



Stability indicating RP-HPLC method for the simultaneous estimation of Quercetin and Rutin in bulk drug

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ABSTRACT

A stability-indicating HPLC technique has been developed and validated for Quercetin and Rutin under different stability environments of acid, base, neutral, oxidative, and photolytic degradation. The stability study were executed according to the ICH guidelines. The separation was accomplished utilizing Phenomenex Luna C₁₈ (250 x 4.6mm i.d, 5 μ) column at a detection wavelength of 259 nm utilizing the mobile phase established of 20mM Ammonium acetate (pH 3.0) and acetonitrile 60: 40, v/v at the flow rate of 1.0 ml/min and injection volume of 20 μ l. The linearity of the developed technique was obtained over the range of 1-5 μ g/ml and 0.1-0.5 μ g/ml for Quercetin and Rutin, respectively, with a correlation coefficient of more than 0.99. Further, the stress degradation studies were performed for which the results obtained stated that Quercetin and Rutin were more susceptible to acidic and oxidative conditions. The developed method provides good sensitivity and excellent reproducibility and can be further used to study the stability in formulations.

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INTRODUCTION

Quercetin and Rutin is a plant pigment which is a category in the class of flavonoids found in many fruits and vegetables. The IUPAC name for Quercetin and Rutin are 3, 3', 4', 5, 7-pentahydroxyflavone, and α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranose, respectively (Figure 1). Both of these compounds have been extensively studied and reported for

their anti-carcinogenic and anti-inflammatory effects (Drug information, 2019). Quercetin and rutin possess antioxidant activity and reduce low-density lipoproteins oxidation (Whalley *et al.*, 1990; Subramanian *et al.*, 2014).

In the production of herbal medicines, stability plays an important role in determining its physical and chemical stability in the formulation stage. Hence, it becomes essential to determine the stability of the product. A thorough literature review stated that the couple of methods have been accounted for the determination of both Quercetin and Rutin individually through high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Abdelkawy *et al.*, 2017; Ang *et al.*, 2014; Sanghavi *et al.*, 2014; Šatínský *et al.*, 2013). HPLC has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability for high throughput screening. HPLC method is a

suitable method for estimation and stability indicating studies of chemical constituents present in plant materials (Kumar, 2015; Crozier *et al.*, 1997; Kumar *et al.*, 2009; Willy *et al.*, 2010; Wang and Huang, 2004; Blessy *et al.*, 2014; Hamrapurkar *et al.*, 2011). However, till date, no stability method has been reported for the simultaneous estimation of quercetin and rutin obtained from the plant *Aganosma dichotoma*. Subsequently, the point of the present work is to build up a simultaneous HPLC stability indication technique to determine the stability of Quercetin and Rutin under different conditions according to the International Conference of Harmonization guidelines (ICH, Q1A (R2), 2003).

MATERIALS AND METHODS

Chemicals

Quercetin and Rutin standards were procured from Natural Remedies, Bangalore. Solvents of HPLC grade and reagents of analytical grade were procured from SD fine chemicals. The water of HPLC grade were procured from Milli-Q-RO system.

Instrumentation and conditions

AHPLC system (Shimadzu, Japan) equipped with SPD M10 A ultraviolet (UV) detector, LC-10 AT-VP solvent data station delivery system, HT autosampler with loop volume of 100 μl and LC-2010 and class VP data station was used. The separation was achieved using Phenomenex Luna C_{18} (250 x 4.6mm i.d, 5 μ) column at a detection wavelength of 259 nm using the mobile phase constituted of 20mM Ammonium acetate (pH 3.0) and acetonitrile 60: 40, v/v at the flow rate of 1.0 ml/min and injection volume of 20 μl (Figure 2).

