Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2019; 11(2): 49-56

ISSN- 0975 1556

Review Article

Analytical Method Development and Validation of Quercetin: A Review

Kaur Pawanpreet[1*], Singh Baljeet[2]

Department of Pharmaceutical Chemistry, A.S.B.A.S.J.S.M. College of Pharmacy, Bela (Ropar)- 140111 (Punjab)

Available Online:25th May, 2019

ABSTRACT

Analytical method development and validation is the inherent part of drug discovery and development of drug substance in pharmaceuticals. Analytical method development and validation are interconnected task related to research and development of new drug products and their combination and quality control of pharmaceuticals. Analytical method is the procedure which determines drug content, presence of impurities and degraded products and validation proves that the method is correct and used in quality control process. As new drugs are emerging day by day, need of analytical method development is increasing as for these drugs standard methods are not available in pharmacopoeias. Designing of new analytical method helps in improvement of accuracy, precision, cost and time consumption for analysis. This review article focus on literature findings of method development and validation of quercetin from 2012-2018 which helps in developing new method for the determination of quercetin in bulk and pharmaceutical dosage forms.

Keywords: Quercetin, method development, spectroscopic, HPLC, validation.

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, pancreatic cancer. The IUPAC name of quercetin is 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one. 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[1].

METHOD DEVELOPMENT OF QUERCETIN

Shaikh *et al*.; (2018); developed a novel, precise, and accurate RP-HPLC method for simultaneous qualitative and quantitative estimation of quercetin, ellagic acid and rutin in an ayurvedic formulation Triphala Churna. Isocratic conditions were used for separation using shim-pack HPLC C_{18} column (4.6×250 mm, 5µm) with temperature maintained at 35 °C. The mobile phase consist of 0.02 M potassium dihydrogen orthophosphate and methanol in the ratio 55:45 at flow rate of 1 mL/min. The run time was 10 min. The components were detected at 254 nm wavelength using UV-visible detector. The method results in elution of quercetin at 7.52 min, ellagic acid at 9.10 min and rutin at 12.47 min. The method was found to be linear over the concentration range of 8-12 ppm, 9-17 ppm and 7-11 ppm for quercetin, ellagic acid and rutin respectively. The r^2 value of quercetin, ellagic acid and rutin was found to be 0.997, 0.999 and 0.999 respectively. The method was found to be precise with %RSD values less than 2. The method was accurate with % recovery values of 98-102% which are in acceptable range. At deliberately changed experimental conditions method was found to be robust and employed for analysis of herbal drugs[2].

Rajpuria (2018); developed a simple RP-HPLC method for the simultaneous identification and quantification of phenolic antioxidants in seaweed. For development of method the column employed was Atlantis C_{18} (250×4.6 mm, 5 µm particle size) fitted with suitable C_{18} (4.0×3.0 mm) Phenomenex guard column. The gradient conditions were used with varying ratio of mobile phase which consist of 0.25% aqueous acetic acid and acetonitrile/water (80/20 v/v) with flow rate and column temperature of 1.0 mL/min and 25°C respectively. Absorbance measures were recorded at 254 nm, 280 nm and 320 nmusing a diode array detector. Quantitative analysis of these compounds revealed the presence of phloroglucinol (394.1±4.33µg/g), gallic acid (96.3±3.12µg/g), chlorogenic acid (38.8±1.94µg/g), caffeic acid (44.4±2.72µg/g), ferulic acid (17.6±0.85µg/g), myricetin (8.6±0.85µg/g) and quercetin (4.2±0.15µg/g), in the extract of H. elongate. The method was then validated for all parameters as per ISH guidelines and the results obtained were found to be satisfactory and the method could be successfully employed for analysis of poly phenols in see weeds[3].

Sandhu et al.; (2017); developed a bioanalytical RP-HPLC method for the analysis of quercetin dehydrate using Quality by Design (QbD) approach. Chromatographic separation was accomplished on a C_{18} column using acetonitrile and ammonium acetate buffer in ratio 35:65 as

**Author for Correspondence: pawandhaliwal600@gmail.com*

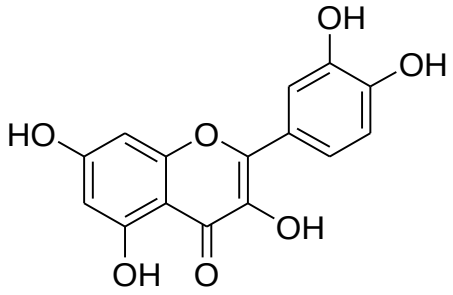

Figure 1: Structure of Quercetin

mobile phase at 0.7 mL/min flow rate. Isocratic mode of elution was used and detection was carried out using PDA detector at 237 nm. Then the method was validated for various parameters and results were found to be satisfactory for linearity, precision, accuracy, robustness which indicates that method could be used for ebioanalytical estimation of quercetin[4].

Das *et al*.; (2017); developed a simple and precise method for the estimation of quercetin and catechin using RP-HPLC. The development of method was carried out using Lichrosper 100 C_{18} (250×4.6 mm, 5µm particle size) column. Gradient elution technique was employed with mobile phase consisting of acetonitrile and buffer at a flow rate of 1.6 µL. The detection was carried out using UV-visible detector at a wavelength of 280nm and 360nm for catechin and quercetin. The retention time of Catechin and Quercetin was 12.24 min and 15.96 min respectively. The values of correlation coefficient for catechin and quercetin were 0.978 and 0.999 respectively. The method was precise and accurate with %RSD values of 0.13 and 0.26 and % recovery of 99% and 98% for catechin and quercetin respectively.LOD values were 0.57 µg/mL and 0.52 µg/mL and LOQ values were 1.74 µg/mL and 1.51 µg/mL for catechin and quercetin respectively. Thus the developed method was accurate, simple, precise and economical and could be employed for routine analysis of marketed formulations[5].

