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Research Article

RP-HPLC DETERMINATION OF RUTIN AND ISOQUERCITRIN FROM LEAVES OF *JASMINUM SAMBAC* AIT

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ABSTRACT

Introduction: A reverse phase high performance liquid chromatography (HPLC) method has been developed and validated for simultaneous quantitative determination of two flavonoids viz. rutin and isoquercitrin from dried leaf powder of *Jasminum sambac* Ait. Method: The chromatographic separation was performed on a Phenomenex C18 column (250 x 4.6 mm, i.d. 5 µm) with mobile phase, comprising 0.2 % tri fluoro acetic acid in distilled water and acetonitrile (75:25 v/v). Results: The detector response was linear for concentrations ranging from 0.1µg/mL to 100.0µg/mL and 0.050µg/mL to 250.0µg/mL for rutin and isoquercitrin respectively with correlation coefficient of 0.999 for both the components. The method was precise as the value of percent relative standard deviation was found to be less than 2. The amounts of rutin and isoquercitrin in the dried leaf powder of *Jasminum sambac* Ait. were found to be 0.4959mg/g and 0.6481mg/g respectively. The accuracy of the developed HPLC method was checked by carrying out the recovery experiment at three different levels, by using standard addition method. The values of percent recovery were found to be 98.62 and 98.80 for rutin and isoquercitrin respectively. Conclusion: This developed HPLC method for simultaneous determination and quantitation of rutin and isoquercitrin present in the dried leaf powder of *Jasminum sambac* Ait. is simple, rapid, precise and can be used for routine quality control.

Keywords: High performance liquid chromatography, *Jasminum sambac* Ait., Rutin, Isoquercitrin.

INTRODUCTION

Jasminum sambac Ait. (Family- Oleaceae) is commonly known as Mogra. It is a famous fragrant plant widely cultivated in all over the world. The flowers of this plant are used in the preparation of an essential oil and for making jasmine tea[1]. In addition to this, the plant has many medicinal properties. The flowers are useful as a drug for the treatment of diarrhea, abdominal pain, conjunctivitis and dermatitis[1]. The leaves are used to heal the wounds[2]. Charak included this herb for the treatment of insanity and epilepsy[3]. Phyto-chemicals such as rutin, quercitrin, isoquercitrin, quercitrin-3-dirhamnoglycoside, and kaempherol-3-rhamnoglycosides, α-amyrin and β-sitisterol are reported to be present in its leaves[3].

Flavonoids are a group of polyphenolic compounds widely distributed throughout the plant kingdom. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens[4]. They show anti-allergic[5], anti-inflammatory[6], anti-microbial[7] and anticancer activity[6].

Rutin is reported to have anti hyperglycemic, anti hyperlipidemic and also antioxidant activity. Rutin exhibits pharmacological activities like anti-bacterial, anti-tumor, anti-inflammatory, anti-diarrheal, anti-ulcer, anti-mutagenic, immunomodulator and hepatoprotective activity[8].

Anti-malarial activity[9] and anti-inflammatory activity[10] of isoquercitrin has been reported in the literature.

In the present research work two flavonoids viz. rutin and isoquercitrin are simultaneously quantitated from dried leaf powder of *Jasminum sambac* Ait. by HPLC technique.

In literature, HPLC methods have been reported for simultaneous analysis of rutin and isoquercitrin from leaves of *Morus alba* Linn.[4], *Morus australis* Poir.[4], *Crataegus pinnatifida* Bge. var. major.[11] and *Scutia buxifolia* Reiss.[12]

However, no HPLC method is reported for the simultaneous quantitation of rutin and isoquercitrin from leaves of *Jasminum sambac* Ait.

Thus, precise and accurate HPLC method has been developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous determination and quantitation of rutin and isoquercitrin from dried leaf powder of *Jasminum sambac* Ait.

MATERIALS AND METHODS

Experimental reagents

Standard, Reagents and Chemicals

The reference standards rutin hydrate (purity 94.0% HPLC Grade) and isoquercitrin (purity 90.0% HPLC Grade) were purchased from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany). All solvents used were of HPLC grade. Acetonitrile (purity-99.8 %), trifluoro acetic acid, distilled water used were procured from LiChrosolv Merck, India.