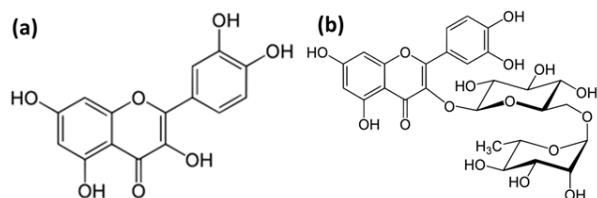


Figure 1: Structure of (a) Quercetin and (b)Rutin

Standard and working solution-Preparation Method

The standard stock arrangement (1 mg/ml) of Quercetin and Rutin were set up in acetonitrile. Further, the working standards were set up by diluting the stock solution to produce the concentration of 1, 3, and 5 $\mu\text{g}/\text{ml}$ for Quercetin and 0.1, 0.2, and 0.5 $\mu\text{g}/\text{ml}$ for Rutin, respectively. The solutions were stored at -8°C until examination.

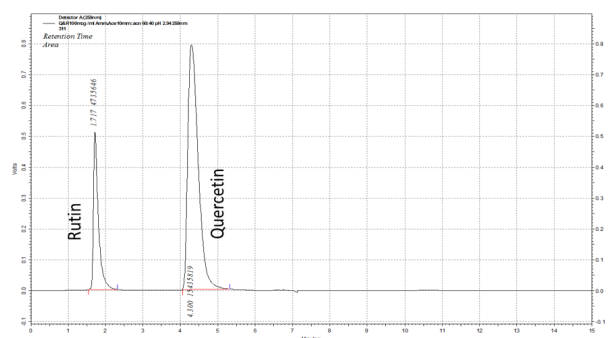


Figure 2: Typical HPLC Chromatogram of Quercetin and Rutin

Degradation studies of Quercetin and Rutin

The degradation studies for the bulk drug were done according to the ICH guidelines Q1A (R2) for different conditions of acid (0.1 N HCl), basic (0.1N NaCl), neutral (water), oxidative (3 % H_2O_2) and photodegradation for a concentration of 100 $\mu\text{g}/\text{ml}$. The samples were withdrawn at a time intervals of 0, 2, 4, 8, 12, and 24 hours and diluted with the mobile phase before investigation (ICH, Q1A (R2), 2003).

Validation

As per ICH guidelines, parameters like specificity, accuracy, linearity, precision studies, the limit of detection (LOD) and limit of quantification (LOQ) were studied for the developed method (ICH, Q2 (R1), 2005).

Linearity for Quercetin and Rutin were analyzed at five concentrations levels ranging from 1 to 5 $\mu\text{g}/\text{ml}$ and 0.1 to 0.5 $\mu\text{g}/\text{ml}$ for Quercetin and Rutin, respectively. The linearity graph was used to calculate the standard deviation, regression coefficient. Recovery studies were performed to determine the method accuracy at three concentrations at replicates for each concentration was examined. For determining the precision of the method, three different concentrations were taken and analyzed on the same day and on different days, and the results were represented as percentage relative standard deviation (% RSD) for each concentration. The detection limit and quantitation limit for Quercetin and Rutin were calculated by signal to noise ratio. Signal to noise ratio of 3:1 was used to determine the LOD and 10:1 signal to noise ratio was used to determine the LOQ. Robustness of the method depends upon experimental conditions (operators, mobile phase used, and types of columns). The parameters like flow rate, mobile phase, and wavelength were studied for robustness. System suitability is an important parameter for method development, and various factor were studied like tailing factor, linearity range.

