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METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF QUERCETIN USING UV AND RP-HPLC IN BULK AND FORMULATION

M. K. Patel*[1], S. K. Shah[1], C. K. Tyagi[1] and Md. Rageeb Md. Usman[2]

[1]College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M.P.) India 466002

[2]Smt. S. S. Patil College of Pharmacy Chopda, Maharashtra 425107

Corresponding Author : Mukesh Kumar Patel

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences,

Email : mukeshpatel84007@gmail.com

Abstract

To develop a novel, accurate, precise and linear reverse phase high performance liquid chromatographic (RP-HPLC) and UV spectrophotometric methods for quantitative determination of quercetin in bulk drug and quercetin loaded microspheres. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on a reversed-phase Thermo C18 (250 × 4.6 mm, 5µm) column with mobile phase consisting of methanol: acetonitrile (50:50v/v) at flow rate of 1.0 ml/min. The retention time for quercetin was 4.186±0.3min. The UV spectrophotometric determinations were performed at 256 nm using phosphate buffer pH 7.4 and 0.1 N HCl as a solvent. The linearity range for quercetin was 5-25µg/ml for HPLC and 10-50µg/ml for UV method. The linearity of the calibration curves for each analyte in the desired concentration range was good (r2 >0.999) by both the HPLC and UV methods. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. Moreover, the accuracy and precision obtained with HPLC co-related well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis. The proposed methods are highly sensitive, precise and accurate and hence successfully applied for determining the assay and in vitro dissolution of a marketed formulation.

***Keywords*:** HPLC, UV Spectrophotometry, Quercetin, Pharmaceutical formulation, Method validation, Quantitative analysis.

Introduction

Quercetin is a plant flavonol and it is found in many fruits, vegetables, leaves, and grains. It acts as antioxidant. It is a non-specific protein kinase enzyme inhibitor. It has also been reported to have estrogenic activities by activating estrogen receptors. It is used in treatment of heart diseases; exercise induced respiratory problems, high cholesterol, diabetes, asthma, gout, cancers such as lung cancer, ovarian cancer, and pancreatic cancer. The IUPAC name of quercetin is 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy4H-chromen-4-one (Fig. 1). It is yellow crystalline powder having molecular formula $C_{15}H_{10}O_7$ and molecular weight 302.236 g/mol. The melting point of quercetin is 316 °C. It is very soluble in ether, methanol; soluble in ethanol, acetone, pyridine, acetic acid (Kaur & Singh 2019; Sandhu *et al.,* 2017). The literature survey reveals that various analytical methods for estimation of quercetin were reported alone and in combination with other drugs (Walid *et al.,* 2015; Lee *et al.,* 2014; Das *et al.,* 2017; Baghel *et al.,* 2107; Sharifuldin *et al.,* 2016), also simultaneous estimation of gallic acid, catechin, rutin, ellagic acid and quercetin in flower extract of Michelia alba, Caesalpinia pulcherrima and Nelumbo Nucifera by HPLC reported (Weerasak & Vorarat 2007) but to the best of our knowledge there is no such reported UV and HPLC analysis method for estimation of quercetin in bulk drug and microspheres. In the present investigation, we have developed a simple, optimized and validated UV and HPLC analysis method for estimation of quercetin in bulk drug and microspheres. The method was validated as per the international conference on harmonization (ICH) guidelines (ICH 2005). This novel validated method has applicability in an industry as well as in academia.

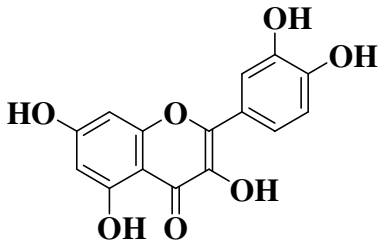

Fig. 1 : Chemical structure of quercetin

Materials and Methods

Reagents and chemicals

HPLC grade quercetin (purity 99%) was procured as gift sample from Yucca Enterprises, Mumbai, India. Acetonitrile, methanol (HPLC Grade), supplied by Merck Ltd, New Delhi, India. Triple distilled water was generated in house. Quercetin loaded microspheres was prepared in house.