Baghel *et al*.; (2017); developed fast, sensitive, precise and selective high performance liquid chromatography and high performance thin layer chromatography methods for the simultaneous estimation of curcumin and quercetin in a polyherbal formulation. The column chromatography was performed on a HPLC system equipped with binary prominence LC-20 AD liquid pump, SPD-20 A prominence UV/Vis detector and a 77251 Rheodyne injector. Chromatographic method conditions were optimized by using Inertsil C18 column having dimensions 250×4.6 mm, 5 µ particle size. For HPLC analysis methanol and water in ratio of 75:25 v/v isocratic elution mode with the flow rate of 1 mL/min was used. Detection was carried out at 397 nm using UV-Vis detector. The data processing was done using LC solution 1.22 version single channel software. Linearity was established within concentration range of 2-12 µg/mL with r^2 values of 0.996 and 0.997 for curcumin and quercetin respectively. The data obtained from precision and % recovery indicate that method was accurate, precise, and reproducible. The robustness of method was also determined by making small deliberate changes[6].

Sharifuldin *et al*.; (2016); developed a RP-HPLC method for the quantification of rutin, quercitrin and quercetin in *Cosmos caudatus* Kunth. Method development was performed on Dionex-Ultimate® 3000 Rapid Separation LC system equipped with auto sampler, quaternary pump, degasser, column oven, and a DAD-3000RS diode array detector (DAD). Separation of components was achieved using Acclaim Polar Advantage II column (150×4.6 mm, 5.0 µm; Dionex) maintained at 40° C temperature. Gradient elution method was used with mobile phase consisting of 0.3% formic acid in water and acetonitrile maintained at a flow rate of 1.0 mL/min. Injection volume was 10 µL and the spectral data from the DAD was collected at 254 nm and data acquisition was performed by Chromeleon software version 6.8. Calibration curves were found to be linear over the concentration range of 0.5-500 µg/mL. The %RSD values of precision for rutin, quercitrin and quercetin were 0.17, 0.28, and 0.31 which indicates the reproducibility of method. the method was proved to be accurate with satisfactory % recovery values. LOD values of rutin, quercitrin and quercetin were 0.0496, 0.0777, and 0.1581 respectively. LOQ of rutin, quercitrin and quercetin were found to be 0.1503, 0.2355, and 0.4792 respectively. As the proposed method was selective, accurate, reproducible, and robust so it could be considered as an analytical tool for quality control assurance of *C. caudatus* herbal products[7].

Seo *et al*.; (2016); developed a RP-HPLC method and validated it for the separation and quantification of flavonoids and isocoumarin present in *Coryloposis coreana*. The chromatographic conditions used for separation include Alliance 2695 HPLC system equipped with a PDA detector and Agilent Zorbax extended C_{18} (5 µm, 150 mm×5 mm) column maintained at 25° C. The mobile phase used for gradient elution consists of mixture of solvent A (acetonitrile) and B (water containing 0.2% phosphoric acid) maintained at a flow rate of 0.8 mL/min. 10 µL of sample was injected and components were detected at 270 nm for bergenin, at 350 nm for quercetin, quercitrin and isosalipurposide using PDA detector for total run time of 40 min. The results of system suitability show % RSD value between 0.25–0.43 for all components which indicates that method is reproducible and suitable. Linearity of method was assured from the calibration curves which are linear over the desired concentration range and r^2 value was found to be 0.999 for all the components. The values of precision for all components were less than 2 and method was accurate with % recovery in range of 98-102 %. The low values of LOD and LOQ indicate that proposed method was accurate, precise, robust, and selective[8].

Kumar *et al*.; (2015); developed a stability indicating simplified HPLC method for simultaneous analysis of resveratrol and quercetin in nanoparticles and human plasma due to their synergistic potential and combination delivery applications. For development of method Shimadzu HPLC system (LC-2010C HT, Japan) consisting LC-20AT pump and a SIL-20ACHT auto

sampler, equipped with a SPD-20A UV/VIS detector and SPD-M20A photodiode array detector was used. The analysis was carried out using two different phenomenex C_{18} analytical columns Synergi 4l Hydro-RP 80A and Luna 5l 100A having size of 250×4.60 mm. The mobile phase consisted of methanol: ACN: 0.1% phosphoric acid in water (60:10:30 v/v) pumped at a flow rate of 0.8 mL/min. The injection volume for all the samples was 20 µL. For detection of compounds isobestic point of both drugs i.e. 279 nm was selected. Under these chromatographic conditions, the obtained chromatograms for both compounds were found to be quite sharp and well separated with retention times of 4.34 and 5.91 min for resveratrol and quercetin, respectively. Then the developed method was validated according to ICH and USP guidelines. Parameters such as theoretical plates, retention factor, tailing factor, resolution, and peak symmetry factor were determined to verify the system suitability. Excellent linear relationship between peak area and concentration was obtained for both analytes with a correlation coefficient of 0.999. LOD value was found to be 0.95 and 1.24 ng/mL for resveratrol and quercetin, respectively. LOQ was found to be 2.87 ng/mL for resveratrol and 3.75 ng/mL for quercetin. These results prove that method was highly sensitive. The % recovery values and %RSD values of precision were found to be in limits indicating the efficiency of method. Forced degradation studies were also performed which revealed that the drugs were able to withstand acidic, alkaline as well as high temperature conditions, but prone to degrade in oxidative and photolytic conditions[9].

