Quality control and standardization of Quercetin in herbal medicines by spectroscopic and chromatographic techniques

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Abstract
Background: Herbal medicines and their preparations have been mostly used since from thousands of years in all developing and developed countries in the primary health care of society and community. Flavonoids are the class of polyphenolic compounds, which are mainly distributed throughout the plant kingdom. Quercetin is a flavonoid which shows major pharmacological activities and effectively used for the management and treatment of various diseases and disorders. Many herbal medicines and their formulations containing Quercetin are available in market and hence quality control of Quercetin in is very important and essential in manufacturing industries.

Main body of the abstract: We have reviewed various scientific research published on quality control analysis and standardization of Quercetin in its isolated form, extract or any other herbal or polyherbal preparation. We have mainly focused on the spectroscopic and chromatographic methods for qualitative and quantitative analysis of Quercetin and they were comprehensively presented in the present review work.

Short conclusion: The present review concludes that the spectroscopic and chromatographic methods play great role in the quality control and standardization of Quercetin in its isolated form, extract and in its herbal and polyherbal preparation.

Keywords: Quality control, Standardization, Herbal medicines, Quercetin, Flavonoids

Background
Herbal medicine and most of their preparations have been widely used from the thousands of years in all developing and developed countries in the primary health care of society and community. They have great demand due its safety, efficacy with minimum side or adverse effects. India is considered as well recorded as well practiced knowledge country of traditional herbal medicine. The Indian systems of medicine mainly composed of Ayurveda, Siddha and Unani system of medicines. Traditional herbal medicines are generally naturally occurring and derived from plants with very less or no requirement of industrial methodology in its final production and used to treat illness, dieses or disorders in local or regional healing practices [1].

Many herbal medicines and phytomedicines are introduced into the market for primary health care of communities without any mandatory safety or toxicological evaluation and quality control parameters regarding the effect of drug. Many countries who are dealing with manufacturing and production of herbal medicines are lacking with availability of effective machineries, laboratories for effective quality control testing to regulate manufacturing practices and quality...

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standards of the herbal preparations [2]. Quality control is one of the very important and essential steps in the manufacturing of herbal preparations as quality of product affects the safety and efficacy of medicines. Quality control is mainly applied for both raw materials along with excipients used and finished product. The quality of the raw materials and other material used as excipients used in the manufacturing of phytomedicines need to be tested and evaluated before production of its finished product. Modern analytical instruments play great role in the quality control and standardization of Herbal Medicines. Especially the spectroscopic and chromatographic methods play essential role in quality control and analytical validation of herbal preparations [3].

Flavonoids are the class of polyphenolic compounds, which are mainly distributed throughout the plant kingdom. There are number of flavonoids are known. Flavonoids, as a major active constituent, shows best various pharmacological activities Rutin and logical activities like anti-inflammatory, anti-allergic anti-oxidant effects. Quercetin possesses antioxidant activity and reduces low density lipoproteins oxidation level. Quercetin and rutin are the some important flavonoids known for its, anti-inflammatory, anti-allergic, anti-thrombic, anti-spasmodic anti-cancer, and hepatoprotective properties [4]. Main flavonoid constituents like quercetin (Fig. 1) quercetin is a flavonoid chemically known as 5,7,3′-4′ tetra hydroxy flavanol, which shows a major pharmacological activity like hepatoprotective activity, anti-spasmodic, anti-inflammatory, etc. And mainly belongs to polyphenolic derivatives [5].

Main text
Many herbal medicines and their formulations containing Quercetin are available in market. We have reviewed various scientific research published on quality control analysis and standardization of Quercetin in its isolated form, extract or any other herbal or polyherbal preparation. In the present review article, we have mainly focused on the spectroscopic and chromatographic methods. The details of methodology and interpretation of results were presented as below.

Spectroscopic techniques
V.C. Yeligar et al., have reported the validation of UV spectrophotometric method for simultaneous estimation of Melatonin and Quercetin in liposome formulation by calibration curve method using methanol as a solvent. The UV spectra run in the range of 200–400 nm. The λmax of standard solution of melatonin (10 µg/ml) and Quercetin (10 µg/ml) was found to be 276 nm and 372 nm. The absorption and absorptivity coefficient were found by simultaneous equation method [6].

Singh Upendra et al., have validated UV spectrometric methods for the simultaneous estimation of Quercetin and Silymarin using double beam UV–Vis spectrophotometer by preparing the standard solution of 0.01 g of each drug in methanol scanned in the range of 200–400 nm. The maximum absorption is found at 256 and 288 nm for quercetin and silymarin respectively [7].

Ginpreet Aneja et al., have validated the method for simultaneous estimation of Piperine, Quercetin, and Curcumin by UV spectrometry by making standard stock solution of 1000 ppm of each drug in methanol and scanned in the range of 200–400 nm and λmax of quercetin, curcumin and piperine were found to be 371.31 nm, 424.68 nm, 343.76 nm respectively [8].

A Viswanath et al., have reported the UV spectrometry method for the estimation of Quercetin from Ipomea sepiaria Koenig. The two-extraction scanned for the absorption maxima. Quercetin standard absorbs at 350 nm, acetone extract at 328.2 nm and methanol extracts absorbs at 323.4 nm [9].

