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Original Article

Marker Based Standardization of Quercetin in Marketed Capsules by Novel Zero Order Spectroscopic and Area Under Curve Spectroscopic Methods

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ABSTRACT

Background: Quality control and standardization of phytomedicines play a vital role in herbal drug industries. Marker based quantification and standardization is one of the essential approaches used in quality evaluation of herbal products. The Quercetin is an important flavonoid having variety of therapeutic activities and many formulations are available in the market. Capsule dosage form of Quercetin is largely marketed. The main objective for analytical development of proposed research is to develop and validate marker-based quantification of analytical methods to carry out quality control of Quercetin along with its standardization in marketed Ayurvedic capsule formulation. **Materials and Methods:** In the proposed analytical research work two novel UV-spectrophotometric methods mainly Area Under Curve (AUC) spectroscopic and zero order spectroscopic methods were developed and also validated for marker-based quantification of Quercetin. The development of both UV-spectrophotometric methods was carried out by using water: methanol (50:50%v/v) as a solvent. In zero order spectroscopic method absorbance of Quercetin was measured at 372nm. The detection of Quercetin was carried out at 372nm. The area covered between 325nm to 402nm was used for calculation. **Results**: Quercetin exhibits λ_{max} at 372nm obeying Beer's law at concentration ranges lying between 2-10µg/mL giving r^2 values of 0.9997 and 0.9992 respectively using absorbance and area under curve method. The validation parameters for all these methods were found to be well within the acceptance values. Both UV-spectrophotometric methods showed excellent recovery values between 98 to 107%. Both newly developed and validated marker-based quantification methods were successfully applied for estimation of Quercetin in its marketed capsule dosage form. **Conclusion:** The proposed research work concludes that these two developed UV-Spectrophotometric methods were new, simple, precise, and accurate for marker-based quantification of Quercetin in marketed formulation.

Keywords: Area Under Curve, Marker Based Quantification, Quality Control, Quercetin, Zero Order Spectroscopy.

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INTRODUCTION

Quality control and Standardization of phytomedicines is very important and essential. Herbal medicinal preparations have been widely used from time immemorial in all developing as well as developed countries for the primary health care of people. They are widely in use as they provide many added advantages in terms of safety, efficacy showing minimum or no side effects. Traditional herbal medicines are prepared from naturally occurring plants showing potent medicinal properties. These preparations may not require lengthy industrial procedures for their production and are now in a great demand for treatment of various ailments including severe health conditions. India is a well-known country for its knowledge in herbal medicine. The Indian systems of medicine mainly comprises of Siddha, Ayurveda and Unani systems forming the pillars of Indian traditional system of medicine.[1]

The herbal medicines may not require evaluating parameters for toxicity or safety profile of the phytochemical. Currently many countries are engaged in the manufacture and production of phyto medicines, but are lacking effective laboratories and equipments for the quality control standardization of herbal formulations.[2]

Quality control for any formulation to be marketed plays an important role as quality of product indicates the safety and

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effectiveness of medicines. Quality control is compulsorily applied for both raw materials, excipients and finished produce. The quality of the materials used in the manufacturing of phytomedicines need to be monitored before subjecting them for production of the final product. This can be achieved with the help of various present-day analytical tools made available in the market which play an important role in the quality control and standardization. These include various electrochemical, chromatographic and spectroscopic methods which are in wide use.[3]

Extensive rise in the demand of herbal medicines and phytopharmaceuticals worldwide expands its rapid global market. Hence the safety and quality evaluation of phytopharmaceuticals is a concernment for health organizations, pharmaceutical companies and the public. There is great requirement in the establishment of consistent quality, safety, efficacy and potency from one batch to another batch. "Standardization of Phytopharmaceuticals based on Marker" is one of the great accepted methods worldwide. The method is based on the analysis of phytochemical markers using sophisticated analytical methods along with the tools and using its profile for establishing the quality control data of herbal products. Recently, the concept of Standardization of Herbal drugs using marker-based is gaining momentum. Marker compounds are recognized as analytical tools internationally for assessing the quality of phytopharmaceuticals.[4,5]

Generally, marker acts as a reference for standardizing test materials which includes raw material, extracts and finished products and therefore used for the quality assurance of phytopharmaceuticals in herbal industries. A marker compound can be defined as a constituent of an herbal drug with or without therapeutic activity. Markers are reference substances that are chemically defined phyto-constituents which may or even may not be responsible to exert any biological activity. Markers are used to quantify the active constituents of phytopharmaceuticals. Marker compounds are secondary metabolites which are single isolated pure compounds.[6,7]

We have many modern instruments and analytical techniques which can be used to control the quality of medicines or drugs from natural origin. The analytical techniques[8] are classified into various categories as Chromatographic Techniques, Optical Methods or Spectral Techniques, Physical Methods or Thermo-analytical Methods, Electrochemical Techniques, Biological and Microbiological Methods, Titrimetric Methods, Radioactive Methods and Miscellaneous Methods.

At present, spectrum techniques play very important role in the structural characterization of compounds from natural as well as from synthetic source. Spectroscopic methods are also used for quantitative as well as qualitative analysis of raw materials and medicinal formulations. In herbal drug development, spectroscopic techniques are widely used in the characterization of isolated fractions, identification of bioactive fractions, quantitative estimation of bioactive compounds etc. UV-spectroscopy is mainly used for the qualitative and quantitative analysis of drug or molecules from natural and synthetic origin.[9]

Flavonoids form the major active constituent of plants that belong to a class of polyphenols, which are distributed throughout the plant kingdom. Different flavonoids are well known for their potent therapeutic activities. Few of the important flavonoids like Quercetin, Apigenin, and Rutin are well known for their antiallergic, antithrombic, antispasmodic, anticancer, hepatoprotective, anti-inflammatory, antiallergic and antioxidant activities.[2]

Quercetin (Figure 1) is a well-known flavonoid chemically identified as 5,7,3′-4′ tetra hydroxy flavanol, is a polyphenolic derivative found in many plants and exert various therapeutic activities such as anti-inflammatory, hepatoprotective and anti-spasmodic properties.[2] A number of medicinal plants and their marketed formulations containing Quercetin are available and hence quality control of these is very important and essential in manufacturing industries.

Chemical Structure of Quercetin

Literature review on the quality control analysis and standardization of Quercetin in its isolated form, extract or any other herbal or polyherbal preparation was done. Review of scientific publications has been added. A complete and detailed summary of spectroscopic[10-14] and chromatographic standardization of Quercetin[15-51] is presented below in Tables 1 and 2 respectively.

Literature search revealed that Quercetin and Berberine Hydrochloride were analyzed by various analytical methods in their herbal formulations, extracts, and pharmaceutical preparations. The reported methods were having their own advantages and disadvantages. To overcome the disadvantages, there is a need for development and subsequent validation of marker-based quantification methods for the estimation, quality control along with the standardization of selected marker in its herbal marketed formulations.[10-51]

The main aim of the proposed research is to carry out marker-based quantification of selected phytochemical, Quercetin in herbal medicines using newly developed and validated analytical techniques. The main objectives of proposed research is the development and validation of novel UV-spectrophotometric methods for the marker-based quantification of Quercetin in its marketed formulations.

Table 1: Spectroscopic standardization of Quercetin in Herbal Medicines

Sl. No.	Author Name	Title of the Work	Description of Analysis
1	V.C. Yeligar *et al.*,	Development and Validation of UV-spectrophotometric Method for Simultaneous Estimation of Melatonin and Quercetin in Liposome Formulation.	The UV-spectra are between 200 and 400 nm. Melatonin (10 µg/ml) and quercetin (10 µg/ml) standard solutions were found to have λ_{max} values of 276 nm and 372 nm, respectively.
2	Singh Upendra *et al.*,	Simultaneous Estimation of Quercetin and Silymarin: Method Development and Validation.	The wavelengths of the UV-spectrum are 400–200 nm. For quercetin and silymarin, the peak absorption was recorded at 256 nm and 288 nm respectively.
3	Ginpreet Aneja *et al.*,	Simultaneous Estimation of Piperine, Quercetin, and Curcumin in A Mixture using UV-visible spectrophotometer and Method Validation.	The maximum absorbance of quercetin, curcumin, and piperine were determined to be 371.31 nm, 424.68 nm, and 343.76 nm, respectively, in the standard stock solution of 1000ppm of each medication in methanol and scanned in the range of 200-400 nm.
4	A Viswanath *et al.*,	UV-spectrometry method for the estimation of Quercetin from *Ipomoea sepiaria* Koenig.	The standard absorbance of quercetin is 350 nm, acetone extract is 328.2 nm, and methanol extract is 323.4 nm.
5	Marzanna Kurzawa *et al.*,	Determination of Quercetin and Rutin in Selected Herbs and Pharmaceutical Preparations.	In methanol and ethanol, respectively, the standard solutions of rutin and quercetin were created. For quercetin and rutin, the absorbance was reported at 425 nm and 362 nm, respectively.