Subramanian et al.; (2015); developed a rapid, simple, accurate, specific and efficient liquid chromatography-mass spectrometry method for the simultaneous estimation of quercetin and rutin from *Aganosma dichotoma* and validated as per ICH guidelines. Experiment was performed on an Agilent 1290 infinity UHPLC-ESI-QTOF system. For separation, the samples were analyzed on Phenomenex C_{18} column (150×4.6 mm, 5µ) set at 30° C temperature. For elution of components isocratic mode of separation was used consisting of mobile phase 10 mM ammonium acetate and methanol (20:80 v/v) at a flow rate of 0.4 mL/min. The retention time of quercetin and rutin was found to be 4.09 min and 3.31 min respectively. Quercetin and rutin exhibited linearity in the concentration range of 1-5 ng/mL and 10-50 ng/mL respectively and the value of correlation coefficient was found to be 0.997 for quercetin and 0.995 for rutin. The recovery range for quercetin and rutin was found to be 99.7% - 100.2% and 100.4% - 101% respectively which stipulate the accuracy of method. The RSD values obtained for quercetin and rutin were 0.6 % and 0.7 % respectively for precision which show that the method was reproducible and precise. For quercetin and rutin, LOD values were found to be 0.3 ng/mL, 3 ng/mL and LOQ values were 1 ng/mL and 10 ng/mL respectively which show the system suitability of method and it could be used for further analysis[10].

Boligon *et al*.; (2015); developed and validated HPLC-DAD analysis for flavonoids in the gel of *Scutia buxifolia*. The chromatographic analysis was carried out under gradient conditions using a C_{18} column (4.6×250 mm) packed with 5 µm diameter particles. The mobile phase consist of water and acetonitrile (70:30 v/v) containing 0.5% (v/v) phosphoric acid (pH 2.8). The injection volume was 40 mL and the flow rate was maintained at 0.8 mL/min. The quercetin and rutin were quantified at 356 nm. Linearity was indicated by r^2 value of 0.999 and 0.998 for quercetin and rutin respectively. The % recovery was found satisfactory i.e. 99.75-100.86 % for quercetin and 99.81- 101.81 % for rutin. The value of %RSD for precision was found to be 0.85 and 0.93 for quercetin and rutin respectively. There was no significant change observed with the change in the mobile phase pH and flow rate, showing that the method is robust under the experimental conditions. The proposed method was found to be linear, sensitive, precise, specific, accurate, and robust. It could be used for the quantification as well as quality control of polyherbal formulations, which contain *S. buxifolia* fractions as their ingredients[11].

Salunkhe *et al*.; (2014); developed a rapid, sensitive, simple, precise, economic and reproducible RP-HPLC method for the quantitative determination of curcumin and quercetin in *Madhujeevan churna* and validated it for its intended use. Analysis was carried out using an Agilent LC-P-1120 provided with pump, degasser, photo diode array detector and autosampler. The analytical signals initiated by detector were monitored and integrated using E Z chrome Elite. The chromatographic separation was achieved using C_{18} Intersil 4.6×250 mm column at oven temperature of 35° C. Isocratic elution was carried out using methanol: acetonitrile: phosphate buffer (pH 5) in the ratio of 42.5 : 42.5: 15 % v/v/v as the mobile phase at a flow rate of 1.2 mL/min. Detection was carried out at 265 nm. Retention time was found to be 3.220 min and 4.287 min for quercetin and curcumin respectively. Then method was validated for different parameters to confirm its effectiveness. Linearity was confirmed by correlation coefficient value of 0.999 in concentration range of 10-150 µg/mL. Results from recovery studies were within acceptable limits i.e. 98.26-99.35% and 98.32-99.27% for quercetin and curcumin respectively indicating good accuracy of method. The %RSD values for intra-day and inter-day precision were 1.78, 1.43 for quercetin and 1.13, 1.22 for curcumin. Adequate sensitivity of method was determined by good LOD and LOQ values about 0.26 µg/mL, 0.804 µg/mL for quercetin and 0.25 µg/mL, 0.78 µg/mL for curcumin. Low values of % RSD obtained after introducing small changes in the developed HPLC method show the robustness of method. Thus developed method was acceptable and can be used for routine analysis of curcumin and quercetin in pharmaceutical dosage forms[12].

Rajendran *et al*.; (2014); developed HPTLC method for the simultaneous estimation of biomarkers in *Barleria cristata* and bio analysis of the selected marker in plasma. Camag HPTLC system with Linomat 5 applicator, Camag TLC scanner 3 and WinCATS software was used for experiment. The stationary phase was pre-coated plates containing silica gel 60F254 on aluminium sheets and mobile phase was methanol: ethyl acetate: toluene: formic acid (1: 1: 7.5: 0.2 %v/v/v/v). Detection was carried out at

308 nm. Calibration curves were plotted for determination of linearity for the mixture of querctin, apigenin and naringenin. The concentration range of 100 -800 ng/spot for quercetin, 10 - 80ng/spot for apigenin and 50- 400 ng/spot for naringenin was found to be linear with r^2 value of 0.99044, 0.99480 and 0.99965 respectively. The values of precision, LOD, and LOQ were found in acceptable range and therefore method could be used for further analysis[13].