Marzanna Kurzawa et al., have determined the quercetin and rutin in selected herbs and pharmaceutical preparations by UV–Vis double beam spectrometer using 1 cm quartz cell. The standard solution of quercetin and rutin was prepared in methanol and ethanol. The absorbance at maximum absorbance of 425 nm for quercetin and 362 for rutin [10].

The detailed summery of spectroscopic standardization of Quercetin is presented in Table 1.

Chromatographic techniques
Sunita Shailajan et al., have reported the validated method for estimation of Quercetin content from Cuscuta reflexa Roxb by the HPLC using 0.025 M NaH2PO4: ACN as mobile phase. The analysis was performed using C18 column (150 mm × 4.6 mm × 5 µm) peaks were recorded at 378 nm [11].

Asma’a Ai-Rifai et al., have describes the HPLC method for analysis of Quercetin and Kaempferol of alcoholic extraction of convolvulus pilosellifolius by...
using isocratic mixture of methanol and water containing 0.1% v/v formic acid (80:20) using BETASIL C18 column (150 mm × 4.6 mm, 3 µm) and peaks were found at 258 nm [12].

Lee Fung Ang et al., have describes the quantitative detection of quercetin and curcuminoids (dimethoxycurcumin, bis-demethoxycurcumin, curcumin) in traditional Chinese medicines by HPLC. The analysis is carried out by using thermo erisol Gold column (250 mm × 4.6 mm ID:5 mm) and a C-18 cartridge guard column (12.5 mm × 4.6mm ID:5 mm) with a mobile phase system of Acetonitrile and 2%v/v acetic acid (40:60) at the detection wavelength 370 nm.In this method the simultaneous estimation of quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin for the concentration range of 0.00488-200 µg/ml, 0.625-320 µg/ml, 0.07813-320 µg/ml and 0.03906-320 µg/ml respectively [13].

Ujjwala supe et al., have reported the HPLC method for analysis of quercetin in Monordica charanta. Merck C-18 Bondapack was used which is maintained at a temperature of 27°C and methanol:ACN:water (60:20:20v/v) were used as mobile phase. At 260 and 262 nm wavelength the investigation carried out for flavonoid, phenols and Quercetin [14].

A. Srinivasa Rao et al., have accessed the HPLC method for the simultaneous determination of Rutin, Quercetin and Kaempferol in Catharanthus roseus by using Athena C18 column and phosphate buffer (pH = 5.8) and ACN as mobile phase 55:45 Ratio and maximum absorbance were measured at 254 nm. The retention time of Rutin, Quercetin, Kaempferol in the extract was found 2.403, 6.143, 8.903 respectively [15].

Deepak Mundkinajeddu et al., have developed a method for estimation of the flavonoid glycoside in Withania somnifera by HPLC using Phenomenex Luna C18 column (5µ, 250 × 4.6 mm) and the mobile system consist of potassium dihydrogen orthophosphate (0.136 g) dissolved in 900 ml of HPLC grade water to that 0.5 ml of orthophosphoric acid added volume made up to 1000 ml. the method for the estimation of 3 flavonoid glycosides that are quercetin-3-orobinoside-7-O-glucoside (1), quercetin-3-O-rutinoside-7-O-glucoside (2), kaempferol 3-O-robinobioside-7-O-glucoside [16].

Aline Augusti Boligon et al., havevalidate the HPLC method for the estimation of flavonoid in gel of Scutia buxifolia using C18 column and mixture of Acetonitrile: water (70:30, v/v) as a mobile phase at 356 nm of maximum absorbance wavelength the quercetin and rutin are quantified.In this method the concentration of quercetin and rutin were found to be 28.15 ± 0.04 and 59.73 ± 0.13 mg/g respectively [17].

Gomathy Subramanian et al., have reported the development and validation of HPLC method for the simultaneous estimation of quercetin and rutin in Aganosma dicotoma. The separation is achieved on C18 column (150 × 4.6 mm) and mobile phase containing acetonitrile: ammonium acetate (40:60v/v) at the flow rate 1 ml/min. The analysis monitored at 259 nm [4].

Haritha Krishna prasad et al., havedeveloped and validated the RP-HPLC method for simultaneous estimation of Resveratrol and Quercetin in bulk and pharmaceutical dosage form. The estimation is performed by using Sunfire C18 (150 × 3.0 mm 1D5µm particle size) column having Rhodyne injector using Methanol:Water/Formic acid: Triethylaminein the ratio 10:70:15:5 as mobile phase at the wavelength 277 nm. resveratrol and Quercetin eluted at retention time 1.24 and 2.14 respectively [18].
Table 2 Chromatographic standardization of Quercetin in herbal medicines