MATERIALS AND METHODS

Materials

Instruments and apparatus

An electronic weighing balance (SARTORIUS) has been used for weighing the analyte, UV-spectrophotometer (Shimadzu 1800) was used to measure the Area Under Curve and absorbance, Ultrasonic Bath Sonicator (Ultrasonic cleaner) was used for sonication.

Reagents and chemicals

All chemicals used were of AR grade and were procured from the chemicals storehouse of KLE College of Pharmacy, Belagavi. Millipore water was taken from a direct-Q UV water purification system from the Department of Pharmacognosy, KLE College of Pharmacy.

Marker

Quercetin was used as a standard marker for analytical development.

Marketed Formulation

The marketed formulation containing Quercetin capsules was procured from local market of Belagavi, Karnataka, India.

Development and Validation of Zero Order Spectroscopic and Area Under Curve UV-spectroscopic Methods for Quantification of Quercetin.

Method Development

The method development for UV-spectrophotometry was initiated first with solvent system selection and determining the wavelength for analysis. Solubility of Quercetin was checked in different solvents and it was found out that Quercetin is freely soluble in methanol. Hence in order to prepare the dilutions we have used methanol for first stock. In order to prepare secondary stock solution and working standards of Quercetin we have used Methanol: Distilled water (50:50%v/v) as the solvent. The UV-spectrophotometer Shimadzu 1800 (Figure 2) was used for analysis of Quercetin.[52-57]

UV-spectrophotometer (Shimadzu 1800)

Preparation of stock solution

Standard stock solution for Quercetin containing 1000µg/mL was prepared in 10mL volumetric flask by first dissolving 10mg in sufficient quantity of methanol and then the volume was made up with the same. From this stock solution, further 1mL was taken in 10mL volumetric flask and further adjusting the volume up to the mark using methanol: distilled water (50:50%v/v) as solvent system to get a concentration of 100µg/mL. From this stock

Table 2: Chromatographic Standardization of Quercetin in herbal formulations.

Sl. No.	Author Name	Title of the Work	Description of Analysis
1	Sunita shailajan *et al.*,	A comparative estimation of quercetin content from *Cuscuta reflexa* Roxbusing validated HPTLC and HPLC techniques.	Using 0.025 M NaH_2PO_4 and ACN as the mobile phase, using HPLC. The investigation was carried out on a C_{18} column (150 mm 4.6 mm 5 m), and peaks at 378nm were noted.
2	Asmaʿ aAi-Rifai *et al.*,	Analysis of Quercetin and Kaempferol in an Alcoholic Extract of *Convolvulus pilosellifolius* using HPLC.	Peaks at 258 nm were detected using the HPLC technique with an isocratic combination of methanol and water containing 0.1% v/v formic acid (80:20) on a BETASIL C_{18} column.
3	Lee Fung Ang *et al.*,	HPLC Method for Simultaneous Quantitative Detection of Quercetin and Curcuminoids in Traditional Chinese Medicines.	Using a thermo ersil Gold column, a C_{18} cartridge guard column, and a mobile phase system of acetonitrile and 2% v/v acetic acid (40:60), at the detection wavelength of 370 nm, quantitative detection of quercetin and curcuminoids (dimethoxy curcumin, bis demethoxy curcumin, and curcumin) in traditional Chinese medicines.
4	Ujjwala supe *et al.*,	Preliminary Phytochemical Analysis and Quantitative Analysis of Quercetin by HPLC of *Momordica charantia*.	Utilising Merck C_{18} Bondapack, which is kept at a temperature of 27°C, and methanol: ACN: water (60:20:20v/v) as the mobile phase, HPLC was utilised to analyse the sample. The investigation was done for flavonoids, phenols, and quercetin at 260 and 262 nm wavelength.
5	A. Srinivasa Rao *et al.*,	Simultaneous determination of phenolic compounds in *Catharanthus roseus* leaves by HPLC method.	Maximum absorbance was measured at 254 nm using the HPLC method using the Athena C_{18} column, phosphate buffer (pH=5.8), and ACN as the mobile phase 55:45 Ratio. Rutin, Quercetin, and Kaempferol each had a retention time of 2.403, 6.143, and 8.903 correspondingly.
6	Deepak Mundkinajeddu *et al.*,	Development and Validation of High Performance Liquid Chromatography Method for Simultaneous Estimation of Flavonoid Glycosides in *Withania somnifera* Aerial Parts.	HPLC method by using Phenomenex Luna C_{18} column and the mobile system consist of potassium dihydrogen orthophosphate dissolved in 900ml of HPLC grade water to that 0.5ml of orthophosphoric acid added volume made up to 1000ml, for the estimation of 3 flavonoid glycosides that are quercetin-3-orobinoside-7-O-glucoside, quercetin-3-O-rutinoside-7-O-glucoside, kaempferol 3-O-robinobioside-7-O-glucoside.
7	Aline Augusti Boligon *et al.*,	Development and Validation of an HPLC-DAD Analysis for Flavonoids in the gel of *Scutia buxifolia*.	They have verified the HPLC by quantifying the quercetin and rutin at a maximum absorbance wavelength of 356 nm using a C_{18} column and a mixture of Acetonitrile: water (70:30, v/v) as the mobile phase.
8	Gomathy Subramanian *et al.*,	Development and Validation of HPLC Method for the Simultaneous Estimation of Quercetin and Rutin in *Aganosma dichotoma* [Roth] K. Schum.	For the HPLC procedure, a C_{18} column and mobile phase containing acetonitrile: ammonium acetate (40:60 v/v) at a flow rate of 1 ml/min are used to achieve separation. The 259 nm analysis was seen.

Sl. No.	Author Name	Title of the Work	Description of Analysis
9	Haritha Krishna prasad *et al.*,	Method Development and Validation for the Simultaneous Estimation of Resveratrol and Quercetin in Bulk and Pharmaceutical Dosage Form by RP-HPLC.	RP-HPLC method for the estimation is performed by using Sunfire C_{18} column having Rheodyne injector using Methanol: Water:Formic acid: Triethylamine in the ratio 10:70:15:5 as mobile phase at the wavelength 277nm.resveratrol and Quercetin eluted at retention time 1.24 and 2.14 respectively.
10	Vishal Sharad Chaudhari *et al.*,	Analytical method development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers.	RP-HPLC method for The estimation was carried out by using Hypersil gold C_{18} column and mixture of acetonitrile and HPLC grade water (pH 2.6 adjusted with 2%v/v glacial acetic acid) as a mobile phasewavelength 346nm Quercetin and Piperine is eluted at retention time 2.80 min and 10.36 min respectively.
11	Ashok Kumar *et al.*,	Estimation of Gallic acid, Rutin and Quercetin in *Termenelia chebula* by HPTLC.	HPTLC method by using precoated silica gel GF_{254} as stationary phase and Toluene: acetone: glacial acetic acid (3:2:1 v/v/v/v/v) as mobile phase for of tannins, and ethyl acetate: dichloromethane: formic acid: glacial acetic acid: water (10:2.5:1:1:0.1v/v/v/v/v) the quantification is carried out for Rutin and Quercetin at 366nm and for gallic acid at 254nm densitometrically and R_f value of gallic acid, rutin, and quercetin are 0.30, 0.13, 0.93 respectively.
12	Sachin U. Rakesh *et al.*,	HPTLC Method For Quantitative determination of Quercetin in Hydroalcoholic Extract Of Dried Flower of *Nymphaea stellata* Willd.	In HPTLC method the detection and quantification were carried out by silica gel 60 F_{254} plates and Toluene: ethyl acetate: formic acid in the ratio 5:4:0.2 (v/v/v) as mobile phase. At 380nm a sharp and well-defined peak found at R_f=0.29.
13	Ansul Shakya *et al.*,	A Rapid High-Performance Thin-Layer Chromatographic Method to Estimate Quercetin in *Benin casahispida* (Thunb.) Cogn. Fruit Pulp.	HPTLC for the estimation using alumina plates with silica gel 60 F_{254} were used with Toluene: ethyl acetate: formic acid (5:4:0.2v/v) as mobile phase at absorbance wavelength 262nm the Rf value of Quercetin found R_f=0.392.
14	Barik Laxmi Dhar *et al.*,	HPTLC method for Quantitative estimation of Quercetin in a polyherbal compound for urolithiasis.	By HPTLC method extract chromatogram med on silica gel 60 F_{254} aluminium plates and mixture of chloroform: methanol: formic acid used as mobile phase in the ratio 7.5:1.5:1 v/v/v. The R_f value was 0.51 analysed at wavelength of 254nm.
15	Bindu A R *et al.*,	High Performance Thin Layer Chromatographic Method for Quantitative Determination of Quercetin in Tender Leaves of *Psidium guajava*.	HPTLC method carried out on silica gel 60 F_{254} TLC plates using toluene: acetone: formic acid (30:10:5) as mobile phase. The detection and quantification done at 364nm and R_f value of acetone extract was 0.45.

