Gowthamarajan. K [1*], Priyanka D.L[1], Madhuri.K[2,] Hemnath.E[3], Dhanabal. SP [4]

1.Department of Pharmaceutics, JSS College of Pharmacy (JSS University, Mysore, Karnataka), Rocklands, Ooty- 643001, India.
2.Department of Pharmacology, JSS College of Pharmacy (JSS University, Mysore, Karnataka), Rocklands, Ooty 643001, India.
3. Department of Pharmaceutical chemistry, JSS College of Pharmacy (JSS University, Mysore, Karnataka), Rocklands, Ooty 643001, India.
4.Department of Phytopharmacy & Phytomedicine, JSS College of Pharmacy (JSS University, Mysore, Karnataka), Rocklands, Ooty , India.

Article history: Received: 2 December 2012, revised: 12 January 2013, accepted: 5February 2013, Available online: 3 April 2013

Abstract

Plan: *Quercetin and rutin are the flavonoides possess good therapeutic properties.*
The main objective of our work was to develop a simple, rapid and sensitive UFLC method for simultaneous estimation of quercetin and rutin.
Methodology: *A simple chromatograph separation was done by using a reverse phase C8 column (250 x 4.6 mm) by using isocratic flow with the constituted a mixture of 25 mM potassium dihydrogen ortho phosphate (pH4) and acetonitrile mobile phase at the ratio of 70:30 with the flow rate of l ml/min. Detection was carried out at 230 nm. Different validation parameters such as specificity, sensitivity, linearity, accuracy, precision, ruggedness and robustness were performed.*
Outcome: *Optimized chromatographic conditions were achieved and results showed good peak resolution between quercetin and rutin. The retention time was found at 7.4 min and 2.8 min. The LOD for quercetin and rutin was found to be 10 ng and 15 ng & LOQ was found to be 90 ng and 100 ng. The calibration curve was fixed at 0-100000 ng/ml. With change in analytical method parameters, analyst, laboratory conditions did not affect the results. % CV was calculated for all the parameters and found to be within specification limits. The developed method can be used for the quantitative and qualitative estimation of quercetin and rutin for the herbal medicinal products.*
Keywords: *Herbal medicinal products, ICH, Quercetin, Rutin, UFLC*

1. Introduction

Quercetin, a flavonoid member chemically known as 5, 7, 3^1, 4^1 - tetra hydroxy flavonol possess a lot of therapeutic benefits. It plays an important role in cardiovascular health improvement, cancer reduction, neurodegenerative diseases, aging, osteoporosis, inflammation, hepato protection, allergies, ulcers and viral diseases [1-4].

For Correspondence: gowthamsang@gmail.com , Phone: +91 423 2443393, 2443647, 2443847, Fax: +91 423 2442937

Researcher ID: J-7381-2012

Infact rutin is also a member of biflavonoides family chemically 5, 7, 3^1, 4^1- tetra hydroxy flavanol-3-rhamnoglucoside possess a lot of pharmacological actions including anti-inflammatory, anticarcinogenic, antithrombic, cytoprotective, and vasoprotective activities [5,6].Quercetin and rutin both possess good antioxidant activities [7].Several HPLC [8-13], LC-MS [14], HPTLC [15-19], UV induced Fluorescence [20] were developed for estimation of quercetin and rutin simultaneously in herbs and herbal formulations. Most of these methods revealed only estimation of quercetin and rutin from herbs. A complete validation of HPLC is also significantly lacking. However only few methods have been developed and validated. Moreover a simple, rapid and sensitive method has been not developed so far. Hence, our study reports a simple, rapid and sensitive RP-UFLC method for simultaneous estimation of quercetin and rutin in various mono and polyherbal formulations belonging to Ayurveda, Sidda and Unani systems of medicine as per ICH guidelines [21].

2. Materials and Methods

2.1. Materials

Standard Quercetin and Rutin were purchased from SDFCL, Mumbai. HPLC grade Acetonitrile (ACN), Methanol, Dimethyl Sulfoxide (DMSO), potassium dihydrogen ortho phosphate, triethyl amine and ortho phosphoric acid were obtained from Merck, Germany. Triple distilled water was obtained from Milli Q unit.

