Simultaneous estimation of Ferulic acid and Quercetin in *Sida cordifolia* extract by HPTLC method

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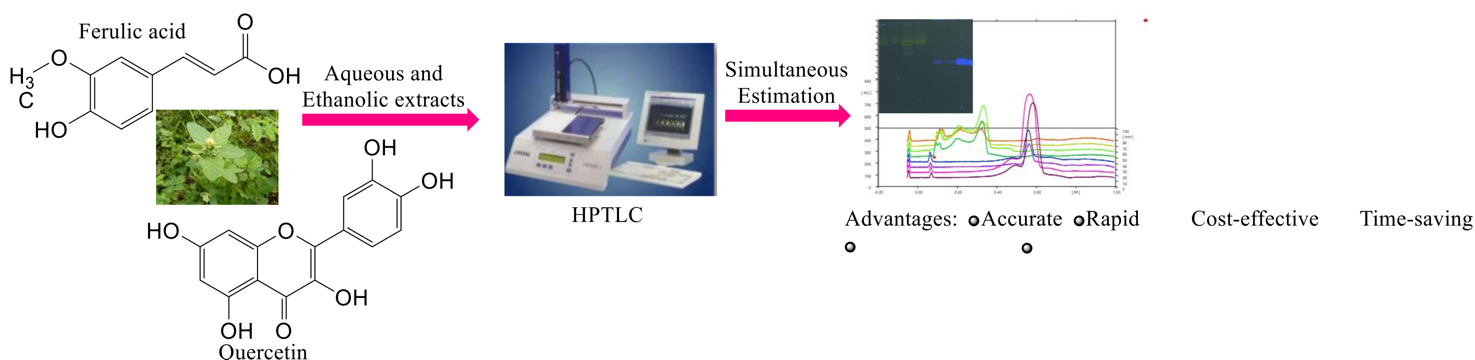
ABSTRACT: Ferulic acid and Quercetin are vital phenolics that are commonly obtained in the leaves, fruits, and seeds of plants. They have the potential against cancer proliferation, chronic inflammation, antiviral, and various other oxidative manifestations. Therefore, the high-performance thin-layer chromatography (HPTLC) method has been established for the simultaneous identification of quercetin and ferulic acid. The aqueous and ethanolic extract of *Sida cordifolia*, a high source of ferulic acid and quercetin, was employed in this investigation. These bioactives were magnificently analyzed at the wavelength of 254 nm using mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v). Further, the method was validated and verified as per the ICH guidelines. This simultaneous identification method of bio-actives will prove to be accurate, rapid, cost-effective, and time-saving for many scientists.



INTRODUCTION

The use of herbal medicines is widespread globally. Approximately 80% of the world's population uses herbal plants and herbal medicines. Humans have been turning to herbal remedies for many years to treat common ailments like allergies, bronchitis, compulsive obsessive pulmonary disorders, rheumatoid arthritis, common cold, snake bites, bug bites, wound healing, skin problems, and ulcerative colitis[1]. Thus, these remedies fascinate increasing attention in many fields up to the present time. However, the huge challenge of this remedies is the analysis of the complex mixture of herbs. Quercetin and ferulic acid are significant bioactives of the plant. They provide defense against a variety of diseases, including the spread of cancer, persistent inflammation, viral infection, etc.[2]

Ferulic acid is a polyphenolic substance with low toxicity and a variety of pharmacological activities including anti-inflammatory, antioxidant, anticancer, antimicrobial, and antidiabetic antiapoptotic and anti-platelet effects. The ferulic acid has also provide the protective effect on the major skin structures, including keratinocytes, fibroblasts, collagen, and elastin. It inhibits melanogenesis, promotes angiogenesis, and hastens wound healing[3]. It is commonly used in skin care formulations as a photoprotective agent, skin photoaging delayer, and brightening component. Many studies have shown that ferulic acid can inhibit the PI3K/AKT pathway, ROS production, and aldose reductase activity. Ferulic acid's anti-inflammatory effect is primarily related to PPAR, CAM, NF-κβ, and p38 MAPK signaling pathways. Ferulic acid not only protects the vascular endothelium via the ERK1/2 and NO/ET-1 signals, but it also inhibits fibrosis via the TGF-β/Smad and MMPs/TIMPs systems.[4]