Sl. No.	Author Name	Title of the Work	Description of Analysis
16	Vaidevi Sethuram *et al.*,	Combinatorial analysis of quercetin and resveratrol by HPTLC in *Sesbania grandiflora* /phyto based nanoformulations.	HPTLC method In this investigation separation achieved by using mobile phase system of toluene: chloroform: ethyl acetate: formic acid (3:2:4.9:0.1 % v/v). The densitometric scanning at 280nm was performed and R_f value was 0.40 (Quercetin) and 0.50 (Resveratrol).
17	Mangesh S. Kharate *et al.*,	Estimation of Quercetin from Crude Leaf Extract, *Mimusops elengi* L. By HPTLC.	Toluene, ethyl acetate, and formic acid were combined in a 5:4:1 ratio as the mobile phase for the detection of quercetin on precoated silica gel plates F_{254}. There was detection and quantification at 365 nm.
18	V Patil *et al.*,	Recent progress in simultaneous estimation of rutin, quercetin and liquiritin in *Cocculus hirsutus* by HPTLC.	The silica gel 60 F_{254} was used as the mobile phase system for the simultaneous estimation together with a mixture of n-butanol, acetic acid, water, and formic acid (7:1:1:0.25). At a wavelength of 254 nm, the R_f values for rutin, quercetin, and liquiditin were, respectively, 0.047, 0.063, and 0.82.Toluene, ethyl acetate, and formic acid were combined in a 5:4:1 ratio as the mobile phase for the detection of quercetin on precoated silica gel plates F_{254}. There was detection and quantification at 365 nm.
19	Omi Laila *et al.*,	Development and Validation of HPTLC Method for Simultaneous Estimation of Diosgenin and Quercetin in Fenugreek Seeds (*Trigonella foenum-graceum*).	Precoated aluminium plates with silica gel G60 F_{254} and a toluene: ethyl acetate: formic acid (5:4:1 v/v/v) mixture as the mobile phase were used for the HPTLC method for validation. The plates were scanned at 275nm, and the R_f values were 0.690.02 (Diosgenin) and 0.570.02 (Quercetin).
20	A. Srinivas Rao *et al.*,	Simultaneous estimation of quercetin and rutin in ethanolic extract of *Melia azedarach*. Linn leaves by HPTLC method.	Precoated silica $60F_{254}$ is used as the stationary phase in the HPTLC method for the analysis, and a combination of toluene, ethyl acetate, and methanol (5:3:2) is used as the mobile phase. Quercetin and rutin have R_f values of 0.65 and 0.15 at 254 nm, respectively.
21	Gundu Sindhu Chakraborty *et al.*,	Determination of Quercetin by HPTLC in "*Calendula officinalis*" Extract.	Silica gel 60GF is used as the stationary phase in the HPTLC process, with precoated aluminium plates serving as the mobile phase solvent system. At a wavelength of 366 nm, scanning was done to estimate the R_f value, which was 0.43.
22	Md. Sarfaraj Hussain *et al.*,	Validation of the method for the simultaneous estimation of bioactive marker gallic acid and quercetin in *Abutilon indicum* by HPTLC.	The analysis was carried out using silica gel $60F_{254}$ HPTLC plates with an aluminium foil backing and a solvent system comprising toluene, ethyl acetate, and formic acid (5:4:1 v/v) as the mobile phase. At 270 nm absorbance, the R_f value was 0.31 for gallic acid and 0.50 for quercetin.

Sl. No.	Author Name	Title of the Work	Description of Analysis
23	Jinan Hussain *et al.*,	Qualitative and quantitative comparison of rutin, quercetin and gallic acid concentrations in Syrian *Capparis spinosa*. L Leaves.	Silica gel $60F_{254}$ was used for the analysis, and the mobile phase was a combination of ethyl acetate, glacial acetic acid, formic acid, and distilled water (100:11:11:25). The R_f values at the 366nm scanning wavelength were 0.39 for quercetin, 0.79 for rutin, and 0.81 for gallic acid.
24	Abhay Prakash Mishra *et al.*,	A Developed and Validated High-Performance Thin-Layer Chromatographic Method for the Quantitative Determination of Quercetin in *Satyrium nepalense* Tubers.	Using silica gel $60F_{254}$ as the stationary phase and the solvent solution toluene: ethyl acetate: formic acid (7:5:1 v/v), HPTLC analysis was performed at 366 nm.
25	Shiv K Yadav *et al.*,	Development and validation of a HPTLC method for simultaneous estimation of quercetin, Chlorogenic acid and trigonelline in polyherbal antibacterial formulation.	Using the solvent system chloroform: ethyl acetate: methanol: formic acid (5:3:1.5:0.5v/v/v/v) as the mobile phase, perform HPTLC analysis on aluminium plates 60 F_{254}. With respect to trigonelline, chlorogenic acid, and quercetin, the well-separated spots were found with R_f values of 0.13, 0.24, and 0.62, respectively.
26	Hiteksha Panchal *et al.*,	Development of Validated High-performance Thin-layer Chromatography Method for Simultaneous Determination of Quercetin and Kaempferol in *Thespesia populnea.*	analysis carried out Using toluene, ethyl acetate, and formic acid (6:4:0.3 v/v/v), silica gel 60 F_{254} was previously precoated on aluminium plates. Quercetin's R_f value at the scanning absorbance wavelength of 366 nm is 0.39, whereas kaempferol's R_f value is 0.50.
27	Khan Dureshahwar *et al.*,	Quantification of Quercetin Obtained from *Allium cepa* Lam. Leaves and its Effects on Streptozotocin-induced Diabetic Neuropathy.	The quantification was performed using precoated GF_{254} silica gel plates, a mobile phase solution of 5:4:1 toluene, ethyl acetate, and formic acid, and a scanning absorbance of 254 nm.
28	Supriya S. Jirge *et al.*,	Simultaneous Estimation of Kaempferol, Rutin, and Quercetin in Various Plant Products and Different Dosage Forms of Bhuiamla and Amla.	The analysis was carried out on precoated silica gel aluminium plates 60 F_{254} using a mobile phase containing a mixture of chloroform, methanol, and formic acid (8.2:1.5:1), and the R_f values of kaempferol, quercetin, and rutin at 254 nm are reported.
29	Shikar Verma *et al.*,	HPTLC Analysis for the Simultaneous Quantification of Gallic Acid, Vanillic Acid, Protocatechuic Acid, and Quercetin in the Methanolic Fraction of *Limonia acidissima* L. Fruits.	They have used silica gel 60 F_{254} plates for the HPTLC technique. The detection was performed at 254 nm using the solvent mixture of toluene, ethyl acetate, and formic acid (5:4:1), and the observed R_f values are 0.30.00 for gallic acid, 0.470.00 for vanillic acid, 0.370.00 for protocatechuic acid, and 0.420.00 for quercetin.
30	Snehal B. Bhandare *et al.*,	Simultaneous Quantification of Kaempferol and Quercetin in Medicinal Plants using HPTLC.	HPTLC method uses RP-18 F_{254} plates and toluene, acetone, and formic acid in the ratio 7:3:0.25 as the mobile phase. At the detection wavelength of 254nm, the R_f values for kaempferol and quercetin were determined to be 0.46 and 0.39, respectively.