| 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 Roxbus-        | By HPLC method using 0.025 M NaH₂PO₄: ACN as mobile phase. The analysis was performed using C18 column (150 mm × 4.6 mm × 5 µm) peaks were recorded at 378 nm |
|       |                                      | ing validated HPTLC and HPLC techniques                                           |                                                                                                                                                           |
| 2     | Asma’Ai-Rifai et al.                 | Analysis of Quercetin and Kaempferol in an Alcoholic Extract of Convolvulus       | HPLC method using isocratic mixture of methanol and water containing 0.1% v/v formic acid (80:20) using BETASIL C18 column (150 mm × 4.6 mm, 3 µm) and peaks were found at 258 nm |
|       |                                      | pilosellifolius using HPLC                                                         |                                                                                                                                                           |
| 3     | Lee Fung Ang et al.                  | HPLC Method for Simultaneous Quantitative Detection of Quercetin and              | Quantitative detection of quercetin and curcuminoids (dimethoxy curcumin, bis demethoxy curcumin, curcumin) in traditional Chinese medicines by HPLC using thermo erasil Gold column (250 mm × 46 mm ID:5 µm) and a C-18 cartridge guard column (12.5 mm × 4.6mmID:5 mm) with a mobile phase system of Acetonitrile and 2%v/v acetic acid (40:60) at the detection wavelength 370 nm |
|       |                                      | Curcuminoids in Traditional Chinese Medicines                                     |                                                                                                                                                           |
| 4     | Ujjwala supe et al.                  | Preliminary Phytochemical Analysis and Quantitative Analysis of Quercetin          | HPLC method performed using Merck C-18 Bondapack which is maintained at a temperature of 27°C and methanol:ACN:water (60:20:20v/v) were used as mobile phase. At 260 and 262 nm wavelength the investigation carried out for flavonoid, phenols and Quercetin [14] |
|       |                                      | by HPLC of Momordica Charantia                                                     |                                                                                                                                                           |
| 5     | A. Srinivasa Rao et al.              | Simultaneous determination of phenolic compounds in Catharanthus roseus leaves    | HPLC method using Athena C18 column and phosphate buffer (pH = 5.8) and ACN as mobile phase 55:45 Ratio and maximum absorbance were measured at 254 nm. The retention time of Rutin, Quercetin, Kaempferol was found 2.403, 6.143, 8.903 respectively |
|       |                                      | by HPLC method                                                                     |                                                                                                                                                           |
| 6     | Deepak Mundkinajeddu et al.          | Development and Validation of High Performance Liquid Chromatography Method       | HPLC method by using Phenomenex Luna C18 column (5µ, 250 × 4.6 mm) and the mobile system consist of potassium dihydrogen orthophosphate (0.136 g) dissolved in 900 ml of HPLC grade water to that 0.5 ml of orthophosphoric acid added volume made up to 1000 ml, for the estimation of 3 flavonoid glycosides that are quercetin-3-orobinoside-7-O-glucoside(1), quercetin-3-O-rutinoside-7-O-glucoside (2), kaempferol 3-O-robinobioside-7-O-glucoside(3) |
|       |                                      | for Simultaneous Estimation of Flavonoid Glycosides in Withania somnifera Aerial   |                                                                                                                                                           |
|       |                                      | Parts                                                                             |                                                                                                                                                           |
| 7     | Aline Augusti Boligon et al.         | Development and Validation of an HPLC–DAD Analysis for Flavonoids in the gel of   | They have validated the HPLC using C18 column and mixture of Acetonitrile: water (70:30, v/v) as a mobile phase at 356 nm of maximum absorbance wavelength the quercetin and rutin are quantified |
|       |                                      | Scuta buxifolia                                                                  |                                                                                                                                                           |
| 8     | Gomathy Subramanian et al.           | Development and Validation of HPLC Method for the Simultaneous Estimation of     | For HPLC method separation is achieved using C18 column (150 × 4.6 mm) and mobile phase containing acetonitrile: ammonium acetate (40:60v/v) at the flow rate 1 ml/min the analysis monitored at 259 nm |
|       |                                      | Quercetin and Rutin in Aganosma Dichotoma [Roth] K. Schum                        |                                                                                                                                                           |
| 9     | Haritha Krishna prasad et al.        | Method Development and Validation for the Simultaneous Estimation of Resveratrol  | RP-HPLC method for the estimation is performed by using Sunfire C18 (150 × 3.0nm:DS5µm particle size) column having Rheodyne injector using Methanol:Water:Formic acid: Triethylamine in the ratio 10:70:15:5 as mobile phase at the wavelength 277 nm:resveratrol and Quercetin eluted at retention time 1.24 and 2.14 respectively |
|       |                                      | and Quercetin in Bulk and Pharmaceutical Dosage Form by RP-HPLC                   |                                                                                                                                                           |