2.2. Instrument and Chromatographic conditions

The Ultra Fast Liquid Chromatography (UFLC) consists of Shimadzu LC-20AD solvent delivery system (pump), Photodiode Array Detector (PDA) with a 7725i rheodyne injector with 20µL loop volume (Kyoto, Japan). The LC Solution software was used for integration. Chromatographic separation was done using Phenomenex C8 column (250 X 4.6 mm, 5µ ID). The mobile phase consist of a mixture of 25 mM potassium dihydrogen ortho phosphate at and acetonitrile mobile phase at the ratio of 70:30 at pH 4 . The flow rate was adjusted to1 mL/min. Quercetin and rutin were quantified at a wavelength of 230 nm. 20µl of injection volume was used for injection. pH meter (Systronics, Mumbai) was used to adjust pH.

2.3. Preparation of Quercetin standard solution

10 mg standard Quercetin was weighed accurately and transferred to a 10 ml volumetric flask and 5 ml of ACN was added and dissolved and the above solution was again made up to volume with ACN to produce 100µg/ml solution. This solution was then stored in the refrigerator at -20^0 C ± 2^0C until analysis.

2.4. Preparation of Rutin standard solution

10 mg standard Rutin was weighed accurately and transferred to a 10 ml volumetric flask and 5 ml of DMSO was added and dissolved and the above solution was again made up to 10 ml with ACN to produce 100µg/ml solution. This solution was then stored in the refrigerator at -20^0 C ± 2^0C until analysis.

2.5. Detection of wavelength

100 µg of both standard solutions of rutin and quercetin were scanned in the UV region of 200 – 400 nm by using PDA detector. Better response was achieved at 230 nm.

2.6. Mobile phase preparation

1.7011gms of potassium dihydrogen ortho phosphate was weighed accurately and dissolved in 500 ml of mill Q water and the pH of the above solution was adjusted to 4 with ortho phosphate (buffer solution A).70% of above buffer solution A and 30% of ACN were mixed together and kept in a sonicator to remove the air bubbles.

2.7. Development of calibration curve for Quercetin and rutin Standards

Working solutions for calibration study were prepared from the stock solution by an adequate dilution using *ACN*. Calibration standards of concentrations 0- 100000 ng/ml were prepared for quercetin and rutin.

2.8. Method Validation

The validation parameters such as accuracy, precision (repeatability and reproducibility), linearity and range, sensitivity (limit of detection and limit of quantitation), robustness/ruggedness, selectivity/specificity and system suitability has be evaluated as per ICH guidelines.

2.8.1. Accuracy

Accuracy is expressed as the closeness of agreement of trueness. It was carried out by recovery studies by adding the known concentration of the standard solution of rutin and quercetin to the samples and the percentages recovery is being determined. The results were shown in the table.

2.8.2. Precision

Precision was carried out by two methods such as interday precision and intraday precision by injecting six injection of three different concentration of rutin (200, 20000, 100000ng/ml) and quercetin (200, 20000,100000 ng/ml) at same prescribed conditions.

$$\%CV = (\text{Standard Deviation} / \text{Mean}) \times 100$$

2.8.3. Linearity

Linearity for rutin and quercetin were prepared from the standard solution from the concentration of 0 ng/ml to 100000mcg/ml which was analyzed to check the linearity response.

2.8.4. Ruggedness and Robustness

Ruggedness of the proposed method was carried out by changing the different instrument, different operators and different column of similar model of c8. Robustness of the method was carried out by small change in flow and small change in the ph value.

2.8.5. Limit of detection (LOD)

Limit of detection of the method was determined by measuring the signal to noise ratio. Based on the signal to noise ratio. LOD for rutin and quercetin was found to be 10 and 15ng respectively.

2.8.6. Limit of Quantification (LOQ)

The smallest concentration of the analyte which can be quantified based on the signal to noise ratio. Based on the signal to noise ratio limit of quantification for rutin and quercetin was found to be 90 and 100 ng.

2.8.7. Specificity

Specificity is the method ability to assess unequivocally the analyte in the presence of compounds which may not be affected by the matrix, degradants, impurities or any other plants matrix. Specificity of the method was carried out by comparing the standard retention time spectra and the sample retention time spectra. Good separation was archived between the two standards and comparision between two standards such as retention time, peak area and spectra are said to be specific.

3. Results & Discussion

Optimization of the chromatographic condition was carried out by changing the buffer concentration, pH, strength and acetonitrile concentration. Optimization of the chromatographic conditions revealed good separation of rutin and quercetin with the mobile phase of 25 mM potassium dihydrogen ortho phosphate: Acetonitrile (70:30) v/v. Retention time for rutin and quercetin were found to be 2.8 and 7.4 min respectively. Increase in acetonitrile concentration shifts the peak to the void volume side.