Ang *et al*.; (2014); developed a rapid, specific, reversed phase HPLC-UV method for the simultaneous quantitative detection of Quercetin and Curcuminoids in traditional Chinese medicines. HPLC analysis was performed using a Shimadzu-LC system equipped with a CBM-20A controller, LC-20AT pump, DGU-20A5 prominence degasser, SIL-20A auto sampler, SPD-20AV detector and CTO-10ASvp column oven. Chromatographic separations were achieved using a Thermo Hypersil Gold column (250 mm×4.6 mm; 5 µm) attached with a guard column Zorbax Eclipse Plus packed with a replaceable C-18 cartridge (12.5 mm×4.6mm; internal diameter 5 mm). An isocratic elution mode was used with a flow rate of 1.3 mL/min, a column temperature of 35°C, and a mobile phase of acetonitrile and 2% v/v acetic acid (pH 2.60) in ratio of 40:60 v/v. The injection volume was 20 µL and the total run time was 18.5 minutes. Detection of components was carried out 370 nm. Data acquisition and processing was permormed using LC-Solution Software. Calibration curves were constructed by plotting the peak area against the concentration in the range from 1.25-200 µg/mL. The linearity was confirmed from correlation coefficient value obtained from the calibration curves i.e. 0.999. The values of % recovery values were in range of 98.292% to 103.617% which indicate the accuracy of the method. The values of precision, LOD, LOQ were less which determines the specificity and selectivity of developed method[14].

Subramanian *et al*.; (2014); developed a simple, specific, accurate and precise high performance liquid chromatography method for the simultaneous estimation of quercetin and rutin in *Aganosma dichotoma*. Analysis was performed on Shimadzu LC system equipped with LC-2010AT VP solvent delivery system, SPD M-10A photodiode array detector and Rheodyne 7725i injector with 20 µL loop volume. The chromatographic conditions for separation include C_{18} Hibar Lichrospher column having dimensions 150×4.6mm and internal diameter of 5µ and mobile phase containing of acetonitrile and 25 mM ammonium acetate pH 3 in ratio of 40:60 v/v maintained at a flow rate of 1 mL/min. Absorbance was measured at 259 nm. The retention time of quercetin and rutin was found to be 4.30 min and 1.71 min respectively. Validation was performed according to ICH guidelines. Response was linear in the concentration range of 1-5 µg/mL, 0.1-0.5 µg/mL for quercetin and rutin respectively and r^2 was found to be 0.997 and 0.995 for quercetin and rutin respectively. Good accuracy of method was indicated by the recovery range for quercetin and rutin that was found to be 99.3 to 101.1 % and 101.3 to 102 % respectively. The RSD values obtained for precision were 0.97 and 1.268 for quercetin and rutin respectively indicating specificity of method. LOD and LOQ value of quercetin was 100 ng/mL and 300 ng/mL which indicated the sensitivity of method. The results of robustness determine that by varying the chromatographic conditions, there was no effect on the chromatographic parameters which satisfies that method was accurate, precise and robust and could be used for analysis of quercetin and rutin in pharmaceutical formulations[15].

Chaudhari et al.; (2014); developed a simple, rapid, accurate, precise, and economic spectrophotometric method for simultaneous estimation of Rutin and Quercetin in niosome formulation. For development of method a double beam UV-spectrophotometer (Shimadzu, UV-1700), attached to a computer software UV Probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm quartz cells. The sample was prepared in methanol and detected over entire UV range i.e. 200-400 nm. As absorbance maximum by rutin and quercetin was shown at 257 and 372 nm respectively, absorbance was measured at these wavelengths for estimation. Then the method was validated according to ICH guidelines. Beer-Lambert's law was obeyed over the concentration range of 2-20 µg/mL for rutin and 2-20 µg/mL for quercetin. The coefficient of correlation for rutin at 257 nm and for quercetin at 372 nm is 0.997 and 0.992 respectively which indicated good linearity of developed method. In both intra and inter day precision study % RSD are not more than 2.0% indicates good repeatability. For rutin and quercetin LOD values were 0.0274 µg/mL and 0.1435 µg/mL and LOQ were 0.0832 µg/mL and 0.4349 µg/mL respectively indicating sensitivity of method. The satisfactory values of recovery studies indicate good accuracy of method and the method was found to be robust if experimental conditions were changed deliberately and experiment performed by different analysts. Thus this method can be successfully employed for simultaneous estimation of rutin and quercetin in niosome formulation[16].

Tuszynska *et al*.; (2014); developed a simple, selective and accurate method for the simultaneous estimation of flavonoids which are obtained from broccoli i.e. quercetin and kaempferol by using the RP-HPLC. The separation was performed by using Zorbax Eclipse XDB-C_{18} column (4.6×150 mm, 5 µm particle size) as stationary phase and the used mobile phase constituent were methanol/water (60/40) and phosphoric acid 0.2%. The flow rate was maintained at about 1 mL/min. The effluent was monitored at 258nm by using DAD. This method showed good linearity with r^2 value of 0.997 for quercetin and 0.999 for kaempferol. The recovery was within the limits 98.07-102.15% for quercetin and for kaempferol was 97.92-101.83%. The RSD percentage for the average recovery for quercetin was 0.94% and for kaempferol was 1.07%. The method is selective as the values of LOD and LOQ were less and quercetin and kaempferol were well separated from other compounds of broccoli with good resolution[17].

Li *et al*,; (2013); developed a simple and rapid method for the simultaneous estimation of salicin, luteolin-7-O-glucoside, myricetin, apigenin-3′-oxyethyl-7-O-glucoside,

rutin, quercetin, luteolin, kaempferol and apigenin obtained from *Salix matsudana* by using RP-HPLC. The separation was performed by using a Nucleosil C_{18} column (250 × 4.6 mm, 5 µm) as stationary phase and methanol in 0.2% phosphoric acid solution as the mobile phase in the linear gradient technique. The flow rate was maintained at about 1 mL/min. The effluent was monitored at 246 nm by using the SPD-20A UV detector. The % RSD was calculated for all the nine compounds and it was less than 1.7% and the recoveries for all the nine compounds were between 95.79% and 99.94% which indicate the good repeatability and accuracy[18].