| Sl. No | Author name                     | Title of the work                                                                                           | Description of analysis                                                                                                                                                                                                                                                                                                                                 |
|-------|---------------------------------|-------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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 (150 mm x 4.6 mm, 5 µm particle size ODS) and mixture of acetonitrile and HPLC grade water (pH 2.6 adjusted with 2%v/v glacial acetic acid) as a mobile phase, wavelength 346 nm. 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 Terminalia chebula by HPTLC                               | HPTLC method by using precoated silica gel GF254 as stationary phase and Toluene: acetone: glacial acetic acid (3:2.1 v/v/v/v/v) as mobile phase of tannins, and ethyl acetate: dichloromethane: formic acid: glacial acetic acid: water (10:2:5:1:10v/v/v/v/v) the quantification is carried out for Rutin and Quercetin at 366 nm and for gallic acid at 254 nm densitometrically and Rf 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 F254 plates and Toluene: ethyl acetate: formic acid in the ratio 5:4:0.2 (v/v/v) as mobile phase. At 380 nm a sharp and well-defined peak found at Rf=0.29. |
| 13    | Ansul Shakya et al              | A Rapid High-Performance Thin-Layer Chromatographic Method to Estimate Quercetin in Benincasa hispida (Thunb.) Cogn. Fruit Pulp | HPTLC for the estimation using alumina plates with silica gel 60 F254 were used with Toluene: ethyl acetate: formic acid (5:4.0:2v/v/v) as mobile phase at absorbance wavelength 262nm the Rf value of Quercetin found Rf=0.392. |
| 14    | Barik Laemi Dhar et al          | HPTLC method for Quantitative estimation of Quercetin in a polyherbal compound for urolithiasis             | By HPTLC method extract chromatogrammed on silica gel 60F254 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 Rf value was 0.51 analysed at wavelength of 254 nm. |
| 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 60F254 TLC plates using toluene: acetone: formic acid (30:10:5) as mobile phase. The detection and quantification done at 364 nm and RF value of acetone extract was 0.45. |
| 16    | Vaidevi Sethuram et al          | Combinatorial analysis of quercetin and resveratrol by HPTLC in Sesbania grandiflora /Phyto based nanofibrillations | By HPTLC method In this investigation separation achieved by using mobile phase system of toluene: chloroform: ethyl acetate: formic acid (32.4:90.1% v/v). The densitometric scanning at 280 nm was performed and Rf 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                               | The estimation of Quercetin carried out on precoated silica gel plates F 254 (10 cm x 10 cm) and mixture of toluene: ethyl acetate: formic acid in the ratio 5:4:1 was used as mobile phase. The detection and quantification done at 365 nm. |
| 18    | V Patil et al                   | Recent progress in simultaneous estimation of rutin, quercetin and liquiritin in Cocculus hirsutus by HPTLC   | The simultaneous estimation carried on silica gel 60F254 and mixture of n-butanol: acetic acid: water: formic acid (7:1.1:0.25) as mobile phase system. The RF value for Rutin, Quercetin and Liquiritin was 0.047 ± 0.03, 0.063 ± 0.03 and 0.82 ± 0.02 respectively at the wavelength 254 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) | HPTLC method for validation carried out using precoated aluminium plates with silica gel G60F254 and mixture of toluene: ethyl acetate: formic acid (6:4.1 v/v/v) as mobile phase. The scanning of plates at 275 nm and the Rf value was 0.69 ± 0.02 (Diosgenin) and 0.57 ± 0.02(Quercetin). |
| Sl. no | Author name | Title of the work | Description of analysis |
|-------|-------------|-------------------|-------------------------|
| 20 | A. Srinivas Rao et al. | Simultaneous estimation of quercetin and rutin in ethanolic extract of *Melia azedarach* Linn leaves by HPTLC method | HPTLC method for the analysis is carried out with precoated silica 60F254 as stationary phase and mixture of toluene: ethyl acetate: methanol (5:3:2) as mobile phase. At 254 nm the RF values of Quercetin and Rutin was 0.65 and 0.15 respectively. |
| 21 | Gundu Sindhu Chakraborty et al. | Determination of Quercetin by HPTLC in *Calendula Officinalis* Extract | HPTLC method using precoated aluminium plates with silica gel 60GF as stationary phase and chloroform: methanol (9:5:0.5) mixture as mobile phase solvent system. The RF value was 0.43 and scanning for estimation was carried out at 366 nm wavelength. |
| 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 performed on aluminium foil-backed silica gel 60F254 HPTLC plates using solvent system of toluene: ethyl acetate: formic acid (5:4:1 v/v) as mobile phase. At absorbance wavelength 270 nm and RF value was 0.31 (gallic acid), 0.50 (Quercetin). |
| 23 | Jinan Hussain et al. | Qualitative and quantitative comparison of rutin, quercetin and gallic acid concentrations in *Syrian Capparis spinosa* L Leaves | The analysis performed on silica gel 60F254 and mixture of ethyl acetate: glacial acetic acid: formic acid: distilled water (100:11:11:25) as mobile phase. At the scanning wavelength of 366 nm the Rf value obtained are 0.39 (Rutin), 0.79 (Quercetin) and at 254 nm the RF value was 0.81 (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 | HPTLC analysis carried with silica gel 60F254 as stationary phase using toluene: ethyl acetate: formic acid (7:5:1) as mobile system 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 | HP TLC analysis with aluminum plates 60F254 using solvent system chloroform: ethyl acetate:methanol:formic acid (5:3:1.5:0.5v/v/v/v) as mobile phase. The well separated spots were obtained with RF values of 0.13, 0.24, 0.62 for trigonelline, chlorogenic acid, and quercetin respectively. |