Plant Material

The leaves of *Jasminum sambac* Ait., were collected from Keshav Srushti, Mumbai, India. Herbarium of the plant was prepared and authenticated from Botanical Survey of India (BSI), Pune, India. A duplicate herbarium was prepared and preserved in Ramnarain Ruia College. The leaves of *Jasminum sambac* Ait., were washed with water to remove soil particles, dried at 45±2º C[13], powdered and then sieved through BSS mesh size 85 and stored in an air tight container at room temperature (25 ± 2º C).

Preparation of solutions

Preparation of stock solution of rutin (1000.0 µg/mL)

About 10.30 mg of rutin hydrate was accurately weighed and transferred to 10.0 mL volumetric flask. 5.0 mL of methanol was added and the contents were sonicated in an ultrasonic bath (Model:TRANS-O-SONIC, Frequency: 50 Hz) for 5 minutes for complete dissolution of rutin. The contents were then diluted up to the mark with methanol to obtain a solution of rutin with concentration of 1000.0 µg/mL.

Preparation of stock solution of isoquercitrin (1000.0 µg/mL)

About 10.0 mg of isoquercitrin was accurately weighed and transferred to 10.0 mL volumetric flask. 5.0 mL of methanol was added and the contents were sonicated for 5 min for complete dissolution of isoquercitrin. The contents were then diluted up to the mark with methanol to obtain a solution of isoquercitrin with concentration of 1000.0 µg/mL.

Preparation of sample solution

About 1.0g of finely powdered leaves of *Jasminum sambac* Ait., was accurately weighed and transferred to a 50.0mL stoppered conical flask. 10.0mL of methanol was added to it and the flask was sonicated in an ultrasonic bath for 15 minutes. The flask was then shaken at 50.0 rpm, on a conical flask shaker overnight at room temperature (25 ± 2°C). Further, sample was filtered through Whatman filter paper no. 41. The filtrate was then finally filtered using 0.45 µm nylon filters (Millipore) before the analysis.

Preparation of mobile phase

The mobile phase used in the present research work is 0.2% trifluoroacetic acid and acetonitrile in the volume ratio of 75: 25. 0.2% trifluoroacetic was prepared by mixing 0.2 mL of trifluoroacetic acid in 100.0 mL of water. Before use, the solvents were degassed in an ultrasonic bath. The flow rate was maintained constant at 1.0 mL/min.

HPLC conditions

HPLC analysis was performed using Shimadzu UFLC Prominence chromatograph, equipped with binary gradient pump (LC-20AD), and fitted with auto sampler (SIL-20 AC HT) and oven (CTO-20 AC) set at 40º C. A reversed phase, phenomenex RP C_{18} (250mm x 4.6mm, i.d. 5µm) column was used for the chromatographic separation. The mobile phase used is 0.2% trifluoroacetic acid and acetonitrile in the volume ratio of 75: 25.The injection volume was 10µL. The detection was done using PDA detector (SPD-M20A) at λ=255nm. LCsolution chromatographic software was used for data acquisition.

METHOD VALIDATION

Linearity

Preparation of calibration curve of rutin

10 µL of each of the standard solutions of rutin, in the concentration range of 0.1µg/mL to 100.0µg/mL, were injected in chromatographic system under optimized chromatographic conditions. The chromatograms were recorded

and peak areas of rutin for each injected concentration of rutin were recorded.

Preparation of calibration curve of isoquercitrin

10 µL of each of the standard solutions of isoquercitrin, in the concentration range of 0.05µg/mL to 250.0µg/mL, were injected in chromatographic system under optimized chromatographic conditions. The chromatograms were recorded and peak areas of isoquercitrin for each injected concentration of isoquercitrin were recorded.

Each concentration of rutin and isoquercitrin were injected in triplicate, under the specified chromatographic conditions described above. The chromatograms were then acquired and the peak areas were recorded for each injected concentration of both rutin and isoquercitrin. The calibration curves of both rutin and isoquercitrin were obtained by plotting graphs of mean peak areas vs. corresponding concentrations. The results listed in Table 1. For both the standards, within the concentration range indicated, there was a good correlation between mean peak area and concentration of standards.

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

The limit of detection (LOD) and limit of quantitation (LOQ) were determined at signal to noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ values obtained for both the components are listed in Table 1.