Savai *et al*.; (2013); developed a simple, accurate and precise HPTLC for simultaneous determination of Quercetin and Rutin in the methanolic extract of bark of Saraca asoca and standard herbal formulation Ashokarishta Silica gel 60 F254(20x20 cm, 0.25 mm) TLC plate with mobile phase of Toluene: Ethyl acetate: Methanol: Formic acid (8:8:3:1) was used for chromatographic separation. Densitometric determination of bio-flavonoids was performed at wavelength 240 nm in reflectance/absorbance mode. The linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range of 100-1000 ng/band with r2 value of 0.993 and 0.99 for quercetin and rutin respectively. The average recovery for Quercetin was 98.593% and for Rutin was 97.66%. The proposed HPTLC method provides good resolution of Quercetin and Rutin from other constituents present in methanolic extract of Bark of Saraca asoca and can be used for quantification of Quercetin and Rutin present in the extract and standard herbal formulation. Method was found to be specific, precise, accurate, sensitive, selective, robust and rugged for determination of bio-flavonoids[19].

Baldi *et al*.; (2013); have developed simple, rapid, accurate, precise, and economic method by UV spectrophotometer for simultaneous estimation of Quercetin and Silymarin. Instrument used was Shimadzu UV-1700 Spectrophotometer with 10mm matched quartz cells having fast scan speed and slit width of 2.0 nm. The estimation was based on solving simultaneous equation in topical formulation. Absorption maxima of Quercetin and Silymarin were detected at 256 nm and 288 nm respectively. Absorbance was measured at these values for Qurecetin and Silymarin and calibration curves were plotted which show linearity at concentration range of 2-10 µg/mL and 8-16 µg/mL respectively. The correlation coefficient value was found to be 0.9998 for Quercetin and 0.9993 for Silymarin. Percentage estimation of quercetin and silymarin in topical formulation was found to be 99.08±0.13 and 99.52±0.47 with standard deviation <2. As this method was valid according to ICH guidelines and can be successfully used for simultaneous estimation of Quercetin and Silymarin in topical formulation[20].

Moorthi *et al*.; (2013); developed a RP-HPLC method for the simultaneous estimation of curcumin and quercetin encapsulated in polymeric nanoparticles. The instrument used for method development was Alliance HPLC (Water Corp.). The chromatographic conditions include Luna C_{18} column having temperature 35°C with mobile phase composed of a degassed mixture of 0.1% ortho phosphoric acid and acetonitrile (50:50 v/v). The separation was carried using isocratic mode with a flow rate of 1.2 mL/min and run time 15 min. Components were detected using UV detector at 292 nm wavelength. The system suitability study has shown the theoretical plate count in the range of 7922-8197 for curcumin and 4389-4638 for quercetin. The encapsulation efficiency in Eudragit E 100 nanoparticles of curcumin was around 79% in sonication approach and around 81% in mechanical stirring approach. Similarly, the encapsulation efficiency of quercetin was around 91% in sonication approach and around 98% in mechanical stirring approach. The %RSD values for robustness and ruggedness were acceptable. Thus this method could be used for routine analysis of curcumin and quercetin[21].

Shanmugam *et al*.; (2013); have developed sensitive RP-UFLC method for quantification of Quercetin in nano formulation in rabbit plasma. Good chromatographic separation was obtained using acetonitrile and potassium dihydrogen ortho phosphate (pH 3.5) in ratio of 65:35 (v/v) as mobile phase and column used was reverse phase Hiber C_{18} (250×4.6 mm, 5µ) with a flow rate of 0.8 mL/min and components were detected at 369 nm. Linearity was performed in concentration range of 10 to 400 µg/mL which results in r^2 value of 0.989. The lower limit, middle limit and higher limit of quantification was found to be 10 µg/mL, 200 µg/mL and 380 µg/mL respectively. The % recovery of drug was found to be in range of 95.91 to 98.59% which show accuracy of method. The low %RSD values indicate that method was accurate, precise and robust and successfully applied for estimation of quercetin in nano-formulation, routine quality control analysis, pharmacokinetic and bioequivalence studies[22].

Savic *et al*.; (2013); have developed a simple and precise RP-HPLC method for the simultaneous determination of quercetin in green tea. Zorbax Eclipse XDB-C8 (250×4.6 mm, 5 µ) column and methanol as mobile phase, which was filtered through a 0.45 µm Millipore filter was used for chromatographic separation. The injected volume of samples was 20 µL and detection wavelength was 370 nm. The retention time for quercetin in standard solution was found to be 2.42 min. The method was found to be linear with r^2 value of 0.998 in concentration range of 10-70 µg/mL. The %RSD values for precision, robustness, ruggedness, LOD and LOQ were in acceptable range i.e. 1.2 and 4 µg/mL. The % recovery for the different concentration of quercetin 40, 50 and 60 µg/mL was 101.3, 98.4 and 98.2% respectively which indicated good accuracy of method. The developed and validated method was successfully applied for the routine analysis of quercetin in green tea extract[23].