| 26 | Hiteksha Panchal et al. | Development of Validated High-performance Thin-layer Chromatography Method for Simultaneous Determination of Quercetin and Kaempferol in *Thepsesa populnea* | Analysis performed on aluminium plates precoated with silica gel 60F254 using toluene: ethyl acetate: formic acid (6:4:0.3 v/v/v) as mobile phase. At the scanning absorbance wavelength 366 nm, the RF value obtained are 0.39 for Quercetin and 0.50 for kaempferol. |
| 27 | Khan Dureshahwar et al. | Quantification of Quercetin Obtained from *Allium cepa* Lam. Leaves and its Effects on Streptozotocin-induced Diabetic Neuropathy | The quantification carried out with precoated silica gel GF254 plates and using mobile phase system toluene: ethyl acetate: formic acid (5:4:1) and the scanning absorbance at 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 performed with precoated silica gel aluminium plates 60F254/20 cm x 10 cm with 250 µm thickness and mixture of chloroform:methanol:formic acid (8:2:1.5:1) used as mobile phase and at 254 nm the RF value of kaempferol-0.69, quercetin-0.53, and rutin-0.12 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 | HPTLC method using silica gel 60F254 plates. The mixture of solvents toluene: ethyl acetate: formic acid (5:4:1) used as mobile phase and the detection carried out at 254 nm and reported RF values are 0.3 ± 0.00 (gallic acid), 0.47 ± 0.005 (vanillic acid), 0.37 ± 0.00 (protocatechuic acid), 0.42 ± 0.005 (quercetin). |
| Sl. no | Author name                  | Title of the work                                                                 | Description of analysis                                                                 |
|-------|------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| 30    | Snehal B. Bhandare et al.    | Simultaneous Quantification of Kaempferol and Quercetin in Medicinal Plants using HPTLC | HPTLC method using toluene: acetone: formic acid in the ratio 7:3:0.25 as mobile phase RP-18 F254 plates. The Rf value for Kaempferol and Quercetin were found 0.46 and 0.39 respectively at the detection wavelength 254 nm |
| 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 Protocatechlic Acid and Quercetin in Carissa carandas L. Fruits | HPTLC analysis using precoated plates with silica gel 60F254 with solvent system toluene: ethyl acetate: formic acid (6:3.1:0.5 v/v). The Rf value obtained as 0.57 and 0.61 for protocatechlic acid and quercetin respectively at the maximum absorbance wavelength 310 nm |
| 32    | Nadeem A. Siddique et al.    | Simultaneous Quantification of Umbelliferone and Quercetin in Polyherbal Formulations of Aegle Marmelos by HPTLC | Analysis performed using precoated alumium plates with silica gel 60F-254 on stationary phase. The solvent system consists of toluene: ethyl acetate: formic acid (6:4:1 v/v) as mobile phase. The Rf value was reported as 0.66 and 0.68 for umbelliferone and quercetin at 300 nm |
| 33    | Pushpendra kumar et al.      | Simultaneous Quantification of Quercetin and Syringic Acid in Methanolic Extract of Leucas lavandulifolia by using Validated HPTLC Densitometric Method | The analysis carried out on Silica gel 60F254 plate using Toluene: ethylacetate: formic acid (7.2:5.0.5 v/v) as mobile phase. The Rf value for quercetin and syringic acid was found to be 0.32 and 0.41 respectively at the detection wavelength 275–370 nm |
| 34    | Thafshila Aafrin Am et al.   | Determination of Quercetin by High Performance Thin Layer Chromatography Method in Achyranthes Aspera (L.) Plant Extract | The determination carried out on silica gel 60F254 plate as stationary phase and mixture of toluene: ethyl acetate: formic acid in the ratio of 5:4:1. The Rf value 0.60 is detected at 254 nm |
| 35    | Avijeet Jain et al.          | Simultaneous estimation of quercetin and rutin in Tephrosia purpurea Pers by high performance thin layer chromatography | The estimation carried out on precoated silica gel RP-18 F254 were used as stationary phase and the solvent system methanol: water: formic acid (40:57.3:0.5 v/v) as mobile phase. At the detection wavelength 254nm the Rf value was 0.07 (quercetin) and 0.17 (rutin) |
| 36    | Girme AS et al.              | Method development, optimization and validation of RP-UFLC method for bioactive flavonoids from Cassia auriculata | The analysis is carried out on acuity C18 column with stepwise gradient elution was carried out with 0.1% formic acid in water and methanol at a flow rate of 0.350 ml/min. The maximum absorbance at 350 nm wavelength was detected for quercetin QUE-3-O-rutinoside. The retention time was found at 3.95 and 5.37 for quercetin and QUE-3-O-rutinoside respectively |
| 37    | Shanmugam R et al.           | Development and Validation of a RP-UFLC Method for Simultaneous Estimation of Quercetin and Rutin | The analysis performed on reverse phase System C8 column (25 x 46 mm). The mobile phase consists of mixture of potassium dihydrogen ortho phosphate and acetonitrile the ratio of 70:30. The detection was done at 230 nm and the retention time was 7.4 and 2.8 min for quercetin and rutin respectively |
| 38    | Khaled Elgendy et al.        | Analysis of Total Flavonoids in Herbal Drugs Expressed as Quercetin by Reversed Phase UHPLC Method | The analysis done on column Phenomenex and methanol and 0.5% phosphoric acid (50:50 v/v) mobile phase with flow rate of 1.3 ml/min. Linearity of the method is established over the concentration range of 240-960 µg/ml for quercetin and retention time were found 7.8 min |
| 39    | Maric santos et al.          | UPLC-MS for Identification of Quercetin Derivatives in Cuphea glutinosa Cham. and Schtidl (Lythraceae) and Evaluation of Antifungal Potential | The analysis performed on fast C18 column analytical column shim pack XR-ODS column (50 x 2 mm, 2.1 µm) the mobile phase consisted of mixture of acetonitrile: methanol (4:1 v/v) as solvent A and water contains 0.1% formic acid as solvent B. The mass spectra was recorded by full scan mode in the range of m/z 200–800 |
Vishal Sharad Chaudhari et al., have reported the simultaneous quantification by RP-HPLC method for Quercetin and Piperine in dual drug loaded nanostructured lipid carriers. The estimation was carried out by using Hypersil gold C-18 column (150 mm × 4.6 mm, 5 µm particle size ODS) and mixture of acetonitrile and HPLC grade water (pH 2.6 adjusted with 2%v/v glacial acetic acid) as a mobile phase at the detection wavelength 346 nm Quercetin and Piperine is eluted at retention time 2.80 min and 10.36 min respectively [19].