Sl. No.	Author Name	Title of the Work	Description of Analysis
31	Shikar Verma *et al.*,	Gas Chromatographic–Mass Spectrometric Profile of Non-Polar Fraction and High-Performance Thin-Layer Chromatographic Analysis of Methanolic Fraction with Simultaneous Quantifications of Proto catechuic Acid and Quercetin in *Carissa carandas* L. Fruits.	HPTLC analysis employing silica gel $60F_{254}$ precoated plates and a solvent solution of toluene, ethyl acetate, and formic acid (6:3:1, v/v). At the highest absorbance wavelength of 310 nm, the R_f values for protocatechuic acid and quercetin, respectively, were 0.57 and 0.61.
32	Nadeem A. Siddique *et al.*,	Simultaneous Quantification of Umbelliferone and Quercetin in Polyherbal Formulations of *Aegle Marmelos* by HPTLC.	Silica gel $60F_{254}$ was used as the stationary phase for the analysis, which was run on pre-coated aluminium plates. Toluene, ethyl acetate, and formic acid (6:4:1 v/v/v) serve as the mobile phase in the solvent system. The R_f values for umbelliferone and quercetin at 300 nm were reported to be 0.66 and 0.68, respectively.
33	Pushpendra kumar *et al.*,	Simultaneous Quantification of Quercetin and Syringic Acid in Methanolic Extract of *Leucas lavandulifolia* by using Validated HPTLCDensitometric Method.	The analysis was performed using toluene, ethylacetate, and formic acid (7:2.5:0.5 v/v) as the mobile phase on a Silica gel $60F_{254}$ plate. At the detection wavelength of 275 to 370 nm, the R_f values for quercetin and syringic acid were determined to be 0.32 and 0.41, respectively.
34	Thafshila Aafrin Am *et al.*,	Determination qf Quercetin by High Performance Thin Layer Chromatography Method in *Achyranthes aspera* (L.) Plant Extract.	The test was conducted using a stationary phase of silica gel $60F_{254}$ and a 5:4:1 combination of toluene, ethyl acetate, and formic acid. At 254 nm, the R_f value 0.60 is found.
35	Avijeet Jain *et al.*,	Simultaneous estimation of quercetin and rutin in *Tephrosia purpurea* Pers by high performance thin layer chromatography.	Precoated silica gel RP-18 F_{254} was used as the stationary phase for the estimate, and the solvent mixture of methanol, water, and formic acid (40:57:3 v/v/v) was utilised as the mobile phase. The R_f values at 254 nm were 0.07 for quercetin and 0.17 for rutin.
36	Girme AS *et al.*,	Method development, optimization and validation of RP-UFLC method for bioactive flavonoids from *Cassia auriculata.*	The analysis was done on an Acquity C18 column using a 0.1% formic acid in water and methanol stepwise gradient elution at a flow rate of 0.350 ml/min. For quercetin QUE-3-O-rutinoside, the highest absorbance at 350 nm wavelength was discovered. For quercetin-3-O-rutinoside and quercetin, the retention times were found to be 3.95 and 5.37, respectively.
37	Shanmugam R *et al.*,	Development and Validation of a RP- UFLC Method for Simultaneous Estimation of Quercetin and Rutin	The reverse phase System C8 column underwent investigation. The mixture of potassium dihydrogen ortho phosphate and acetonitrile in the mobile phase has a 70:30 ratio. For quercetin and rutin, the retention times were 7.4 and 2.8 minutes, respectively, and the detection was carried out at 230 nm.

Sl. No.	Author Name	Title of the Work	Description of Analysis
38	Khaled Elgendy *et al.*,	Analysis of Total Flavonoids in Herbal Drugs Expressed as Quercetin by Reversed Phase-UHPLC Method.	The analysis was carried out using a Phenomenex column with 0.5% phosphoric acid and methanol as the mobile phase with a flow rate of 1.3ml/min. For quercetin, the method's linearity is established over the concentration range of 240-960 mcg/ml and the retention time was 7.8 minutes.
39	Maric santos *et al.*,	UPLC-MS for Identification of Quercetin Derivatives in Cuphea glutinosa Cham. and Schltdl (Lythraceae) and Evaluation of Antifungal Potential.	The quick C_{18} analytical column shim pack XR-ODS column underwent analysis. A mixture of acetonitrile: methanol (4:1 v/v) as solvent A and water with 0.1% formic acid as solvent B made up the mobile phase. Full scan mode was used to record the mass spectra in the *m/z* 200–800 range.

solution, serial dilutions were made to obtain 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL concentrations.

Selection of solvent and wavelength for analysis

By literature survey and solubility studies and it was revealed that Quercetin shows complete solubility in methanol. Many trials were carried out in different proportions of water and methanol to get an alternate solvent system which can be economically feasible. Finally, a solvent system comprising of methanol: distilled water (50:50%v/v) was chosen for UV-spectrophotometric studies for analysis of Quercetin. In order to obtain the wavelength for analysis, solution containing 2µg/mL was scanned in the UV region of 200-400nm. A spectrum was obtained and the wavelength for maximum absorbance was set at 372nm.

Measurement of Area Under Curve (AUC) and absorbance for ZOS methods

For Area Under Curve (AUC) method, two wavelengths 325-402 nm were selected and AUC between these two was used for the measurement.

Method Standardization

The developed UV-spectrophotometric method was validated as per ICH guidelines in terms of specificity, selectivity, linearity, LOD, LOQ, precision, ruggedness and accuracy.[55-60]

Selectivity and Specificity

Specificity was performed to eradicate the possibilities of interference due to solvent system in the region of maximum absorbance peak of Quercetin. The specificity and selectivity were evaluated by running the solvent system and comparing the spectrum of Quercetin.

Linearity and Range

Dilution series were prepared from the standard stock solution of Quercetin to obtain even concentrations from 2µg/mL – 10µg/mL of Quercetin. For AUC method, the two wavelengths 325-402nm were selected for the determination. The calibration plot was constructed on the basis of AUC *vs* Concentration parameters.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of Quercetin were determined using calibration standard and statistical formulas by the proposed method.

Precision

Precision was studied in order to evaluate the accuracy of proposed methods. The system precision was evaluated by measuring AUC and absorbance given by Quercetin solution at three different concentrations. In the same manner, intraday and interday precisions were evaluated by performing analysis on same day at two different intervals and on three different days. After every analysis, %RSD was calculated.

Ruggedness

The ruggedness expresses the variation that might arise due to the change in laboratory conditions (different analyst). It was performed by repeating the same analysis by different analyst on the same instrument. After analysis been carried out, % RSD was calculated.

Accuracy

The accuracy of developed method was set on by recovery studies at three different levels. The samples were spiked with 50%, 100% and 150% of the mixed standard solution. The mixture was analyzed and recoveries were determined. The recovery study was carried out in triplicate and hence the mean % recovery was calculated.

Table 3: Developed UV-Spectrophotometric method parameters.

Sl. No.	Parameters	Specifications
1	Method	Spectrophotometric
2	Instrument	UV-spectrophotometer
3	Model	Shimadzu
4	Make	UV-1800
5	Software	UV-Probe
6	Analyte	Quercetin
7	Solvent	Methanol:Water (50:50)
8	Lambda Max.	372 nm

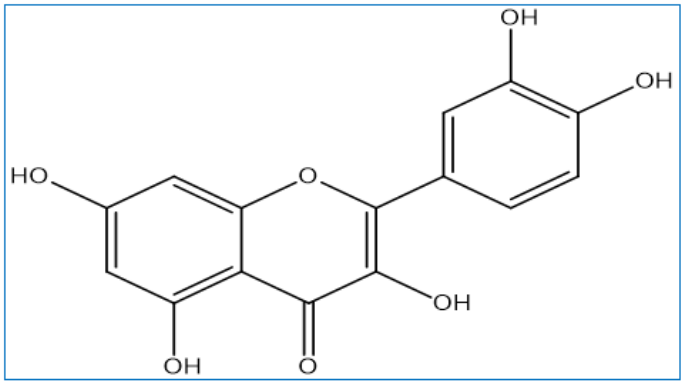

Figure 1: Chemical Structure of Quercetin.