Decrease in the acetonitrile leads to the loss of resolution. Increase or decrease in the buffer concentration leads to the peak splitting. Change in the pH value is not much affected. Calibration curve for the standard rutin and quercetin was plotted from 0-100000 ng/ml and it was found to be linear in range. Regression equation for rutin and quercetin was found to be 0.9987 and 0.9998 respectively.

Specificity of the method show ed good separation between the two standards quercetin and rutin and it is not affected by the other plants constituents and matrix. Accuracy results indicate that the recovery of rutin and quercetin was consistent at all levels.

Precision studies were carried out by intraday and interday method. Six injection of three different concentration of 200, 20000 and 100000 ng/ml was injected and the percentage RSD was calculated and found to be within the limits. The method was found to be rugged and robust, since there were no changes in the chromatogram by changing the optimized chromatographic conditions, instruments, operator and column. Limit of detection for the rutin and quercetin were found to be 10 and 15ng respectively. Limit of quantification for rutin and quercetin were found to be 90 and 100ng. System suitability, such as column efficiency, resolution and peak asymmetry, were calculated from the standard solution which was shown in the tables.

5. Conclusion

A simple, sensitive and rapid method for simultaneous estimation for rutin and quercetin have been developed and validated as per the ICH guidelines. This method was specific, sensitive, accurate, precise, robust and reproducible can be applied for routine analysis. The developed method can be used for the quantitative and qualitative estimation of rutin and quercetin in herbal formulations.

Acknowledgements

The authors are thankful to JSS University, Mysore and The Principal, JSS college of Pharmacy, Ooty for providing necessary facilities.

Table 1 Accuracy studies**.**

S.No.	*Quercetin (ng/ml)*			*Rutin (ng/ml)*		
	Measured concentration	Actual Concentration	% Nominal	Measured concentration	Actual Concentration	% Nominal
1	18809	18876	99.6451	2872	2840	101.127
2	18976	18876	100.53	2986	2840	105.141
3	18896	18876	100.106	2864	2840	100.845
4	18123	18876	96.0108	2753	2840	96.9366
5	18765	18876	99.412	2845	2840	100.176
6	17976	18876	95.232	2811	2840	98.9789
Mean		98.48926			100.534	
SD		2.267898			2.726127	
%CV		2.302686			2.711646	

Table 2 Precision studies: intra-day precision

S.No.	*Quercetin (ng/ml)*			*Rutin (ng/ml)*		
	200	20000	100000	200	20000	100000
1	199.96	20628.9	99976.72	196.9989	19702.19	96667.38
2	196.699	19998.47	100615.2	201.657	19952.36	97517
3	204.1705	19833.36	99748.23	198.029	19902.78	96671
4	191.84	20704.78	99153.49	197.044	19433.08	100598
5	192.28	19855.36	97992.94	203.494	19997.08	102050
6	197.22	19984.95	98188.04	200.314	19406.72	97523.8
Mean	197.02825	20167.64	99279.1	199.589	19732.37	98504.5
SD	4.67366843	393.0835	1034.629	2.67098	262.3127	2263.99
%C/V	2.372080363	1.94907989	1.04214223	1.338238	1.32935243	2.298358

Table. 3. Precision studies: inter-day precision-1

S.No.	*Quercetin (ng/ml)*			*Rutin (ng/ml)*		
	200	20000	100000	200	20000	100000
1	192.34	19279.6	99548.45	195.12	20146.48	95795.91
2	199.1	19926.64	100207.8	200.99	19610.9	101210
3	205.78	20432.85	98575.56	194.71	20064.6	96289.5
4	195.11	20488.74	99398.84	199.46	20038.3	101211
5	192.16	19769.95	100224.3	193.73	19613.6	96271.4
6	195.19	19415.48	100141.6	204.39	20089.6	100731
Mean	196.613	19885.54	99682.76	198.067	19927.2	98584.9
SD	5.14958	503.3148	648.6955	4.22442	246.592	2712.76
%C/V	2.619139	2.53105997	0.65075997	2.132826	1.237462	2.751699