Instrument

In UV-spectrophotometric method, Labindia model-3000 + series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

UV spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) of quercetin

Wavelength of maximum absorption was determined by scanning 10µg/ml solution of quercetin using UV spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 256.0 nm.

Preparation of calibration curve of quercetin

Calibration curve in phosphate buffer pH 7.4: 10 mg of quercetin was weighed accurately and dissolved in 5 ml of phosphate buffer pH 7.4 in a 100 ml of volumetric flask and volume was made up to 100 ml with the phosphate buffer pH 7.4. Aliquot of 1 ml of this solution was withdrawn and transferred to 10 ml volumetric flask and diluted to 10 ml with pH 7.4 phosphate buffer to obtain a stock solution of 10µg/ml. From this stock solution, aliquots of 1ml, 2ml, 3ml, 4 ml and 5 ml were transferred to 10 ml volumetric flasks and volume was made upto 10 ml phosphate buffer pH 7.4. The absorbances of these solutions were measured at 256nm against a blank phosphate buffer pH 7.4. The calibration curve was obtained by plotting the absorbance of quercetin versus the concentration of quercetin. The straight line of best fit was obtained by using linear regression analysis program (Fig. 2).

Calibration curve in 0.1 N HCl: Quercetin solution was scanned in the U.V. range of 200-400 nm using Labindia 3000+ UV-Visible spectrophotometer. 10 mg of quercetin was weighed accurately and dissolved in 5ml of 0.1 N HCl in a 100 ml of volumetric flask and volume was made up to 100 ml with the 0.1 N HCl solutions. Aliquot of 1 ml of this solution was withdrawn and transferred to 10 ml volumetric flask and diluted to 10ml with 0.1 N HCl to obtain a stock solution of 10 µg/ml. From this stock solution, aliquots of 1 ml, 2ml, 3ml, 4ml and 5ml were transferred to 10 ml volumetric flasks and volume was made up to 10 ml 0.1 N HCl. The absorbances of these solutions were measured at 256 nm against a blank 0.1 N HCl. The calibration curve was obtained by plotting the absorbance of quercetin versus the concentration of quercetin (Fig. 3).