Table 1: Recovery and precision results of Quercetin and Rutin

| Compound | Conc. ($\mu\text{g/ml}$) | Recovery* | | Intra-day* | | Inter-day* | |
|-----------|----------------------------|---------------|-------------------|--------------|-------------------|--------------|-------------------|
| | | Accuracy (%) | Precision (% RSD) | Accuracy (%) | Precision (% RSD) | Accuracy (%) | Precision (% RSD) |
| Quercetin | 1 | 99.3 0.18 | \pm | 99.56 | 1.11 | 98.34 | 3.11 |
| | 3 | 100.3 0.44 | \pm | 100.88 | 1.03 | 99.44 | 2.70 |
| | 5 | 101.1 0.52 | \pm | 101.44 | 0.67 | 100.27 | 1.92 |
| Rutin | 0.1 | 101.7 1.21 | \pm | 100.1 | 1.30 | 99.8 | 1.82 |
| | 0.2 | 102.0 1.10 | \pm | 100.2 | 1.14 | 99.7 | 1.41 |
| | 0.5 | 101.3 0.87 | \pm | 99.7 | 0.63 | 99.1 | 1.02 |

*The results represents mean of three injections

Table 2: System suitability studies of Quercetin and Rutin by HPLC

| S.No | Description | Quercetin | Rutin |
|------|-------------------------|----------------------|--------------------------|
| 1 | Linearity range | 1-5 $\mu\text{g/ml}$ | 0.1-0.5 $\mu\text{g/ml}$ |
| 2 | Regression equation | $Y = 88980x - 5306$ | $Y = 41670x - 333.3$ |
| 3 | Correlation coefficient | 0.997 | 0.995 |
| 4 | Asymmetric factor | 1.2 | 1.1 |
| 5 | LOD (ng/ml) | 100 | 10 |
| 6 | LOQ (ng/ml) | 300 | 30 |

Table 3: Stress degradation of Quercetin and Rutin

| Time (hrs) | Acidic degradation (%) | | Basic Degradation (%) | | Oxidative Degradation (%) | | Neutral Degradation (%) | | Photo Degradation (%) | |
|------------|------------------------|-------|-----------------------|-------|---------------------------|-------|-------------------------|-------|-----------------------|-------|
| | Quercetin | Rutin | Quercetin | Rutin | Quercetin | Rutin | Quercetin | Rutin | Quercetin | Rutin |
| 0 | 10.12 | 7.67 | 9.67 | 11.06 | 14.78 | 10.15 | 2.45 | 2.05 | 1.05 | 1.25 |
| 2 | 31.50 | 15.07 | 17.17 | 21.47 | 13.85 | 29.00 | 6.38 | 9.18 | 5.18 | 5.48 |
| 4 | 50.34 | 21.77 | 22.87 | 38.28 | 33.45 | 55.94 | 10.99 | 10.09 | 9.09 | 11.09 |
| 8 | 75.12 | 39.18 | 39.78 | 47.02 | 40.05 | 76.72 | 15.00 | 13.70 | 14.00 | 13.00 |
| 12 | 88.25 | 44.22 | 45.12 | 55.00 | 48.85 | 89.15 | 18.14 | 19.85 | 19.15 | 17.94 |
| 24 | 98.62 | 50.00 | 51.00 | 60.25 | 55.21 | 98.24 | 20.00 | 25.00 | 25.00 | 29.00 |

RESULTS AND DISCUSSION

The linearity curve was determined for Quercetin and Rutin drug at five concentration levels ranging from 1 to 5 $\mu\text{g/ml}$ and 0.1 to 0.5 $\mu\text{g/ml}$ for Quercetin and Rutin, respectively. The linearity curve shows a correlation coefficient of 0.9956 and 0.997 with regression equation of $y = 88980x - 5306$ and $y = 41670x + 333.33$ for Quercetin and Rutin, respectively. The standard addition method was used

to determine the method accuracy at three levels. The percentage recovery of Quercetin and Rutin ranged between 99.3 and 101.1 % and 101.3 to 102 % for Quercetin and Rutin, respectively. The percentage relative standard deviation of intra-day and inter-day precision results was seen below 1.41 % and 1.63 % for Quercetin and Rutin, respectively (Table 1). Detection limit and quantitation limit for Quercetin and Rutin were calculated by signal to