Table 4: Linearity and range data of Quercetin using UV-spectroscopic methods.

Sl. No.	Concentration	Absorbance at 372nm	Area Under Curve (325 nm– 402 nm)
1	2µg/mL	0.148	3.542
2	4µg/mL	0.27	6.669
3	6µg/mL	0.404	10.060
4	8µg/mL	0.540	13.289
5	10µg/mL	0.668	16.140
r^2		0.9997	0.9992
LOD		0.01µg/mL	0.53µg/mL
LOQ		0.04µg/mL	1.61µg/mL

Assay

Twenty marketed capsules of Quercetin were weighed and the average weight was calculated for the same. Capsules containing powder which was equivalent to 10mg of Quercetin was weighed and transferred to 10 mL volumetric flask. Quercetin was extracted from powder using methanol: water (50:50 v/v) system and was sonicated for 15 min. After extraction, serial dilutions were made to obtain concentration of 10µg/mL and finally the absorbance was measured.

RESULTS

Development and Validation of Absorbance/Zero Order Spectroscopic and Area Under Curve UV-Spectroscopic Methods for Quantification of Quercetin

Development

The method development step for solvent involves the use of methanol: water (50:50) in which Quercetin showed spectrum with maximum absorbance at 372nm. Parameters for the developed method are presented in Table 3.

Method Standardization

Specificity and Selectivity: Solvent spectrum obtained showed no interference and gave an absorbance at 372 nm which show the specificity and selectivity of developed method. UV spectrum of solvent and Quercetin were showed in Figure 3 and Figure 4.

UV-Spectrum of Solvent

Linearity and Range: Standard calibration curve was plotted using concentration *vs* absorbance obtained by serial dilution of Quercetin. Each concentration shows linear absorbance between the even concentration ranges of 2, 4, 6, 8, 10µg/mL with regression equation of 0.9997 for Quercetin. Linearity data was presented in Table 4. The standard calibration curve was presented in Figure 5 and Figure 6. The overlay spectrum for linearity of Quercetin was showed in Figure 7 and AUC graphs was presented in Figure 8.

Limit of Detection (LOD) and Limit of Quantification(LOQ)

The LOD and LOQ were found to be 0.01µg/mL and 0.04µg/mL respectively by using absorbance method and by AUC method, the LOD and LOQ were found to be 0.53µg/mL and 1.61µg/mL respectively.

Precision

Ruggedness: The %RSD for each concentration performed by different analyst was within the acceptance ranges and hence the method was found to be rugged. Table 7 represents Ruggedness data of Quercetin using UV methods.

Table 5: Interday precision data of Quercetin using UV-spectroscopic methods.

Concentration (µg/mL)	Days	Absorbance	SD	% RSD	Area Under Curve	SD	% RSD
2	1st day	0.132	0.0015	1.15	3.258	0.0236	0.73
	2ndday	0.131	0.0020	1.53	3.228	0.0282	0.87
	3rd day	0.147	0.0006	0.39	3.775	0.0092	0.24
6	1st day	0.483	0.0052	1.08	12.373	0.1189	0.95
	2nd day 3rd day	0.404	0.0036	0.89	10.318	0.0512	0.49
		0.403	0.0006	0.57	10.361	0.0106	0.10
10	1st day	0.664	0.0040	0.61	16.705	0.0781	0.48
	2nd day	0.660	0.0080	1.21	16.514	0.2329	1.41
	3rd day	0.669	0.0038	0.57	17.336	0.0365	0.21

Table 6: Interday precision data of Quercetin using UV-spectroscopic methods.

Concentration (µg/mL)	Days	Absorbance	SD	% RSD	Area Under Curve	SD	% RSD
2	Morning	0.127	0.0020	1.57	3.199	0.0178	0.56
	Afternoon	0.142	0.0006	0.41	3.493	0.0056	0.16
	Evening	0.132	0.0015	1.15	3.258	0.0236	0.73
6	Morning	0.406	0.0006	0.14	10.334	0.0636	0.61
	Afternoon	0.404	0.0015	0.38	10.287	0.1166	1.12
	Evening	0.403	0.0025	0.62	10.083	0.0450	0.45
10	Morning	0.671	0.0067	0.99	17.427	0.1997	1.15
	Afternoon	0.635	0.0010	0.16	15.845	0.0695	0.44
	Evening	0.667	0.0006	0.09	16.400	0.0779	0.47

Table 7: Ruggedness data of Quercetin using UV-spectroscopic methods.

Concentration (µg/mL)	Absorbance	SD	%RSD	Area under curve	SD	%RSD
2	0.149	0.0017	1.15	3.694	0.0318	0.87
	0.152			3.679		
	0.152			3.633		
6	0.441	0.0012	0.26	10.879	0.1192	1.11
	0.438			10.675		
	0.439			10.670		
10	0.679	0.0052	0.37	16.391	0.1544	0.95
	0.676			16.106		
	0.674			16.154		

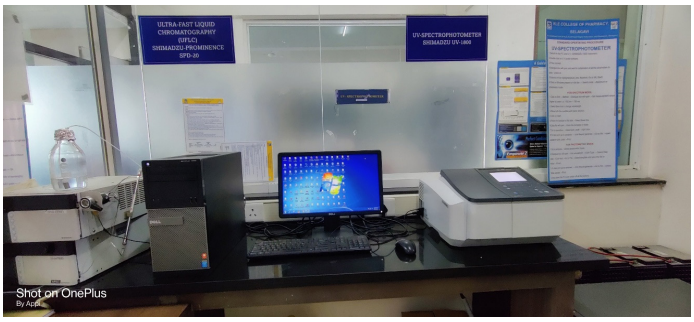

Figure 2: UV-Spectrophotometer (Shimadzu 1800).

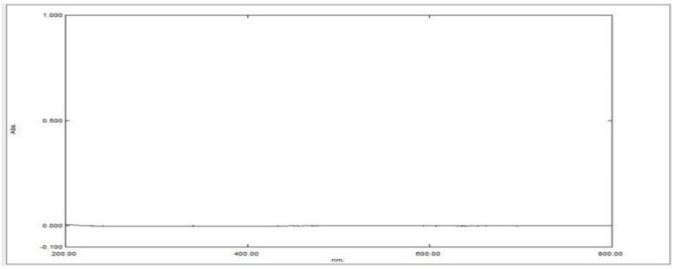

Figure 3: UV-Spectrum of Solvent.

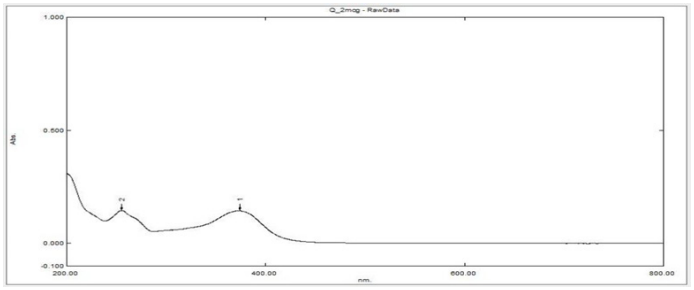

Figure 4: UV-Spectrum of Quercetin (2µg/mL).

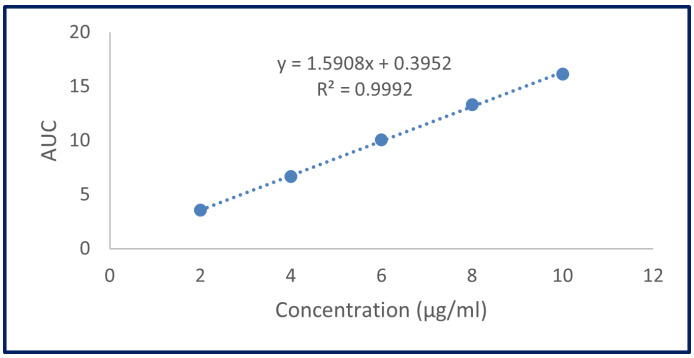

Figure 6: Standard calibration curve Quercetin using AUC method.