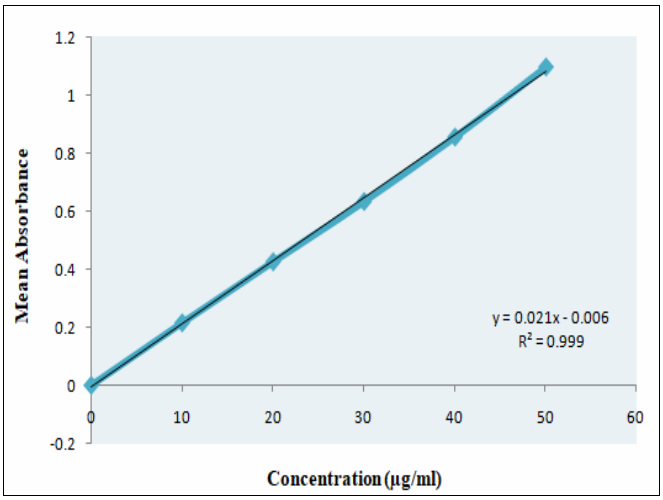

Fig. 2 : Calibration curve of quercetin in phosphate buffer pH 7.4 at 256nm

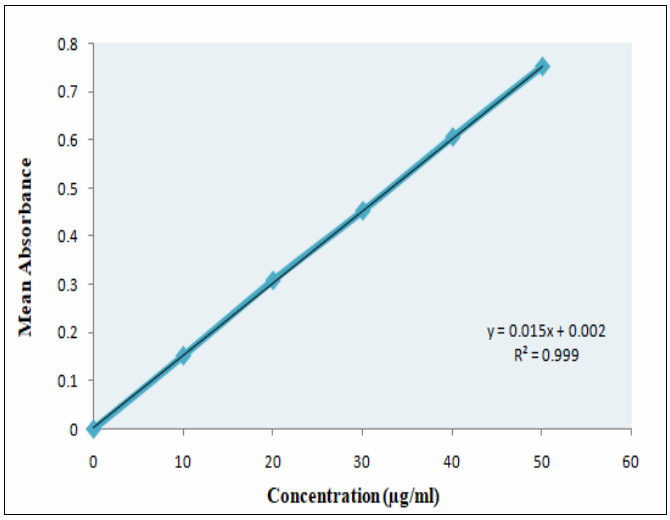

Fig. 3 : Calibration curve of quercetin in 0.1 N HCl at 256nm

RP-HPLC method

Chromatographic condition

The isocratic mobile phase consisted of methanol: acetonitrile in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 256 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Preparation of standard stock solution : 10mg of quercetin was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the mobile phase to give a stock solution of 1000ppm.

Preparation of working standard solution : From stock solutions of quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 5-25 µg/ml for quercetin solutions manually in triplicate to the HPLC system at detection wavelength of 256 nm. Mean of n =5 determinations was plotted as the standard curve (Fig.4). The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter day and intra-day measurements.

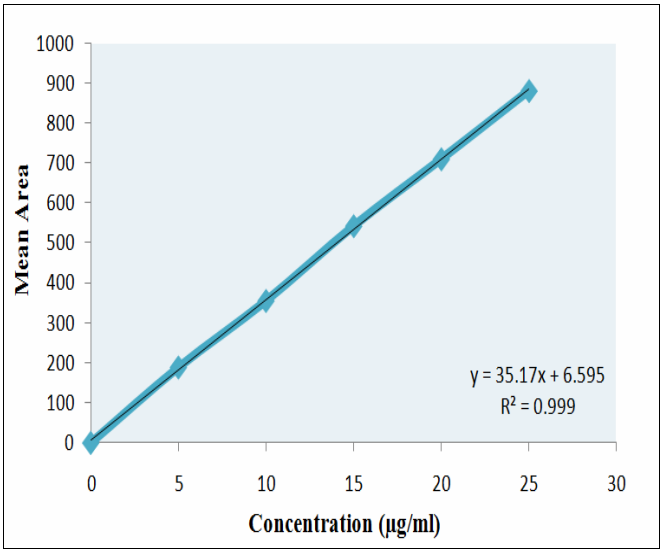

Fig. 4 : Calibration graph of quercetin

System Suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 µg/ml of quercetin were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in Table1.

Table 1 : Results of system suitability parameters

Parameters	Quercetin
AUC	355.337
No. of Theoretical Plates	3470.000
Tailing Factor	1.153
Retention time	4.186

Validation Parameters

Linearity

Linearity was studied by analyzing five standard solutions (n=5) in the range of 5-25 and 10-50µg /ml of quercetin for HPLC and UV spectrophotometric method respectively. Calibration curves with concentration verses absorbance or peak area was plotted for each method and the obtained data were subjected to regression analysis using the least squares method. Linearity of quercetin was established by response ratios of drug. Response ratio of drug was calculated by dividing the absorbance or peak area with respective concentration (Table 2).

Accuracy

The validity and reliability of the proposed methods was assessed by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The recovery studies were carried out by adding known amount of standard solution of quercetin to preanalysed formulation solutions. The resulting solutions were then re-analysed by proposed methods. In UV Spectrophotometric method, the value of mean recoveries was found to be in the range of 99.25 % to 99.42 % for quercetin. The value of SD and %RSD less than 2 indicate the accuracy of the method. In RP-HPLC method, the value of mean recoveries was found in the range of 97.73% to 98.89 % for quercetin. Total amount of drug found and percentage recovery was calculated. Results of recovery studies are reported in Table 3.