System Suitability

System suitability was carried out to verify that resolution and reproducibility of the system were acceptable for the analysis.

10 µL of standard solutions of rutin and isoquercitrin of concentrations 50.0 µg/mL each were injected in the chromatographic system in six replicates under specified conditions. The chromatograms were recorded. The values of percent relative standard deviations of peak area and retention time of standards were taken as an indicator of system suitability and are less than 2, indicating that the method is suitable for analysis.

Precision

The method was validated in terms of repeatability and intermediate precision.

The repeatability was evaluated by triplicate analysis of three sample solutions i.e. 10 µL of methanolic extract of the dried leaf powder of *Jasminum sambac* Ait., was injected in the chromatographic system in triplicate on the same day in the same laboratory under the specified chromatographic conditions. The peak areas of rutin and isoquercitrin were recorded.

The Intermediate precision of the method was evaluated by analyzing the sample solution in triplicate on three different days, in the chromatographic system, under the specified chromatographic conditions. The peak areas of rutin and isoquercitrin were recorded.

The precision results were expressed as percentage relative standard deviations of peak areas of rutin and isoquercitrin and are listed in Table 1. The results indicate that the proposed method is precise and reproducible.

Specificity

The specificity of the proposed HPLC method was ascertained by injecting 10.0µL of blank solution to observe for interference, if any, with the peaks of interest in the chromatogram of the sample solution. It was observed that there is no interference from the blank solution. Methanol was taken as blank solution since standard and sample solutions were prepared in methanol.

Solution Stability

The stabilities of standard rutin and isoquercitrin solution were determined by comparing the peak areas of rutin and isoquercitrin solution, of concentration 50.0µg/mL each, at different time intervals, for a period of minimum 48 hrs, at room temperature. The results showed that the peak areas of rutin and isoquercitrin almost remained unchanged (values of percent relative standard deviation were less than 2) over a period of 48 hrs, and no significant degradation was observed within the given period, indicating the stability of standard solutions of rutin and isoquercitrin for minimum 48 hrs.

Robustness

Robustness tests examine the effect of the operational parameters on the analysis results. By introducing small changes in the mobile phase composition, the effects on the results were examined.

The mobile phase composition was altered to 0.2% trifluoroacetic acid: acetonitrile (73.0:27.0 v/v) and 0.2% trifluoroacetic acid: acetonitrile (77.0:23.0 v/v) and flow rate was changed to 0.9 mL /min and 1.1 mL /min.

The resolution between rutin and isoquercitrin and tailing factors of both components in sample solution did not change much due to alteration in the methods.

The amounts of rutin and isoquercitrin from dried leaf powder of *Jasminum sambac* Ait. obtained by altered method and that obtained by normal method was found to be almost similar. The modifications did not affect the system suitability criteria. However, slight variation in the retention time was observed, which was due to changes made in the mobile phase composition and flow rate. From the above observations, it was concluded that the method is robust as the above mentioned deliberate changes made the method did not affect the results.

Assay procedure

The developed and validated HPLC method was used for quantitation of rutin and isoquercitrin from the methanolic extract of dried leaf powder of *Jasminum sambac* Ait. 10µL of methanolic extract of the dried leaf powder of *Jasminum sambac* Ait., was injected (n=7) under the specified chromatographic conditions. The chromatograms were recorded. Amounts of rutin and isoquercitrin present in the sample solution were determined from the calibration curve, by using the peak area of rutin and isoquercitrin in the sample. Mean contents of rutin and isoquercitrin in methanolic extract of the dried leaf powder of *Jasminum sambac* Ait., are found to be 0.4959mg/g and 0.6481mg/g respectively.

Accuracy

The accuracy of the method was established by performing recovery experiment by using standard addition method at three different levels. To accurately weighed 1.0g of dried leaf powder of *Jasminum sambac* Ait., known amounts of standard rutin and isoquercitrin i.e. 0.1mg, 0.2mg, 0.3mg were added, and extracted using methanol. Each of the three different levels containing sample solution and standard was injected in seven replicates; under the specified chromatographic conditions, as described above. The rutin and isoquercitrin contents were quantified by the proposed method and the percentage recovery was calculated. The values of percent recoveries obtained were 98.62 and 98.80 for rutin and isoquercitrin respectively. The results of accuracy are listed in Table 2.