Yang *et al.;* (2013); developed a simple, rapid and precise method for the simultaneous estimation of rutin, quercetin, luteolin, genistein, galangin and curcumin in propolis by using RP-HPLC method. For development of method, Finnigan series LC-DAD system consisting of a surveyor pump, a diode array detector system, a column oven, a six-way rotary valve for sample introduction with a 20 µL sample loop and controlled by a Xcalibur software, were used. The separation was performed by using an Eclipse

XDB-C_{18} column (150mm×4.6 mm, 5µm) stationary column and the isocratic mobile phase used was phosphate buffered saline (pH = 4.5) and methanol (40/60, v/v). The flow rate of the mobile phase was set at 0.8 mL/min. The effluent was monitored using a DAD at 260 nm. Excellent linear correlation coefficient was observed in the range of 0.9977 to 0.9997. The reproducibility of the method was 1.58 % (RSD) and accuracy was confirmed with average recovery ranging from 95.71 to 104.26 %. The proposed method showed good sensitivity, linearity, repeatability and accuracy[24].

Shah et al.; (2012); developed a rapid, accurate and sensitive method for the simultaneous estimation of the methanolic extract of Kaempferol and Quercetin of the plant *Tridax procumbens.* The chromatographic separation was performed by using the Kromasil (4.6×250 mm) type of analytical column and the mobile phase, a mixture of Acetonitrile and 0.01N Potassium phosphate buffer (pH=2.5) adjusted with orthophosphoric acid was used in gradient system. The flow rate of the mobile phase was set at 1 mL/min and injection volume was 20 µL. The effluent was monitored using a UV Spectrophotometer at 240 nm. Quercetin and Kaempferol was observed with retention time of about 8.9 min and 10.6 min respectively. Correlation coefficient of linear regression analysis was observed to be 0.999 shows that method was linear. Detection limits and quantitation limits for Quercetin were 0.164 µg/mL and 0.289 µg/mL and for Kaempferol were 0.497 µg/mL and 0.816 µg/mL respectively. The values of %RSD for precision were in acceptable limit indicating selectivity and reproducibility of method. As the method complies to the validation parameters it can be used as quality control of Quercetin and Kaempferol in the extract of *Tridax procumbens* plant[25].

Landim *et al*.; (2012); developed and validated a method for separation and quantification of three flavonoids by reverse-phase high performance liquid chromatography in a crude extract of *Dimorphandra gardneriana*. For calibration standards rutin, isoquercitrin and quercetin were used. The experiment was carried out using an HPLC system (Shimadzu, USA) consisting of a solvent delivery pump (Model LC-10 ADvp), a variable wavelength UV/VIS detector (Model SPD 10 AVP), a manual injection valve (Rheodyne®, USA) with a 20 µL loop, and degasser (DGU 14A). Data collection and integration was performed using CLASS-VPTM System Software. The analysis was performed using a Thermo Scientific Hypersil C_{18} column (250×4.0 mm i.d., 5 µm particle size), as stationary phase and mobile phase was composed of two different solutions, solution A and solution B and both solutions was composed of tetrahydrofuran (THF) and sodium dihydrogen phosphate buffer (15.6 g/L), 5:95 for solution A and 40:60 for solution B adjusted to pH 3.0 with phosphoric acid with a flow rate of 0.8 mL/min. The detection was carried out at a wavelength of 356 nm. For the purpose of validation various parameters like specificity, linearity, sensitivity, accuracy, precision and robustness were used. Linearity was determined by regression coefficient the value of which was found to be greater than 0.998 indicating that method was linear. LOD values were 0.09 µg/mL, 0.02 µg/mL and 0.02 µg/mL for the compounds rutin, isoquercetin and quercetin, respectively while the LOQ values were 0.29 µg/mL, 0.07 µg/mL and 0.05 µg/mL respectively showing the good sensitivity of method. Small changes in the experimental conditions have no marked effect on the resolution of peaks and thus the method developed was robust[26].

Patil *et al*.; (2012); developed a simple and reproducible method for simultaneous estimation of quercetin and curcumin in Madhujeevan churna, an ayurvedic proprietary medicine by UV spectrophotometry which was then validated as per ICH guidelines by using limit of quantization and limit of detection and other parameters like precision and accuracy. Instrument used for analysis was an UV/Visible double beam spectrophotometer Shimadzu Model no. 1800 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm quartz cell. Madhujeevan churna was extracted with a mixture of 95% ethanol and water in ratio of 75:25 and then the stock solution was prepared in ethanol and the further dilutions were also prepared in ethanol. Absorbance of solution was measured at 256 nm for quercetin and 263 nm absorption maxima for curcumin. Calibration curve was plotted and the linearity in range of 2-20 µg/mL for quercetin and 4-36 µg/mL for curcumin. Then the value of correlation coefficient was found to be 0.999 for quercetin and 0.997 for curcumin which showed good linearity. The % recovery for quercetin in range of 98.50-99.50 % and for curcumin 99.01-99.37 % indicated that the accuracy of method was good. The %RSD value for inter-day precision was 0.51 and 0.76, for intra-day precision 0.43 and 0.56 for quercetin and curcumin respectively. Low value indicated that method was precise. For quercetin LOD and LOq values were 0.9 and 0.27 respectively, for curcumin 0.105 and 0.318 respectively. The data obtained from validation revealed that this UV spectrophotometric method for determination of quercetin and curcumin was accurate, simple, precise, and sensitive for routine analysis of Madhujeevan churna[27].