Precision

Precision was determined by repeatability and intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 4.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and / or concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of quercetin was studied Table 5.

LOD and LOQ

LOD and LOQ of described method were observed as 0.145µg/ml and 0.450µg/ml for quercetin in UV spectrophotometric method and 0.570 µg/ml and 0.500 µg/ml for quercetin in RP-HPLC method, based on the SD of response and slope, which meet the requirement of new method.

Analysis of in house microspheres formulation

10mg Quercetin were weighed and ground to a fine powder. An equivalent amount to 10 mg of Quercetin was taken in 10 ml volumetric flask. This was dissolve in 5 ml of diluents by sonication for about 10 minutes. The volume was made up to the mark by diluents as per the UV spectrophotometry method and RP-HPLC method. The solutions were filtered (whatman filter paper no.41). The filtrate was used to prepare samples of different concentration. The statistical evaluation of tablet analysis by both methods is reported in Table 6.

Table 2: Response ratios of quercetin

Concentration (µg/ml)	HPLC Method		Concentration (µg/ml)	UV Method Phosphate buffer pH 7.4		UV Method 0.1 N HCl	
Quercetin	AUC	RR	Quercetin	ABS	RR	ABS	RR
5	188.768	37.754	10	0.215	0.021	0.152	0.015
10	355.337	35.534	20	0.425	0.021	0.308	0.015
15	542.515	36.168	30	0.632	0.021	0.452	0.015
20	709.997	35.500	40	0.854	0.021	0.605	0.015
25	880.841	35.234	50	1.095	0.021	0.751	0.015

Table 3: Results of recovery study

Recovery Level%	% Mean±SD*	
	U.V Method	RP-HPLC Method
80%	99.25±0.573	98.08±0.878
100%	99.40±0.781	97.73±1.270
120%	99.42±0.210	98.89±0.631

- Value of three replicate and three concentrations
-

Table 4: Results of precision

Parameters	UV Method		RP-HPLC Method	
Precision (Mean± SD)*	Quercetin	%RSD	Quercetin	%RSD
Repeatability	99.96±0.017	0.017	99.90±0.050	0.050
Day to Day	99.95±0.153	0.153	99.555±0.181	0.182
Analyst to Analyst	99.17±0.666	0.671	99.80±0.256	0.245

*Average of 5 replicate and 5 concentration.

Table 5: Result of robustness of formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 °C	+ 5 °C
Quercetin	0.45	0.87	0.99
Flow rate		(-10%)	(+10%)
Quercetin	0.52	0.98	0.99
Mobile phase ratio		- 2 %	+ 2 %
Quercetin	0.36	0.92	0.82

Table 6: Results and statistical parameters for microspheres formulation

S.No	Drug	Label claim	Amount found	% Label claimed	SD*	%RSD*
UV Method	**Quercetin**	10	9.98	99.96	0.017	0.017
RP-HPLC	**Quercetin**	10	9.90	99.90	0.050	0.050

*Average of five determination

Result and Discussion

RP-HPLC and UV-Spectrophotometric methods were developed for quercetin which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (Rt) of quercetin was 4.186±0.3min. The chromatograms have been shown in Fig. 5. A five point calibration curve was constructed with working standards and was found linear (r2 = 0.999) for each of the analytes over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram. The developed HPLC method was accurate, precise, reproducible and very sensitive.