Ashok Kumar et al., have investigated the HPTLC method for the estimation of Gallic acid, Rutin and Quercetin in *Termenelia chebula*. The investigation is carried out by using precoated silica gel GF254 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 (10:2.5:1:0.1:0v/v/v) the quantification is carried out for Rutin and Quercetin at 366 nm and for gallic acid at 254 nm densitometrically and Rf value of gallic acid, rutin, and quercetin are 0.30, 0.13, 0.93 respectively [20].

Sachin U. Rakesh et al., have determined the HPTLC method for the quantitative estimation of Quercetin in Hydrochloric extract of dried flower of *Nymphaea stellate* willd. The detection and quantification were carried out by silica gel 60 F254 plates and Toluene: ethylacetate: formic acid (5:4:2v/v) as mobile phase. The densitometric scanning at absorbance wavelength 380 nm was done and a sharp and well-defined peak found at Rf = 0.29 [21].

Ansl Shakya et al., have reported the method to estimate Quercetin in *Benincasahispida* Cong fruit pulp by HPTLC. The estimation is carried out using alumina plates with silica gel 60 F254 and Toluene: ethylacetate: formic acid (5:4:2v/v) as mobile phase at absorbance wavelength 262nm the Rf value of Quercetin found Rf = 0.392 [22].

Barik Laxmi Dhar et al., have reported the HPTLC method for the quantitative estimation of Quercetin in a polyherbal compound for urolithiasis. *Cra-taeva nurvala* and *Bryophyllum pinnatum* extracted with methanol and extracts are chromatographed on silica gel 60F254 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 Rf value was 0.51 analysed at wavelength of 254 nm [23].

Bindu A R et al., have described the HPTLC method for the Quantitative determination of Quercetin in tender leaves of *Psidium guajava* on silica gel 60F254 TLC plates using toluene: acetone: formic acid (30:10:5) as mobile phase. The detection and quantification done at 364 nm and Rf value of acetone extract was 0.45 [24].

Vaidevi Sethuram et al., have reported the HPTLC method for combinatorial analysis of quercetin and resveratrol in Sesbania grandiflora/Phyto based nanoparticles. In this investigation separation achieved by using mobile phase system of toluene:chloroform: ethyl acetate: formic acid (3:2:4:0.1% v/v). The densitometric scanning at 280 nm was performed and Rf value was 0.40 (Quercetin) and 0.50(Resveratrol) [25].

Mangesh S. Kharate et al., have reported the HPTLC method for estimation of quercetin from crude leaf extract *Mimusops elengi* L. The estimation of Quercetin carried out on precoated silica gel plates F 254 (10 cm × 10 cm) and mixture of toluene: ethyl acetate: formic acid in the ratio 5:4:1 was used as mobile phase. The detection and quantification done at 365 nm [26].

V Patil et al., have evaluated the simultaneous estimation of Rutin, Quercetin and Liquiritin in *Cocculus hirsutus* by HPTLC using silica gel 60F254 and mixture of n-butanol: acetic acid: water: formic acid (7:1:1:0.25) as mobile phase system. The Rf value for Rutin, Quercetin and Liquiritin was 0.047 ± 0.03, 0.063 ± 0.03 and 0.82 ± 0.02 respectively which was scanned at the wavelength 254 nm [27].