On other hand, quercetin (Figure 1) is also used to treat cancer and viruses, as well as allergies, metabolic and inflammatory disorders, eye and cardiovascular diseases, and arthritis. Antioxidant activity of Quercetin is primarily shown by its effect on glutathione (GSH), enzymatic activity, signal transduction pathways, and reactive oxygen species (ROS) caused by environmental and toxicological factors. By maintaining oxidative balance, quercetin exhibits significant antioxidant properties.[5] However, because of its poor solubility, low bioavailability, poor permeability, and instability, its application in the

pharmaceutical field is limited. The newer formulations such as quercetin-loaded nanoparticles, polymeric micelles of quercetin, quercetin-loaded mucoadhesive nanoemulsion and quercetin-loaded gels are improving the clinical efficacy by increasing the solubility and bioavailability of quercetin.[6]

Currently, there are no analytical methods available for simultaneous estimation of ferulic acid and quercetin. Therefore, the objective of this study is to develop a new HPTLC method for simultaneous identification of these two bio-active components. *Sida cordifolia* has been used for the development of this method as this plant is rich in various chemical constituents including ephedrine, pseudoephedrine, ferulic acid, quercetin, fatty acids, sterculic, malvalic, and coronaric acid, folic acid, saponin, indole alkaloids, etc. It also known as “Bala” (Hindi and Sanskrit) and country mallow (English), belongs to the Maleaceae family. This plant has been used as hepatoprotective, hypotensive, anti-inflammatory, analgesics, and CNS depressants. Hydroalcoholic extract of S. cordifolia is found to produce sedation, analgesia, and other CNS depressant properties. The whole part of S. cordifolia contains a large amount of ephedrine which is known to stimulate CNS, but the hydroalcoholic extract is found to give CNS depressant results. Alongside S. cordifolia extract also have free radical scavenging activity. This activity found to be present due to flavonoids ferulic acid and quercetin.

RESULT AND DISCUSSION

HPTLC Analysis

A mixture of toluene/ethyl acetate/ formic acid (5:4:1 v/v/v) mobile phase gave a good resolution of the marker and reproducible peak at an Rf value of 0.50 and 0.26 for ferulic acid and quercetin, respectively (Figure 2). The LOD values of ferulic acid and quercetin by HPTLC were determined by repeated scanning of the lowest detectable standard solution, multiplying the standard deviation of the peak area by three, and converting from area to concentration. The recovery rates were determined at three different concentrations of the marker to the both extracts and analyzed quantitatively in triplicate. Mean recoveries for ferulic acid from the aqueous extract and quercetin from the ethanolic extract of S. cordifolia by HPTLC assay were found to be 97.8–99.7%, which indicates the accuracy of the method. The precision of the HPTLC instrumentation was checked by repeated scanning of the same spots of the markers three times on the same day (intra-day precision) and on three consecutive days (inter-day precision) and the relative standard deviation values were calculated. The results showed acceptable precision with the method as revealed by the relative standard deviation data. The UV–Vis absorption spectra recorded on the CAMAG TLC scanner at the start, middle, and end position of the AP bands were superimposable indicating the purity of the ferulic acid and quercetin peak. The HPTLC procedure was used as a fast-screening method identification of ferulic acid and quercetin in aqueous and ethanolic extract of *Sida cordifolia* extract respectively. Qualitatively, similar HPTLC fingerprints were obtained for both the extracts i.e., aqueous and ethanolic. Ferulic acid and quercetin were well separated in the extracts by the HPTLC method and detected at an Rf value of 0.50 and 0.26 approximately. Using the techniques of the HPTLC we found that ferulic acid is present in aqueous extract and quercetin is present in ethanolic extract of *Sida cordifolia*. There was no interference from the other components present in the extracts. These components appeared in the chromatogram at significantly different Rf values.

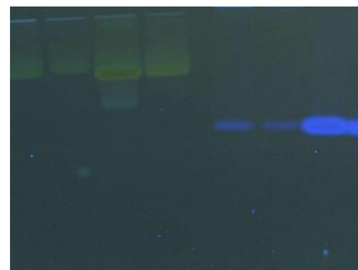


Figure 2. High-performance thin layer chromatogram showing Rf of 0.50 (ferulic acid) and 0.26 (quercetin), respectively.