Table. 4. Precision studies: inter-day precision-2

S.No.	*Quercetin (ng/ml)*			*Rutin (ng/ml)*		
	200	20000	100000	200	20000	100000
1	199.7	20430.9	99360.2	195.12	19660.74	97017.39
2	202.7	19642.41	99747.88	200.99	20109.8	101028
3	200.84	19246.27	100169.3	190.64	19710.1	101198
4	194.84	19713.61	99327.07	195.12	20095.9	96266.4
5	195.11	19785.47	100057	205.55	19610.5	101474
6	195.11	19584.09	99549.87	196.77	20109.3	97671.9
Mean	198.05	19733.79	99701.887	197.365	19882.7	99109.2
SD	3.45602	389.2204	354.13062	5.21142	245.557	2373.1
%C/V	1.745024	1.97235505	0.35518948	2.640499	1.235026	2.394433

Table. 5. Calibration and linearity

Concentration (ng/ml)	*Perk area of Quercetin*	*Perk area of Rutin*
0	0	0
200	25992	4465
600	80543	13189
1000	133597	21978
10000	1346060	225114
20000	3089867	445386
40000	5461788	901325
60000	7998643	1348965
80000	10345287	1791203
100000	13459565	2251381

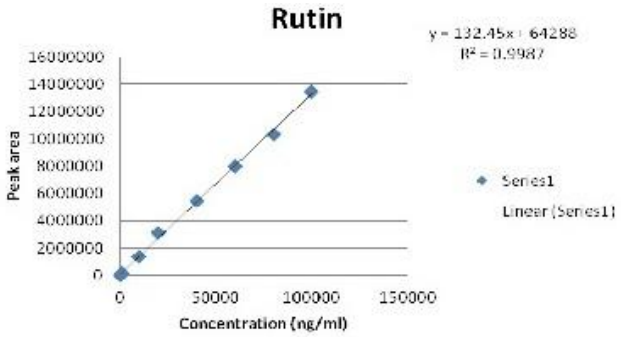

Fig.1. Calibration curve of standard Rutin

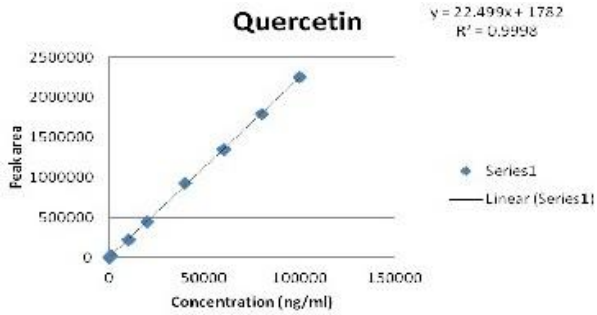

Fig.2. Calibration curve of standard Quercetin

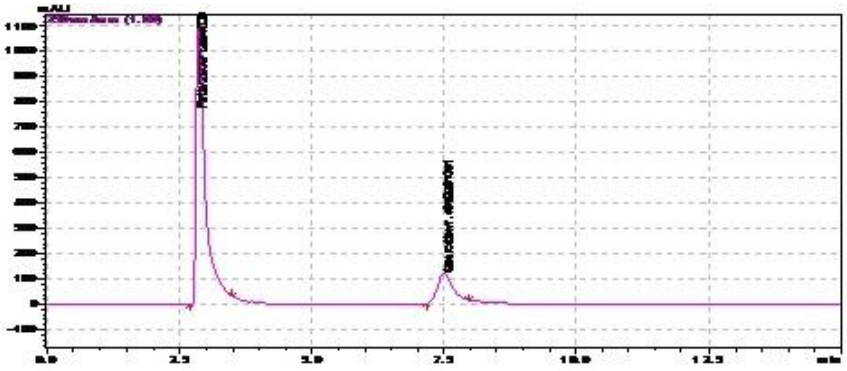

Figure 3: Typical HPLC chromatogram of Standard Quercetin and Standard Rutin

References

1. Charoenlarp P, Radomyos P, Harinasuta T, Treatment of taeniasis with Puag Haad: a crude extract of Artocarpus lakoocha wood, *Southeast Asian J Trop Med Public Health* **981**;12: 568-570.
2. Salguero CP, A Thai, Herbal Traditional Recipes for Health and Harmony, (Findhorn Press, Scotland) **2003**,119.
3. Cesarone MR, Laurora G, Ricci A, Belcaco G, Pomante P, Acute effects of hydroxyethylrutosides on capillary filtration in normal volunteers, patients with various hypotensions and in patients with diabetic micro angiopathy, *J Vas Disease* **1992**;21: 76-80.
4. Clack W, Heller W, Michel C, Saran M, Effect of flavonoid substances on histamine toxicity, anaphylactic shock and histamine enhanced capacity to dye, *J Allergy* **1950**; 21:133-147.
5. Colergie Smith PO, Thomas P, Scurry JH, Dormandy JA, Causes of various ulceration, a New Hypothesis, *Br Med J* **1980**; 296: 1726-1727.
6. Hertog MGL, Hollman PCH, Katan Klohout D, Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands, *Nutr Cancer* **1993**;20: 21-29.
7. De Whalley C, Rankin S M, Houct JRS, Jessup W, Leake DS, Flavonoids inhibit the oxidative modification of low-density lipoproteins by macrophages, *Biochem Pharmacol* **1990**; 39: 1743-1750.
8. Yuangang Z, Chunying L, Yujie F, Chunjian Z, Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD, *J. Pharmaceut. Biomed. Anal* **2006**;41: 714–719.
9. Paśko P, Sajewicz M, Gorinstein S, Zachwieja Z, Analysis of Selected Phenolic Acids and Flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* Seeds and Sprouts by HPLC, *Acta Chromatogr* **2008**;4: 661–672.