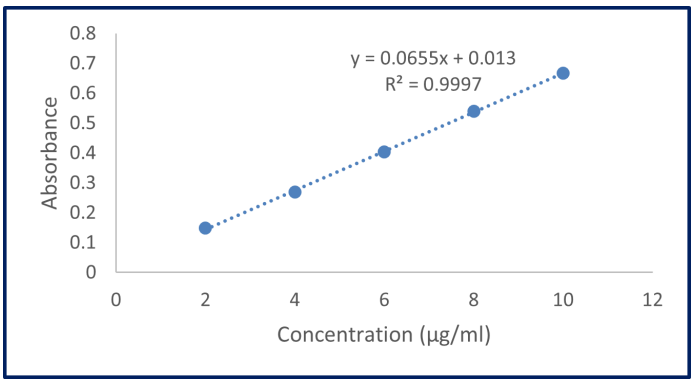

Figure 5: Standard calibration curve of Quercetin using Absorbance method.

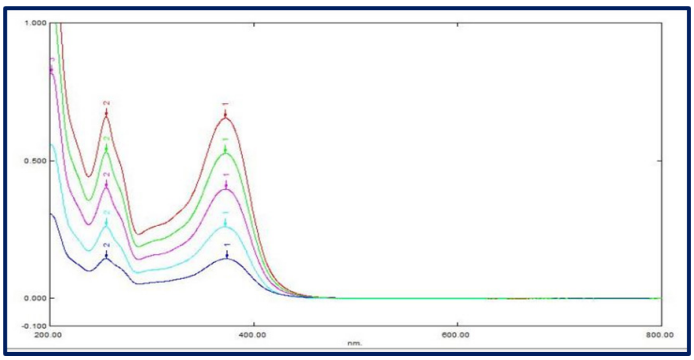

Figure 7: Linearity overlay spectrum of Quercetin.

Accuracy: The method was found to be accurate as all the level recovery values were well within the acceptance ranges. Accuracy of Quercetin was found to be within the acceptance values for both methods and data was presented in Tables 8 and 9.

Assay: The assay values of Quercetin in capsule dosage form by AUC and ZOS methods were found to be 99.21% and 98.76% respectively.

Method was found to be precise as the calculated % RSD for six replicate solutions of Quercetin at each precision level were found to be less than 2%. Data for precision was presented in Tables 5 and 6.

DISCUSSION

Quercetin is a well-known flavonoid found in many plants and exert various therapeutic activities. A number of medicinal plants and their marketed formulations containing Quercetin are available and hence quality control is essential in the manufacturing industries. Marker based quantification is one of the essential approaches used in quality evaluation of herbal products. Capsule dosage form of Quercetin is largely marketed. In the proposed analytical research work two novel UV-spectrophotometric methods mainly area under curve

Table 8: Accuracy data of Quercetin using UV-spectroscopic ZOS method.

Level	Total Concentration Added (Standard + Sample)	Absorbance	Concentration Obtained	Sample Concentration Difference	% Recovery
50%	3µg/mL	0.184	3.032 µg/mL	1.032 µg/mL	101.09%
		0.187	3.082 µg/mL	1.082 µg/mL	102.74%
		0.189	3.115 µg/mL	1.115 µg/mL	103.84%
100%	6µg/mL	0.401	5.926 µg/mL	3.926 µg/mL	98.76%
		0.412	6.088 µg/mL	4.088 µg/mL	101.47%
		0.415	6.133 µg/mL	4.133 µg/mL	102.21%
150%	9µg/mL	0.551	9.463 µg/mL	7.463 µg/mL	105.15%
		0.555	9.532 µg/mL	7.532 µg/mL	105.91%
		0.551	9.463 µg/mL	7.463 µg/mL	105.15%

Table 9: Accuracy data of Quercetin using UV-spectroscopic AUC method.

Level	Total Concentration Added (Standard + Sample)	AUC	Concentration Obtained	Sample Concentration Difference	% Recovery
50%	3µg/mL	4.601	3.229 µg/mL	1.229	107.65%
		4.623	3.244 µg/mL	1.244	108.16%
		4.557	3.198 µg/mL	1.198	106.62%
100%	6µg/mL	9.912	5.953 µg/mL	3.953	99.21%
		10.149	6.095 µg/mL	4.095	101.59%
		10.197	6.124 µg/mL	4.124	102.07%
150%	9µg/mL	13.574	9.322 µg/mL	7.322	103.57%
		13.726	9.426 µg/mL	7.426	104.73%
		13.601	9.340 µg/mL	7.34	103.78%

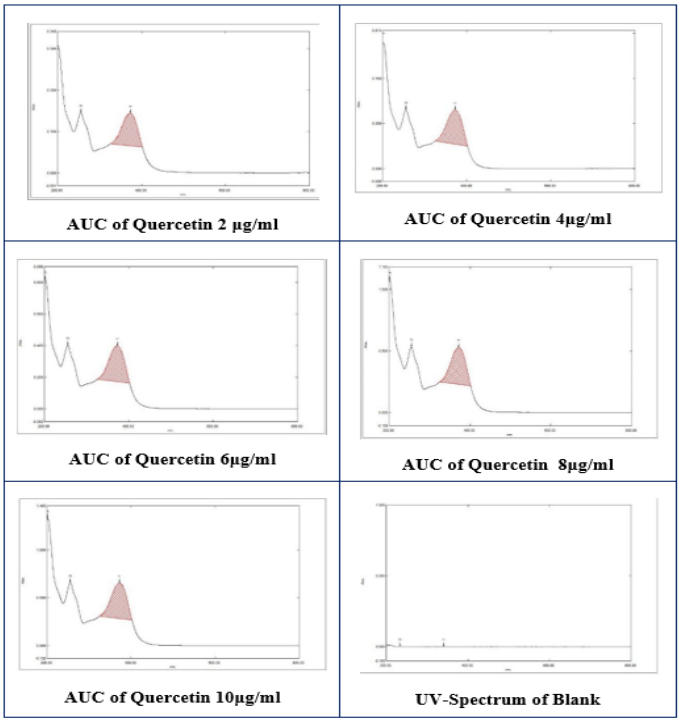

Figure 8: Area Under Curve Spectrum of Quercetin.

spectroscopic and zero order spectroscopic methods were developed and also validated for marker-based quantification of Quercetin. The development of both UV-spectrophotometric methods was carried out by using water: methanol as a solvent. In zero order spectroscopic method absorbance of Quercetin was measured at 372 nm. The detection of Quercetin was carried out at 372 nm. The area covered between 325nm to 402nm was used for calculation. The validation parameters for all these methods were found to be well within the acceptance values. Both UV-spectrophotometric methods showed excellent recovery values. Both newly developed and validated marker-based quantification methods were successfully applied for estimation of Quercetin in its marketed capsule dosage form.

CONCLUSION

Quality control and standardization of phytomedicines is essential and an important step in the production industries. Marker based quantification and standardization is one of the essential approaches used in quality evaluation of herbal products. The main objective of analytical development for proposed research is to develop and validate marker-based quantification analytical methods for quality control along with standardization of

Quercetin in marketed formulations. In the proposed analytical research work two UV-spectrophotometric methods, mainly zero order spectroscopy and Area Under Curve (AUC) were developed and validated for marker based quantification of Quercetin. The development of both UV-spectrophotometric methods was carried out by using water: methanol (50:50%v/v) as a solvent. In zero order spectroscopic method absorbance of Quercetin was measured at 372 nm. The detection of Quercetin was carried out at 372 nm. The area covered between 325 nm to 402 nm was used for calculation. The proposed research work concludes that the two newly developed and validated UV-spectrophotometric methods were found to simple, precise and accurate for marker-based quantification of Quercetin in marketed capsule formulation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High-performance liquid chromatography; **HPTLC:** High-performance thin layer chromatography; **RP-UFLC:** Reverse phase ultra-fast liquid chromatography; **UHPLC:** Ultra high-pressure liquid chromatography; **ZOS:** Zero Order Spectroscopy; **AUC:** Area Under Curve; **UV:** Ultra Violet;

SUMMARY

The quality evaluation of herbal formulations is essential step in the manufacturing companies. Marker based standardization is essential tool used in quality assessment of herbal drugs. The main objective of proposed research is to develop and validate marker-based quantification analytical methods for quality control of Quercetin in marketed formulations. In the proposed analytical research work two spectrophotometric techniques, mainly zero order spectroscopy and area under curve tools were developed and validated for quantification of Quercetin. The developed methods involved the utilization of water: methanol as a solvent. In zero order spectroscopic method absorbance of Quercetin was measured at 372 nm. The detection of Quercetin was carried out at 372 nm. The area covered between 325 nm to 402 nm was used for calculation. The proposed research work concludes that the two newly developed and validated UV-Spectrophotometric methods were found to simple, precise and accurate for quantification of Quercetin in marketed capsule dosage form.