Omi Laila et al., have reported the method development and validation of HPTLC for simultaneous estimation of Diosgenin and Quercetin in fenugreek seeds, using precoated aluminium plates with silica gel GF60F254 and mixture of toluene: ethylacetate: formic acid (5:4:1 v/v/v) as mobile phase. The densitometric scanning of plates done at 275 nm and the Rf value was 0.69 ± 0.02 (Diosgenin) and 0.57 ± 0.02(Quercetin) [28].

A. Srinivas Rao et al., have described the simultaneous estimation of Quercetin and Rutin in ethanolic extract of *Melia azedarach*. Linn leaves by HPTLC method. The analysis is carried out with precoated silica gel 60F254 as stationary phase and mixture of toluene: ethylacetate: formic acid (5:4:1 v/v/v) as mobile phase. The scanning for estimation was carried out at 254 nm and the Rf values of Quercetin and Rutin was 0.65 and 0.15 respectively [29].

GunduSindhuChakraborty et al., have described the method for determination of Quercetin by HPTLC in *Calendula officinalis* extract using precoated aluminium plates with silica gel 60GF as stationary phase and chloroform: methanol (9.5:0.5) mixture as mobile phase solvent system. The Rf value was 0.43 and scanning for estimation was carried out at 366 nm wavelength [30].

Md. Sarfaraj Hussain et al., have validated the HPTLC method for simultaneous estimation of bioactive marker Gallic acid and Quercetin in *Abutilon indicum*. The analysis performed on aluminium foil-backed silica gel 60F254 HPTLC plates using solvent system of toluene: ethyl acetate: formic acid (5:4:1 v/v) as mobile phase. The densitometric scanning performed at absorbance
wavelength 270 nm and Rf value was 0.31 (gallic acid), 0.50 (Quercetin) [31].

Jinan Hussain et al., have reported the qualitative and quantitative comparison of rutin, quercetin and gallic acid concentration in Syrian Capsaris Spinosa L leaves using aluminium plates with silica gel 60F254 and mixture of ethyl acetate: glacial acetic acid: formic acid (5:3:1:5/v/v/v) as mobile phase. At the scanning wavelength of 366 nm the Rf value obtained are 0.47 (Rutin), 0.79 (Quercetin) and at 254 nm the Rf value was 0.81 (gallic acid) [32].

Abhay Prakash Mishra et al., have developed and validated method for the Quantitative determination of Quercetin in Satyrium nepalense tubers by HPTLC with silica gel 60F254 as stationary phase using toluene: ethyl acetate: formic acid (7:5:1 v/v/v) as solvent system at absorbance wavelength 366 nm [33].

Shiv K Yadav et al., have developed and validated method for simultaneous estimation of Quercetin, chlorogenic acid and trigonelline in polyherbal antibacterial formulation by HPTLC with aluminium plates 60F254 using solvent system chloroform: ethyl acetate:methanol: formic acid (5:3:1:5:0.5v/v/v/v) as mobile phase. The well separated spots were obtained with Rf values of 0.13, 0.24, 0.62 for trigonelline, chlorogenic acid, and quercetin respectively [34].

Hiteksha Panchal et al., have developed and validated HPTLC method for simultaneous determination of Quercetin and Kaempferol in Spesia populena. On aluminium plates precoated with silica gel 60F254 using toluene: ethyl acetate: formic acid (6:4:1v/v/v) as mobile phase and the detection carried out at 254 nm [35].

Khan Dureshahwar et al., have reported method for the quantification of Quercetin from Allium cepa lam. Leaves by HPTLC. The quantification carried out with precoated silica gel GF254 plates and using mobile phase system toluene: ethyl acetate: formic acid (5:4:1) and the scanning absorbance at 254 nm [36].

Supriya S. Jirge et al., have discussed the HPTLC method for the simultaneous estimation of kaempferol, rutin, quercetin in various plant products. The analysis performed with precoated silica gel aluminium plates 60F254(20 cm × 10 cm with 250 μm thickness) and mixture of chloroform:methanol: formic acid (8:2:1:5:1) used as mobile phase and the absorbance mode kept at 254 nm. Rf value of kaempferol-0.69, quercetin-0.53, and rutin-0.12 are reported [37].

Shikar Verma et al., have reported the simultaneous quantification of gallic acid, vanillic acid, protocatechuic acid and quercetin in Limonia acidissima by HPTLC method using silica gel 60F254 plates. The mixture of solvents toluene:ethyl acetate: formic acid (5:4:1) used as mobile phase and the detection carried out at 254 nm and reported Rf values are 0.3±0.00 (gallic acid), 0.47±0.005 (vanillic acid), 0.37±0.00 (protocatechuic acid), 0.42±0.005 (quercetin) [38].

Snehal B. Bhandare et al., have estimated the simultaneous quantification of kaempferol and Quercetin in medicinal plants by HPTLC method using toluene:acetone: formic acid in the ratio 7:3:0.25 as mobile phase on RP-18 F254 plates. The Rf value for Kaempferol and Quercetin were found 0.46 and 0.39 respectively at the detection wavelength 254 nm [39].