10. Ashok Kumar BS, Lakshman K., Jayaveera KN, Vamshi Krishna N, Manjunath M, Suresh MV, Estimation of Rutin and Quercetin in *Amaranthus viridis Linn* by HPLC, *Asian J. Exp. Sci* **2009**;23: 51-54.
11. Swapnadeep P, Jain DC, Joshi SB, Identification and quantification by RPHPLC analysis of secondary metabolites in extracts of *Artemisia annua., J Pharm Res* **2009**;2: 1552-1556.
12. Nitra N, Ank HL, Kornkanok I, Separation and Detection of the Antioxidant Flavonoids, Rutin and Quercetin, Using HPLC Coupled on-line With Colorimetric Detection of Antioxidant Activity, *Naresuan University J* **2004**;12: 25-37.
13. Weerasak S, Suwanna V, Simultaneous Determination of Gallic acid, Catechin, Rutin, Ellagic Acid and Quercetin in Flower Extracts of *Michelia alba, Caesalpinia pulcherrima* and *Nelumbo nucifera* by HPLC, *Thai Pharm Health Sci J* **2007**;2: 131-137.
14. Ashish R, Arvind J, Rajesh T, Vikas, Liquid Chromatography- Tandem Mass Spectrometric Method For Simultaneous Determination Of Rutin And Quercetin From Leaves Of *Artocarpus Lakoocha* Roxb, *International journal of Pharma and Bio sciences* **2011**;2: 848-853.
15. Avijeet J, Santram L, Singhai AK, Simultaneous estimation of quercetin and rutin in *Tephrosia purpurea* Pers by high performance thin layer chromatography. *Asian J Tradit Med* **2009**; 4:104-109.
16. Sajeeth CI, Manna PK, Manavalan R, Jolly C, Quantitative estimation of Gallic Acid, Rutin and Quercetin in certain herbal plants by hptlc method, *Der Chemica Sinica*, **2010**; 1:80-85.
17. Zhuohong X, Yang Z, Pei C, Pu J, Jin Y, Liangli Y, Chromatographic Fingerprint Analysis and Rutin and Quercetin Compositions in the Leaf and Whole Plant Samples of Di and Tetraploid Gynostemma pentaphyllum, *J Agric Food Chem* **2011**;59: 3042–3049.
18. Preeti T, Rakesh KP, Development and Validation of HPTLC method for quantification of Quercetin and Rutin in Draksharishta, *AJPSR* **2012**; 2:7-18.
19. Pradeep HA, Saleemulla K, Raghu C, Mohammed I, Development of Validated HPTLC Method for simultaneous quantification of Rutin and Quercetin from bark of *Anogeissus Latifolia, Int J Pharm* **2012**; 2:33-38.
20. Komori A, Naoto I, Kaori F, Shinya K, Asakazu H, Measurement of Rutin and Quercetin in Tartary Buckwheat Flour by Ultraviolet-Induced Fluorescence, Proceedings of the l0th international symposium on buckwheat, 401-409
21. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology **2005**, Available at: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html (accessed Jan 18, 2011).

Conflict of interest: None

Shanmugam. R, Gowthamarajan. K , Priyanka D.L, Madhuri.K, ' Hemnath.E, Dhanabal. SP. Development and Validation of a RP UFLC Method for Simultaneous Estimation of Quercetin and Rutin .*Hygeia.J.D.Med*. **2013**; 5(1):113-120. Available at http://www.hygeiajournal.com / Article ID- Hygeia.J.D.Med/95/13**.**