Method Validation

Calibration graphs were constructed by plotting peak area vs. concentration of quercetin and ferulic acid and the regression equations were calculated. Over the preferred range of the target analytical concentration, an excellent level of linearity was detected which signifies a highly acceptable degree of linearity. The accuracy aspects of the analytical HPTLC method were estimated for the recovery data by utilizing the calibration curve where the Y-intercept and the slope represented a fundamental function in determining the % recovery. The perceived % RSD were <2% at three specific concentrations; 50%, 100% and 150% of the target concentration which was in accordance to the prescribed pharmacopeia acceptance limit of ±2% that indicated brilliant accurateness of this novel analytical method. Over the 50%, 75% and 150% of the target concentration, the % RSD values were detected to be <2% at both intra-day (n = 3) variability as well as under the inter-day variability (n = 3) which represents that the developed HPTLC method possess high precision attributes with reduced variability. The % RSD were observed lie in the recommended pharmacopeia acceptance limit of ±2%. A minuscule variation in the retention value (±0.2) was observed which reflected that the chromatographic method is robust enough to detect even after modifications in the chromatographic environment.

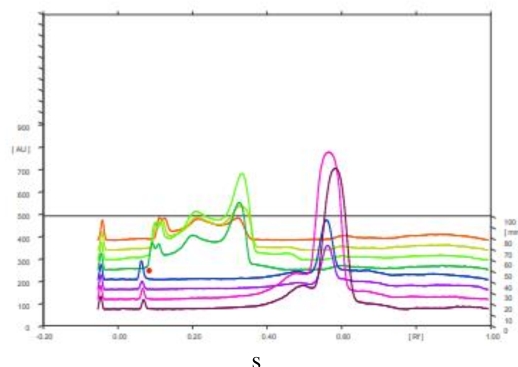


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Figure 3. Different tracks at wavelength 254nm.

MATERIALS AND METHODS

Materials

Aqueous and ethanolic powder extracts of *Sida cordifolia* were taken from AMSAR PVT LTD, Indore, M.P. Standard quercetin and ferulic acid were purchased from Sigma Aldrich. Other solvents and chemicals used in the study were procured from SD Fine Chemical Ltd., Mumbai, India and all are of analytical grade. Precoated silica gel 60 F254 TLC plates were used.

Instrumentation and chromatographic condition

HPTLC equipment: A CAMAG TLC system equipped with CAMAG Linomat V, an automatic TLC sample spotter, CAMAG glass twin trough chamber i.e. 20 x10 cm, CAMAG scanner 3, and integrated winCATS 4 software were used for the analysis. A working standard solution of ferulic acid 1 mg/mL; i.e., 1000 ppm was prepared using methanol. Similarly, for quercetin, a 1 mg/mL; i.e., 1000 ppm working standard solution was prepared using methanol. The planar chromatography was performed on fresh aluminum plates (10×10 cm) precoated with silica gel 60 F254 of layer thickness 0.2 mm (Merck, Germany). Before use, the plates were developed in the mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v) and were activated horizontally for 20 min at 120±0.5°C using an oven to remove elutable components. Samples were applied to the plates as bands 6 mm wide, 10 mm from the bottom, by means of pressurized nitrogen gas (150 kg/cm2) through Camag automatic TLC sampler fitted with a 25 µL syringe.[7] The bands were visualized in Camag UV cabinet at 254 nm. The developing chamber was Camag glass twin through the chamber (10×10 cm). Densitometer consisted of Camag TLC scanner operated by WinCATS software. The scanning speed and data resolution were 20 mm/s and 100 µm/step, respectively. The samples were spotted in the form of bands of 6 mm with Camag microlitre syringe on a pre-coated silica gel aluminum plate 60 F254 (10×10 cm) with 200 µm thickness (Merck, Germany) using a Camag Linomat V sample applicator.[8] A constant application rate of 150 nL/s was employed and the space between the two bands was 12.3 mm. The slit dimension was kept at 5 mm × 0.45 mm and 20 mm/s scanning speed was employed. The composition of the mobile phase is Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v). Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25±2°C). The length of the chromatogram run was 80 mm. Then the plate was allowed to dry at room temperature. The separated bands on the HPTLC plates were scanned over the wavelength of 100–900 nm. The maximum absorbance was found at 254 nm (Figure 3).[9,10]