Shikar Verma et al., have reported the HPTLC method for analysis of methanolic fraction with simultaneous quantification of protocatechuic acid and quercetin in Carissia carandas L. fruits using precoated plates with silica gel 60F254 with solvent system toluene: ethyl acetate: formic acid (6:3:1, v/v). The Rf value obtained as 0.57 and 0.61 for protocatechuic acid and quercetin respectively at the maximum absorbance wavelength 310 nm [40].

Nadeem A. Siddique et al., have discussed the HPTLC method for simultaneous Quantification of umbelliferone and quercetin in polyherbal formulation of Aegle marmelos by using precoated aluminium plates with silica gel 60F-254 as stationary phase. The solvent system consists of toluene: ethyl acetate: formic acid (6:4:1v/v/v) as mobile phase. The Rf value was reported as 0.66 and 0.68 for umbelliferone and quercetin at the detection wavelength 300 nm [41].

Pushpendra kumar et al., have reported the simultaneous quantification of quercetin and syringic acid in methanolic extract of Leucas lavandulifolia by using validated HPTLC densitometric method. The analysis carried out on Silica gel 60F254 plate using Toluene: ethylacetate: formic acid (7:2.5:0.5 v/v) as mobile phase. The Rf value for quercetin and syringic acid was found to be 0.32 and 0.41 at the detection wavelength 275 to 370 nm [42].

Thaflsha Aafrin Am et al., have determined HPTLC method for quercetin in Achyranthes aspera (L) plant extract. The determination carried out on silica gel 60F254 plate as stationary phase and mixture of toluene: ethyl acetate: formic acid in the ratio of 5:4:1. The Rf value 0.60 is detected at 254 nm [43].

Avijeet Jain et al., have reported simultaneous for the estimation of Quercetin and Rutin in Tephrosia purpurea by HPTLC. The estimation carried out on precoated silica gel RP-18 F254 were used as stationary phase and the solvent system methanol:water: formic acid (40:57:3v/v/v) as mobile phase. At the detection wavelength 254nm the Rf value was 0.07(quercetin) and 0.17 (rutin) [44].

Girme AS et al., havedescribed method development, optimization and validation of RP-UFLC method for bioactive flavonoids from Cassia auriculata. The analysis is
carried out on acuity C18 column with stepwise gradient elution was carried out with 0.1% formic acid in water and methanol at a flow rate of 0.350 ml/min. The maximum absorbance at 350 nm wavelength was detected for quercetin QUE-3-O-rutinoside. The retention time was found at 3.95 and 5.37 for quercetin-3-O-rutinoside and quercetin respectively [45].

Shanmugam R et al., have reported the development and validation of RP-UFLC method for simultaneous estimation of quercetin and rutin. The separation was done by using reverse phase System C8 column (25 x 4.6 mm), the mobile phase consists of mixture of potassium dihydrogen ortho phosphate and acetonitrile the ratio of 70:30. The detection was done at 230 nm and the retention time was 7.4 and 2.8 min for quercetin and rutin respectively [5].

Khaled Elgendy et al., have analysed the total flavonoids in herbal drugs expressed as quercetin by reverse phase-UHPLC method. The analysis done on column Phenomenex and methanol and 0.5% phosphoric acid (50:50v/v) as mobile phase with flow rate of 1.3 ml/min. Linearity of the method is established over the concentration range of 240-960 µg/ml for quercetin and retention time were found 7.8 min [46].

Maric santos et al., have reported the UPLC-MS for identification of quercetin derivative in Cuphea glutinosa by using reverse phase system and fast C18 column analytical column shim pack X-ODS column (50 x 2 mm,2.1 µm). the mobile phase consisted of mixture of acetonitrile: methanol (4:1v/v) as solvent A and water contains 0.1% formic acid as solvent B. the mass spectra was recorded by full scan mode in the range of m/z 200–800 [47].

The detailed summery of chromatographic standardization of Quercetin is presented in Table 2.

Conclusions
Herbal medicine and their preparations have been widely used from the thousands of years in developing and developed countries in the primary health care of society and community. Quality control is one of the very important and essential steps in the manufacturing of herbal preparations as quality of product affects the safety and efficacy of medicines. Quality control is mainly applied for both raw materials along with excipients used and finished product. Flavonoids are the class of polyphenolic compounds, which are mainly distributed throughout the plant kingdom. Quercetin is a flavonoid which shows major pharmacological activities like anti-cancer, hepatoprotective activity, anti-spasmodic, and anti-inflammatory activity. Many herbal medicines and their formulations containing Quercetin are available in market. Spectroscopic and Chromatographic methods play great role in the quality control and standardization of Quercetin in its isolated form, extract or any other herbal or polyherbal preparation.

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.

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Authors’ contributions
We have assured that all authors have read and approved the manuscript. All the authors have equal contribution and participation in this review work. PK has reviewed all manuscripts on quality control and standardization of Quercetin in herbal medicines by spectroscopic and chromatographic techniques. SG helped in paraphrasing and writing the review part on spectroscopic methods. KJ has collected the data on chromatographic methods for Quercetin. SH has helped in writing and paraphrasing the review on chromatographic methods of Quercetin. SS has selected the objectives and need of study and also helped in writing, correcting and communicating the proposed review article. All authors read and approved the final manuscript.

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