REFERENCES

1. Jalalpure SS, Kurangi BK, Suryawanshi SS. Quality control and standardization of phytomedicines. Nirali Prakashan. 2021;2021; 9789354512704.
2. Kagawad P, Gharge S, Jivaje K, Hiremath SI, Suryawanshi SS. Quality control and standardization of quercetin in herbal medicines by spectroscopic and chromatographic techniques. Future J Pharm Sci. 2021;7(1):1-12. doi: 10.1186/s43094-021-00327-y.
3. Bhardwaj S, Verma R, Gupta J. Challenges and future prospects of herbal medicine. Int Res Health Sci. 2018;1(1):12-5. doi: 10.36437/irmhs.2018.1.1.D.
4. Aneesh T, Hisham M, Sekhar S, Madhu M, Deepa T. International market scenario of traditional Indian herbal drugs – India declining. Int J Green Pharm. 2009;3(3):184-90. doi: 10.4103/0973-8258.56271.
5. Sharma S. Current status of herbal product: regulatory overview. J Pharm Bioallied Sci. 2015;7(4):293-6. doi: 10.4103/0975-7406.168030, PMID 26681886.
6. Li S, Han Q, Qiao C, Song J, Lung Cheng C, Xu H. Chemical markers for the quality control of herbal medicines: an overview. Chin Med. 2008;3(7):7. doi: 10.1186/1749-8546-3-7, PMID 18588699.
7. WHO guidelines for selecting marker substances of herbal origin for quality control of herbal medicines. In: WHO Expert Committee on Specification for Pharmaceutical Preparations [fifty-first report]. Geneva: World Health Organization; 2017: Annex 1. (WHO Technical Report Series, No. 1003).
8. Available from: https://www.intechopen.com/books/phytochemicals-in-human-health/analytical-methods-of-isolation-and-identification.
9. Siddiqui MR, *et al*. Analytical techniques in pharmaceutical analysis: a review. Arab J Chem. 2013:1-13.
10. Yeligar VC, Rajmane MA, Chougule KB, Chougule VK, Patil SS. Development and validation of UV spectrophotometric method for simultaneous estimation of melatonin and quercetin in liposome formulation. Int J Innov Sci Res Technol. 2017;2(6):189-94.
11. Upendra S, Ashish B. Simultaneous estimation of quercetin and silymarin: method development and validation. Int J Pharm Biol Arch. 2013;4(3):527-31.
12. Aneja G, Dave U, Vadodaria K. Simultaneous estimation of piperine, quercetin and curcumin in a mixture using UV-Visible spectrophotometer and method validation. IJTA. 2012;8:14-7.
13. Viswanath A, Lakshmanarao A, Kumari KBR, Babu PS, Karthikeyan R. Isolation, characterization and estimation of quercetin from *Ipomoea sepiaria* Koenig ex. roxb by UV spectrophotometry. Pharm Anal. Qual Assur. 2016;1:9-16.
14. Kurzawa M. Determination of quercetin and rutin in selected herbs and pharmaceutical preparations. Anal Lett. 2010;43(6):993-1002. doi: 10.1080/00032710903491070.
15. Shailajan S, Joshi H, Tiwari B. A comparative estimation of quercetin content from *Cuscuta reflexa* Roxb. using validated HPTLC and HPLC techniques. J Appl Pharm Sci. 2014;4(7):123.
16. Al-Rifai AA, Aqel A, Awaad A, Alothman ZA. Analysis of quercetin and kaempferol in an Alcoholic Extract of *Convolvulus pilosellifolius* using HPLC. Commun Soil Sci Plant Anal. 2015;46(11):1411-8. doi: 10.1080/00103624.2015.1043454.
17. Ang LF, Yam MF, Fung YT, Kiang PK, Darwin Y. HPLC method for simultaneous quantitative detection of quercetin and curcuminoids in traditional Chinese medicines. J Pharmacopuncture. 2014;17(4):36-49. doi: 10.3831/KPI.2014.17.035, PMID 25780718.
18. Supe U, Daniel P. Preliminary phytochemical analysis and quantitative analysis of quercetin by HPLC of *Momordica charantia*. World J Pharm Res. 2014;4(1):848-53.
19. Rao AS, Ahmed MF. Simultaneous determination of phenolic compounds in *Catharanthus roseus* leaves by HPLC method. Int J Pharm Sci Res. 2014;5(3):977.
20. Mundkinajeddu D, Sawant LP, Koshy R, Akunuri P, Singh VK, Mayachari A, *et al*. Development and validation of high performance liquid chromatography method for simultaneous estimation of flavonoid glycosides in *Withania somnifera* aerial parts. Int Sch Res Not. 2014;2014:1-6. doi: 10.1155/2014/351547.
21. Boligon AA, Jesus Rda S, Piana M, de Brum TF, da Cruz RC, Mossmann N, *et al*. Development and Validation of an HPLC-DAD Analysis for Flavonoids in the gel of *Scutia buxifolia*. J Chromatogr Sci. 2015;53(9):1557-61. doi: 10.1093/chromsci/bmv056, PMID 25977240.
22. Prasad HK, Hariprasad R, Rahman SH. Method development and validation for the simultaneous estimation of resveratrol and quercetin in bulk and pharmaceutical dosage form by RP-HPLC. J Pharm Sci Res. 2019;11(12):3777-81.
23. Chaudhari VS, Borkar RM, Murty US, Banerjee S. Analytical method development and validation of Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers. J Pharm Biomed Anal. 2020;186:113325. doi: 10.1016/j.jpba.2020.113325, PMID 32380356.