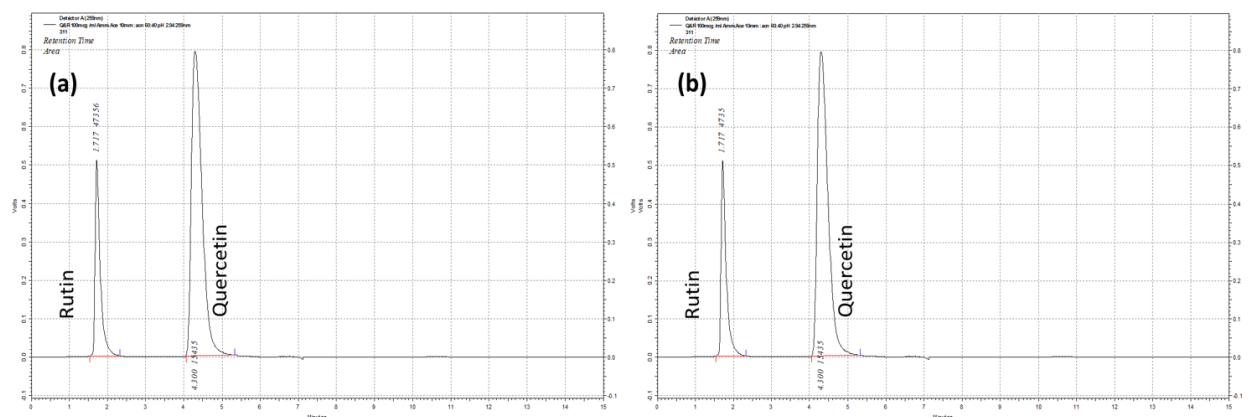


Figure 3: Typical HPLC Chromatogram of Quercetin and Rutin showing (a) acidic oxidative degradation (b)

noise ratio. The LOD for Quercetin and Rutin were obtained as 100 and 10 ng/ml, respectively, and for Quercetin due to its low detection limit, the quantification limit was 300 and 30 ng/ml for Rutin. Robustness of the present study was determined by altering the operation set up (flow rate, mobile phase ratio, and wavelength). The parameters like the mobile phase was checked by changing the ratio (65:35 and 55:65, v/v) flow rate (0.9, 1.0, and 1.1 ml/min) wavelength (264, 259, and 254 nm). The results were represented in terms of % RSD, and the obtained results were very much within the limits of less than 3.0%. The system suitability of the method was determined against parameters such as tailing factor, linearity range, and validation parameters. The obtained results were found to be within the limit indicating the suitability of the developed method (Table 2).

Degradation study

The developed and validated HPLC technique were used to determine the degradation behavior of Quercetin and Rutin. The developed method resolved the degradant and drug peaks, which enabled to identify the degradation rate of Quercetin and Rutin. The results obtained from the degradation study on Quercetin and Rutin revealed that Quercetin degradation rate increased with the addition of 0.1 N HCl (98.62 %, 24 hrs) (Figure 3). The minimal degradation rate was found with the addition of 3 % H₂O₂ (55.21 %, 24 hrs) and 0.1 N NaOH (51.00 %, 24 hrs), whereas photolytic and neutral degradation has less effect on Quercetin (< 25.00 %, 24 hrs). Nevertheless, Rutin was more susceptible to oxidative degradation with a degradation rate of 98.24 % at the end of 24 hrs (Figure 3). The basic, acidic, neutral, and photodegradation study showed moderate degradation behavior with a degradation rate of less than 60.25 % at the end of 24 hrs. The

results of stress degradation studies are summarized in Table 3.

CONCLUSIONS

A stability-indicating high-performance liquid chromatography method has been developed to determine different degradation behavior of Quercetin and Rutin under various stress situations. The results obtained stated that Quercetin and Rutin are more susceptible to acidic and oxidative conditions. It can be concluded that these findings provide an insight and information about the storage and intrinsic stability conditions of Quercetin and Rutin with respect to the advanced formulation aspects.

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