HPTLC method for simultaneous estimation of ferulic acid and quercetin

A mobile phase consisting of toluene/ethyl acetate/ formic acid (5:4:1 v/v/v) was used. The sample was applied using an automatic TLC sampler. Then, the TLC plate was developed over a development distance of 8 cm (migration time of 15 min) in the solvent. After this, the plates were dried at 60±0.5°C and then densitometric analysis was done.

Preparation of standard solution for HPTLC

A common stock solution (1 mg/mL; i.e., 1000 ppm) of ferulic acid and quercetin were prepared separately. Then, by taking 1 mL from stock solution 9 mL methanol was added to make up the volume up to 10 mL and prepared the sub stock solution of 100 ppm for each. Aliquots of the substock solution were transferred to 10 mL volumetric flasks and diluted to volume with methanol to furnish standard solutions containing 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL (ppm).

Preparation of sample solution for HPTLC

The powder of aqueous and ethanolic S. cordifolia extract was dried at room temperature. The combined extracts were filtered, concentrated, and dried on a rotary evaporator and transferred to a 50-volumetric flask, and the volume was made up of methanol to get a stock solution (100 mg/100 mL). The sample solution was prepared using *Sida cordifolia extract*. A quantity of 100 mg dissolved in 100 mL methanol, volume made up with methanol and filtered through a 0.45 µm Millipore nylon membrane filter under vacuum.

Method Validation

Linearity and range were established for both methods. Precision was evaluated by measuring intraday and interday precision. Accuracy was established by performing recovery studies. Robustness was established by making deliberate minor variations in the wavelength and calculating the percent deviation from the original method. Methanol was used as the extraction solvent as the efficiency of the extraction was more with methanol than with other solvents.[11]

Validation of the analysis method

Validation of the present method was done by sensitivity, the limit of detection, the limit of quantification, accuracy, precision, reproducibility, and stability studies.

Limit of detection, and Limit of quantification

These parameters were calculated from the data set obtained from a linear calibration curve in the range of 2-20 µg (two replicates from each standard). For this purpose, a 10 µL of the corresponding methanolic standard solution was applied in duplicate as bands of 2 mm. Application parameters were the same as above, except for delivery speed, which was changed to 50 nL/s to obtain appropriate evaporation. The corresponding slope and regression standard deviation (SY/X) values were used to establish sensitivity (SY/X/b).[8] LOD was calculated with the following equation:

$$LOD = 3(SY/X/b)\ \sqrt{n-2} \div n-1$$

Accuracy

The accuracy of the present method was determined by the standard addition technique. Three different concentrations (50%, 100%, and 150%) of the standard were added to a previously analyzed sample.

Precision

The precision of an analytical method is expressed by S.D. and % R.S.D. of a series of measurements. It was ascertained by replicate estimation of the samples by the proposed method.

Reproducibility

A similar analysis process was repeated under the same set of conditions for three days. The sample and standard solution prepared earlier were used in this study. On the HPTLC plate five spots of sample and two spots of the standard were applied, the plate was developed, scanned and densitograms were recorded. The same analysis procedure was repeated for the next two days and the concentration of the sample was calculated.[8]

CONCLUSION

The developed high-pressure thin layer chromatography method successfully eluted the ferulic acid and quercetin with the Rf of 0.50 and 0.26, respectively. This method was successfully analyzed and validated at the wavelength of 254 nm using mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v). From the above studies, it can be concluded that the HPTLC technique can be successfully used for the simultaneous estimation of ferulic acid and quercetin in aqueous and ethanolic extract of *Sida cordifolia* extract respectively. Statistical analysis proves that the method is accurate, reproducible and selective for the analysis of ferulic acid and quercetin. Furthermore, the proposed method can be helpful to many researchers who wish to simultaneous quantify and isolate ferulic acid and quercetin from plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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