Aneja*et al.;* (2012); developed and validated a method for simultaneous estimation of piperine, quercetin and curcumin in a mixture using UV Visible spectrophotometer. A dual beam PERKIN ELMER, U.V-Visible Spectrophotometer was used with a pair of 1cm quartz cells. Analytical grade methanol was used as solvent for preparation of stock solution. Simultaneous equation method was used for analysis. Three wavelengths 371.31 nm, 424.68nm and 343.76 nm were selected for development of simultaneous equations for quercetin, curcumin and piperine respectively. The method was validated as per ICH guidelines by considering various parameters like accuracy and precision. Quercetin, curcumin, and piperine obeyed Beer's Law in concentration range of 5-30 µg/mL, 1-5 µg/mL and 1-10 µg/mL with correlation coefficient value of 0.984, 0.994 and 0.992 respectively. LOD values were found to be 4.921 µg/mL, 0.492 µg/mL and 0.710 µg/mL for quercetin, curcumin and piperine respectively. LOQ values for quercetin, curcumin and piperine were found to be 13.005 µg/mL, 1.493 µg/mL and 2.913 µg/mL

respectively. The % recovery values were found in acceptable range. Intra and inter-day precision values were less showing the reproducibility of method. The developed method was found to be economical and simple for routine analysis of quercetin, curcumin, and piperine in a mixture[28].

Pawar *et al*.; (2012); developed and validated method for simultaneous estimation of rutin and quercetin in hydroalcoholic extract of Triphala churna by HPTLC method. The Triphala churna was extracted with ethanol and water (70:30) with Soxhlet apparatus. The method was developed on CAMAG HPTLC system consisting of Linomat V applicator, CAMAG twin trough chamber, CAMAG TLC scanner, equipped with Win cats software (version 1.4.6), CAMAG syringe of 100 µL capacity. Separation and identification of quercetin, rutin were performed separately on aluminum backed silica gel 60 F254 (20cm x10cm of plate size, layer thickness 0.2 mm. The standard solutions 200-600 ng was applied on TLC plate and further it was developed and scanned. Stock solution was prepared in methanol. Mobile phase used was composed of ethyl acetate: formic acid: acetic acid: water (10: 1.1: 1.1: 0.6). Good resolution was obtained with Rf value for rutin 0.03 and for quercetin 0.76. For detection 254 nm wavelength was selected. ICH guidelines were followed for the validation of the analytical method developed. For linearity studies, calibration curves were plotted in concentration range of 200-600 ng/mL and correlation coefficient r^2 was found to be 0.999 for both the drugs. The average % recovery for quercetin and rutin was found to be 99.71% and 99.38% respectively. Intra-day and inter-day precision were found to be 1.05, 1.13 and 1.03, 1.11 for rutin and quercetin respectively. The sensitivity of method was indicated by low values of LOD and LOQ. The method was found to be specific and effective for further analysis[29].

Movaliya *et al*.; (2012); developed HPTLC method for estimation of quercetin in the alcoholic extract of *Aerva javanica* root. Stationary phase consisted of TLC Aluminum sheets pre-coated with silica gel 60 F254, thickness 0.2mm, (20×20 cm) and mobile phase consisted of a mixture of toluene: acetone: methanol: formic acid (7: 2: 0.8: 0.2 v/v). Detection was performed at a wavelength of 254 nm and 366 nm. The Rf value of quercetin was found to be 0.45. The minimum detectable amount of quercetin was found to be 0.02196% w/w and the method can be employed for routine analysis[30].

CONCLUSION

In this review article various published analytical methods and their validation for determination of presence of quercetin either single or in combination with other drugs are reported. These methods are valid, accurate, precise, reliable and reproducible as per validation parameters. New analytical method could be developed from these methods by changing the chromatographic conditions.

CONFLICT OF INTEREST

The authors confirm that this article has no conflict of interest.

REFERENCES

1. https://pubchem.ncbi.nlm.nih.gov/compound/quercetin.
2. Shaikh S, Jain V. Development and Validation of a RP-HPLC Method for the Simultaneous Determination of Quercetin, Ellagic Acid and Rutin in Hydroalcoholic Extract of Triphala Churna. International Journal of Applied Pharmaceutics 2018; 10(3): 169-174.
3. Rajpuria G. Optimization and validation of reverse phase HPLC method for qualitative and quantitative assessment of polyphenols in seaweed. Journal of Pharmaceutical and Biomedical Analysis 2018; 148: 230-237.
4. Sandhu PS, Beg S, Kumar R, Katare OP, Singh B. Analytical QbD-Based Systematic Bioanalytical HPLC Method Development for Estimation of Quercetin Dihydrate. Journal of Liquid Chromatography & Related Technologies 2017; 40(10): 506-516.
5. DasP, Pannerselvam T, Prabhu PP. Spectrometric Method Development and Validation of Catechin and Quercetin in Khadirarista. Saudi Journal of Medical and Pharmaceutical Sciences 2017; 3(8): 936-940.
6. Baghel US, Nagar A, Pannu MS, Singh D, Yadav R. HPLC and HPTLC Methods for Simultaneous Estimation of Quercetin and Curcumin in Polyherbal Formulation. Indian Journal of Pharmaceutical Sciences 2017; 79(2): 197-203.
7. Sharifuldin MMA, Ismail Z, Aisha AFA, Seow EK, Beh HK. Quantification of Rrutin, Quercitrin and Quercetin in *Cosmos caudatus* Kunth by Reverse Phase High Performance Liquid Chromatography. Quality Assurance and Safety of Crops & Foods 2016; 8 (4): 617-622.
8. Seo JH, Kim JE, Shim JH, Yoon G, Bang M, Bae CS, Lee KJ, Park DH, Cho SS. HPLC Analysis, Optimization of Extraction Conditions and Biological Evaluation of *Corylopsis coreana* Uyeki Flos. Molecules 2016; 21(94): 1-13.
9. Kumar S, Lather V, Pandita D. Stability indicating simplified HPLC method for simultaneous analysis of resveratrol and quercetin in nanoparticles and human plasma. Food Chemistry 2016; 197: 959–964.
10. Subramanian G, Meyyanathan SN, Byran G, Karthik Y, Palanisamy DS. Development and Validation of Liquid Chromatography-Mass Spectrometric Method for Simultaneous Estimation of Quercetin and Rutin from *Aganosma Dichotoma* [Roth] K. Schum. Indo American Journal of Pharmaceutical Research 2015; 5(11): 3412-3417.
11. Boligon AA, Jesus RDS, Piana M, Brum TFD, Cruz RT, Mossmann NL, Athayde ML. Development and Validation of an HPLC-DAD Analysis for Flavonoids in the gel of *Scutia buxifolia*. Journal of Chromatographic Science 2015; 53: 1557–1561.
12. Salunkhe VR, Patil SJ. UV Spectrophotometric and HPLC Method Development of Quercetin and Curcumin in Polyherbal Churna and its Validation. International Journal of Pharmaceutical and Phytopharmacological Research 2014; 4 (1): 8-12.