For UV Method (Phosphate buffer pH 7.4):

Y = 0.021x - 0.006 (r2= 0.999)

(0.1 N HCl): Y = 0.015x + 0.002(r2= 0.999)

For RP-HPLC: Y = 35.17x +6.595 (r2= 0.999)

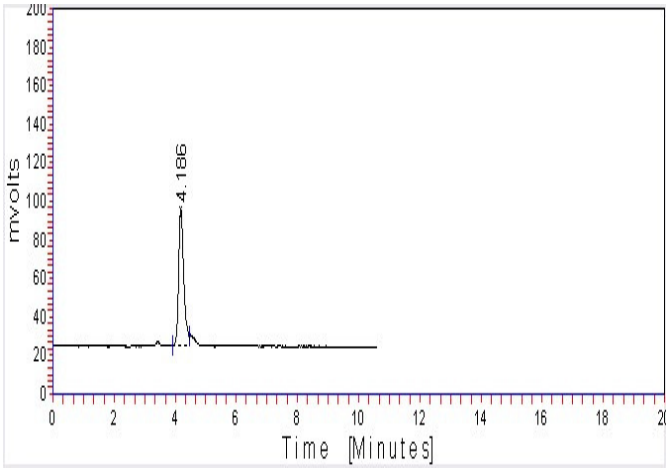

Fig. 5 : Chromatogram of Quercetin

All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines. Table 3 lists the percent recovery (content uniformity) of quercetin in the microspheres formulations by HPLC and UV methods. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 4). Moreover the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD

concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients interfered with the analytes of interest. Hence, the methods were suitably employed for assaying quercetin in microspheres formulation (Table 6).

Conclusion

The advantage of UV method over HPLC method is that the proposed UV method does not require the elaborate treatment and procedures usually associated with chromatographic method. It is less time consuming and economical. A statistical comparison of the quantitative determination of quercetin shows that HPLC method as more accurate and precise than UV method. The results indicate HPLC and UV spectrotometry methods are adequate methods to quantify quercetin in pure form and its dosage form. There was no interference by excipients in the microspheres and the mobile phase is easy to prepare. Since these methods are simple, specific, rapid and accurate, they may be successfully and conveniently adopted for routine quality control analysis of quercetin in bulk and pharmaceutical dosage form.

References

Baghel, U.S.; Nagar, A.; Pannu, M.S.; Singh, D. and Yadav, R. (2017). HPLC and HPTLC Methods for Simultaneous Estimation of Quercetin and Curcumin in Polyherbal Formulation. Indian Journal of Pharmaceutical Sciences, 79(2): 197-203.

Das, P.; Pannerselvam, T. and Prabhu, P.P. (2017). Spectrometric Method Development and Validation of Catechin and Quercetin in Khadirarista. Saudi Journal of Medical and Pharmaceutical Sciences, 3(8): 936-940.

International Conference on Harmonisation. (2005) Validation of analytical procedures: Text and Methodology, Geneva, Switzerland.

Kaur, P. and Singh, B. (2019). Analytical Method Development and Validation of Quercetin: A Review. International Journal of Pharmaceutical and Clinical Research 11(2): 49-56.

Lee, F.A.; Mun, F.Y.; Yvonne, T.; Peh, K. and Yusrida, D. (2014). HPLC method for simultaneous quantitative detection of quercetin and curcuminoids in traditional chinese medicines. J Pharm., 17: 36-49.

Sandhu, P.S.; Begm S.; Kumarm R.; Katarem O.P. and Singh, B. (2017). Analytical QbD-Based Systematic Bioanalytical HPLC Method Development for Estimation of Quercetin Dihydrate. Journal of Liquid Chromatography & Related Technologies, 40(10): 506-516.

Sharifuldin, M.M.A.; Ismail, Z.; Aisha, A.F.A.; Seow, E.K. and Beh, H.K. (2016). Quantification of Rrutin, Quercitrin and Quercetin in Cosmos caudatus Kunth by Reverse Phase High Performance Liquid Chromatography. Quality Assurance and Safety of Crops & Foods, 8(4): 617-622.

Walid, E.; Hassan, M. and Mona, M. (2015). HPLC analysis of quercetin and antimicrobial activity of comparative methanol extracts of *Shinus molle* L. Int J Curr Microbiol Appl Sci., 4: 550-558.

Weerasak, S. and Vorarat, S. (2007). 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 2:131-137.