24. Kumar A, Lakshman K, Jayaveera KN, Tripathi SM, Satish KV. Estimation of gallic acid, rutin and quercetin in Terminalia chebula by HPTLC. Jordan J Pharm Sci. 2010;3(1):63-8.
25. Rakesh SU, Patil PR, Salunkhe VR, Dhabale PN, Burade KB. HPTLC method for quantitative determination of quercetin in hydroalcoholic extract of dried flower of *Nymphaea stellata* willed. Int J ChemTech Res. 2009;1(4):931-6.
26. Shakya A, Chaudhary SK, Bhat HR, Gogoi N, Ghosh SK. A rapid high-performance thin-layer chromatographic method to estimate quercetin in *Benincasa hispida* (Thunb.) Cogn. fruit pulp. JPC J Planar Chromatogr Mod TLC. 2019;32(6):467-74. doi: 10.1556/1006.2019.32.6.4.
27. Bindu AR, Remya K, Aleykutty NA, Sajan J. High performance thin layer chromatographic method for quantitative determination of quercetin in tender leaves of *Psidium guajava*. Pharmacogn J. 2010;2(17):21-3. doi: 10.1016/S0975-3575(10)80005-7.
28. Dhar BL, Kumar RK, Manosi D, Jayram H. HPTLC method for quantitative determination of quercetin in a polyherbal compound for urolithiasis. Int J Pharmacogn Phytochem Res. 2016;8(7):1187-90.
29. Sethuraman V, Janakiraman K, Krishnaswami V, Natesan S, Kandasamy R. Combinatorial analysis of quercetin and resveratrol by HPTLC in *Sesbania grandiflora*/phyto-based nanoformulations. Nat Prod Res. 2019:1-6.
30. Sakshi S, Vineet G, Rajiv G, Shubhini AS. Estimation of quercetin from crude leaf extract, *Mimusops elengi* by HPTLC. Pharm Lett. 2011;3(5):12-9.
31. Patil V, Angadi S, Devdhe S, Wakte P. Recent progress in simultaneous estimation of rutin, quercetin and liquiritin in *Cocculus hirsutus* by HPTLC. Res J Pharmacogn. 2015;2(4):49-55.
32. Laila O, Murtaza I, Abdin MZ, Ahmad S, Ganai NA, Jehangir M. Development and validation of HPTLC method for simultaneous estimation of diosgenin and quercetin in fenugreek seeds (*Trigonella foenum-graceum*). Int Sch Res Not. 2014:1-8.
33. Rao AS, Ahmed MF. Simultaneous estimation of quercetin and rutin in ethanolic extract of *Melia azedarach*. Linn leaves by HPTLC method. Asian J Biomed Pharm Sci. 2013;3(21):56-69.
34. Chakraborthy GS, Ghorpade PM. Determination of quercetin by HPTLC in *Calendula officinalis* extract. Int J Pharm Biol Sci. 2010;1(1):405-25.
35. Hussain MS, Fareed S, Ali M, Rahman MA. Validation of the method for the simultaneous estimation of bioactive marker gallic acid and quercetin in Abutilon indicum by HPTLC. Asian Pac J Trop Dis. 2012;2:S76-83. doi: 10.1016/S2222-1808(12)60127-3.
36. Hussain J, Bassal M, Sarhan H, Issam M, Aga H. Qualitative and quantitative comparison of rutin, quercetin and gallic acid concentrations in Syrian *Capparis spinosa*. L Leaves. J Pharmacogn Phytochem. 2017;6(4):407-15.
37. Mishra AP, Saklani S, Parcha V, Milella L. A developed and validated high-performance thin-layer chromatographic method for the quantitative determination of quercetin in *Satyrium nepalense* tubers. JPC J Planar Chromatogr Mod TLC. 2014;27(6):444-8. doi: 10.1556/JPC.27.2014.6.7.
38. Yadav SK, Jain G, Mazumder A, Khar RK. Development and validation of a HPTLC method for simultaneous estimation of quercetin, chlorogenic acid and trigonelline in polyherbal antibacterial formulation. J Pharm Innov. 2018;7(12):75-81.
39. Panchal H, Amin A, Shah M. Development of validated high-performance thin-layer chromatography method for simultaneous determination of quercetin and kaempferol in *Thespesia populnea*. Pharmacogn Res. 2017;9(3):277-81. doi: 10.4103/0974-8490.210326, PMID 28827970.
40. Dureshahwar K, Mubashir M, Une HD. Quantification of quercetin obtained from *Allium cepa* Lam. leaves and its effects on streptozotocin-induced diabetic neuropathy. Pharmacogn Res. 2017;9(3):287-93. doi: 10.4103/pr.pr_147_16, PMID 28827972.
41. Jirge S, Tatke P, Gabhe S. Simultaneous estimation of kaempferol, rutin, and quercetin in various plant products and different dosage forms of Bhuiamla and Amla. JPC J Planar Chromatogr Mod TLC. 2014;27(4):267-73. doi: 10.1556/JPC.27.2014.4.6.
42. Verma S, Gupta A, Ramana MV, Rawat AKS. High-performance thin-layer chromatographic analysis for the simultaneous quantification of gallic acid, vanillic acid, protocatechuic acid, and quercetin in the methanolic fraction of *Limonia acidissima* L. fruits. JPC J Planar Chromatogr Mod TLC. 2016;29(5):356-60. doi: 10.1556/1006.2016.29.5.5.
43. Bhandare SB, Laddha KS. Simultaneous quantification of kaempferol and quercetin in medicinal plants using HPTLC. Int J Pharm Sci Res. 2016;7(6):2379.
44. Verma S, Rawat AKS, Ramana MV, Dhaneshwar S. Gas chromatographic—mass Spectrometric Profile of Non-Polar Fraction and High-Performance Thin-Layer chromatographic analysis of methanolic Fraction with Simultaneous Quantifications of Protocatechuic Acid and quercetin in *Carissa carandas* L. Fruits. JPC J Planar Chromatogr Mod TLC. 2019;32(3):237-41. doi: 10.1556/1006.2019.32.3.9.
45. Siddique N, Mujeeb M, Aamir M, Husain A. Simultaneous quantification of umbelliferone and quercetin in polyherbal formulations of *Aegle marmelos* by HPTLC. Am J Pharm Tech Res. 2012;2:569.
46. Shukla PK, Srivastava A, Misra A, Srivastava S. Simultaneous Quantification of quercetin and syringic acid in methanolic Extract of *Leucas lavandulifolia* by using Validated HPTLC-Densitometric Method. Indian J Pharm Educ Res. 2020;54(3s):s687-94. doi: 10.5530/ijper.54.3s.169.
47. Thafshila AM, Anuradha R. Determination of quercetin by HPTLC method in *Achyranthes aspera* (L.).plant extract. 2017;10(2):52-6.
48. Jain A, Lodhi S, Singhai AK. Simultaneous estimation of quercetin and rutin in *Tephrosia purpurea* Pers. by high performance thin layer chromatography. Asian. J Tradit Med. 2009;4(3):104-9.
49. Girme AS, Saste GB, Shengule SA, Kunkulol RR, Hingorani LL. Method development, optimization and validation of RP-UFLC method for bioactive flavonoids from *Cassia auriculata*. J Pharmacogn Phytochem. 2019;8(1):77-81.
50. Elgendy K, Zaky M, Eldidamony A, Wahaab B, Amin M. Analysis of total flavonoids in herbal drugs expressed as quercetin by reversed phase-UHPLC method. Bulletin of Faculty of Science. Zagazig University. 2020;1:23-31.
51. Santos MC, Farias LS, Merlugo L, de Oliveira TV, Barbosa FS, Fuentefria AM, *et al*. UPLC-MS for identification of quercetin derivatives in *Cuphea glutinosa* Cham. and Schltd (Lythraceae) and evaluation of antifungal potential. Curr Pharm Anal. 2018;14(6):586-94. doi: 10.2174/1573412913666170918150924.
52. Suryawanshi SS, Kavalapure R, Palled MS, Alegaon SG. Development and validation of UV-spectrophotometric method for determination of ciprofloxacin and curcumin in bulk powder. Int J Pharm Sci Res. 2020;11:1161-6.
53. Pancham YP, Girish B, Suryawanshi SS. UV-spectrophotometric method for quantification of ascorbic acid in bulk powder. J Pharm Innov. 2020;9:5-8. [ICH guidelines], Validation of analytical procedures: text and methodology.
54. International Conference on Harmonization (ICH) Q2. (R^1), Geneva, Switzerland. 2005;11:10
55. Suryawanshi SS, Palled MS. Box-Behnken design assisted optimization and standardization of chromatographic methodology for quality assessment of metformin: analytical quality by design avenue. Indian J Pharm Educ Res. 2022;56(2s):s152-62. doi: 10.5530/ijper.56.2s.86.
56. Tavade S, Patil K, Kurangi B, Suryawanshi S. Development and validation of UV-spectrophotometric method for estimation of berberine hydrochloride in marketed formulation and poly lactic co-glycolic acid nanoparticles. Indian J Pharm Educ Res. 2022;56(3):873-80. doi: 10.5530/ijper.56.3.140.
57. Patil A, M. Maste M, S. Suryawanshi S, Patil N. Green solvent assisted UV-spectrophotometric method for estimation of rosuvastatin in bulk and pharmaceutical dosage forms. Res J Pharm Technol. 2022;15(2):587-90. doi: 10.52711/0974-360X.2022.00096.
58. Yadav RK, Maste MM, Suryawanshi SS, Shastri U. Conductometric method development and validation to estimate acamprosate calcium in API and marketed formulation. J Appl Pharm Sci. 2021;11(11):82-6. doi: 10.7324/JAPS.2021.1101111.
59. Uday TC, Shivabasappa PM, Sanjay SS, Maruti MS. Development and validation of stability indicating UV-spectrophotometric method for the simultaneous estimation of telmisartan and metformin hydrochloride in bulk drugs. Indian J Pharm Educ Res. 2021;55(2):590-7. doi: 10.5530/ijper.55.2.98.
60. Shivabasappa PM, Diksha H, Chouhan MK, Satyappa S, Jalalpure SS. Method development and validation of ciprofloxacin HCl and ornidazole by UFLC in combined dosage form. Indian J Pharm Educ Res. 2019;53(3):S373-9.

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