13. Rajendran J, Jacob L, Kochupappy RT. Development of Validated Analytical Methods for the Simultaneous Estimation of Biomarkers in the leaves of *Barleria cristata* and Bio analysis of the selected marker in Plasma. Journal of Advanced Pharmacy Education & Research 2014; 4(4): 409-416.
14. Ang LF, Yam MF, Fung YTT, Kiang PK, Darwin Y. HPLC Method for Simultaneous Quantitative Detection of Quercetin and Curcuminoids in Traditional Chinese Medicines. Journal of Pharmacopuncture 2014; 17(4): 36-49.
15. Subramanian G, Meyyanathan SN, Karthik Y, Karunakaranair A, Palanisamy DS. Development and Validation of HPLC Method for the Simultaneous Estimation of Quercetin and Rutin in *Aganosma dichotoma* [Roth] K. Schum. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(2): 606-608.
16. Chaudhari SP, Bangar JV, Akuskar GK, Ratnaparkhi MP. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Rutin and Quercetin in Niosome Formulation. Der Pharmacia Lettre 2014; 6(3): 271-276.
17. Tuszynska M. Validation of the Analytical Methodfor the Determination of Flavonoids in Broccoli. Journal of Horticultural Research 2014; 22(1): 131-140.
18. Li W, Shi LL, Han LQ, Zhang J. Development and Validation of a RP-HPLC Method for Simultaneous Determination of Salicin and Eight Flavonoids in Leaves of *Salix Matsudana* Koidz. Acta Chromatographica 2013; 25(4): 735–743.
19. Prajapati B, Savai J, Pandita N. Method Development and Validation for Simultaneous Estimation of Quercetin and Rutin in Bark of *Saraca Asoca* and Herbal Formulation by HPTLC. Indo American Journal of Pharmaceutical Research 2013; 3(4): 3233-3245.
20. Singh U, Baldi A. Simultaneous Estimation of Quercetin and Silymarin: Method Development and Validation. International Journal of Pharmaceutical & Biological Archives 2013; 4(3): 527 – 531.
21. Moorthi C, Kathiresan K. Reversed Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Curcumin and Quercetin in Pharmaceutical Nanoformulation. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(3): 622-625.
22. Shanmugam R, Gowthamarajan K, Priyanka DL, Madhuri K, Narayanareddy KVVS. Bioanalytical Method Development and Validation for Herbal Quercetin in Nano Formulation by RP-UFLC in Rabbit Plasma. Bioequivalence & Bioavailability 2013; 5(5): 191-196.
23. Savic IM, Nikolic VD, Savic IM, Nikolic LB, Stankovic MZ. Development and Validation of a New RP-HPLC Method for Determination of Quercetin in Green Tea. Journal of Analytical Chemistry 2013; 68(10): 906–911.
24. Yang L, Yan QH, Ma JY, Wang Q, Zhang JW, Xi GX. High Performance Liquid Chromatographic Determination of Phenolic Compounds in Propolis. Tropical Journal of Pharmaceutical Research 2013; 12 (5): 771-776.
25. Shah R, Sharma N, Patel V, Savai J, Saraswathy N. Validated HPLC fingerprint analysis for Simultaneous Determination of Quercetin and Kaempferol in Methanolic Extract of *Tridax procumbens*. International Journal of Pharmaceutical and Biological Research 2012; 3(4): 166-175.
26. Landim LP, Feitoza GS, Costa JGMD. Development and Validation of a HPLC method for the quantification of three flavonoids in a crude extract of *Dimorphandra gardneriana*. Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy 2013; 23 (1): 58-64.
27. Patil SJ, Salunkhe VR. Simultaneous Estimation of Cucumin and Quercetin in Ayurvedic Proprietary Medicine by U.V. Spectrometry. International Journal of Research in Ayurveda and Pharmacy 2012; 3 (2): 267-271.
28. Aneja G, Dave U, Vadodaria K. Simultaneous Estimation of Piperine, Quercetin, and Curcumin in a Mixture Using U.V-Visible Spectrophotometer and Method Validation. International Journal of Therapeutic Applications 2012; 8: 14 – 17.
29. Pawar NP, Salunkhe VR. Development and Validation of HPTLC Method for Simultaneous Estimation of Rutin and Quercetin in Hydroalcoholic Extract of Triphala Churna. International Journal of Pharm Tech Research 2012; 4(4): 1457-1463.
30. Movaliya V, Zaveri MN. HPTC Method Development and Estimation of Quercetin in the Alcoholic Extract of *Aerva javanica* Root. Advance Research in Pharmaceuticals and Biologicals 2012; 2: 222-228.