RESULTS

Different mobile phases were tried for simultaneous HPLC separation of rutin and isoquercitrin from other components of the dried leaf powder of *Jasminum sambac* Ait. and good separation was achieved by using 0.2% tri fluoro acetic acid : acetonitrile(75:25 v/v) as mobile phase. Detection was carried out at λ = 255 nm as both rutin and isoquercitrin showed maximum response at this wavelength. The identity of the peaks of rutin and isoquercitrin in the sample solutions was confirmed by comparing their retention times in sample with that of reference standards. The retention time for rutin and isoquercitrin were 4.543 minutes and 5.527 minutes respectively. Figure 1 shows typical HPLC chromatograms of standard rutin, standard isoquercitrin and Figure 2 shows chromatographic separation of rutin and isoquercitrin in methanolic extract of dried leaf powder of *Jasminum sambac* Ait. The developed method provided a good separation of the phyto constituents with the resolution (Rs) of 4.565 whereas the tailing factors are 1.564 and 1.519 for rutin and isoquercitrin respectively. The resolution and tailing factor values lies between the acceptable limits.

A good linear relationship was observed for rutin and isoquercitrin in the concentration in the range of 0.1 µg/mL to 100.0 µg/mL and 0.05 µg/mL to 250.0µg/mL respectively, with correlation coefficient of 0.999 for both the components (Table 1). When the method was validated for instrumental precision, repeatability and intermediate precision, the values of percentage relative standard deviations were less than 2, indicating the proposed method is precise and repeatable (Table 1). The mean amounts of rutin and isoquercitrin from the methanolic extract of dried leaf powder of *Jasminum sambac* Ait. were found to be 0.4959mg/g and 0.6481mg/g respectively. The values of percent recoveries of rutin and isoquercitrin at three levels were 98.62 and 98.80 respectively indicating accuracy of the method (Table 2)

DISCUSSION

A reverse phase HPLC method has been reported for simultaneous quantitation of rutin and isoquercitrin from leaves of *Morus alba* Linn.[4], and *Morus australis* Poir.[4] using Cosmosil C8 column (150 x 4.6 mm, 5 µm) with an isocratic mobile phase, comprising 0.1 % formic acid in distilled water, acetonitrile and methanol (75:15:10 v/v/v) at a flow rate of 1.0 mL/min. Detection was carried out at λ=259nm. The retention times for rutin and isoquercitrin were 6.77 minutes and 8.41 minutes respectively.

Rutin and isoquercitrin were also simultaneously determined from leaves of *Crataegus pinnatifida* Bge. Var. major[11]. The compounds were determined at room temperature on an analytical column Diamonsil C18, (150 × 4.6 mm, i.d., 5µm). The

mobile phase consisted of the solvent (A) acetonitrile–tetrahydrofuran (95:5, v/v) and (B) 1% aqueous phosphoric acid (v/v) using a gradient elution with flow rate of 1 mL/min. The detection was carried out λ=270 nm. The retention times for rutin and isoquercitrin were 22.4 and 25.6 minutes respectively.

Simultaneous quantitation of rutin and isoquercitrin, from leaves of *Scutia buxifolia* Reiss.[12], Was carried out on RP-C18 column (4.6mm x 250 mm, i.d.5µm). The mobile phase was methanol-acetonitrile-water (40:15:45, v/v/v) containing 1.0% acetic acid at 1mL/min flow rate. The detection was carried out at λ=257 nm. The retention times for rutin and isoquercitrin were 4.9 and 5.2 minutes respectively.

The mobile phase selected for the present research study is 0.2% TFA: ACN (75:25 v/v). Rutin and isoquercitrin are strongly polar compounds[14] hence mobile phase comprising of more amount of water which is more polar as compared to acetonitrile was used. The addition of 0.2% TFA to mobile phase helped to improve the peak shapes of rutin and isoquercitrin. The selected mobile phase in the present research work is advantageous as compared to the reported mobile phases. In all the methods reported above, three solvent systems have been used as the mobile phase but in the developed method only two solvents are used. In the present research work, the retention times of rutin and isoquercitrin are less as compared to reported retention times. The developed HPLC method is cheap and fast as compared to the HPLC methods reported in the literature.

CONCLUSION

The developed HPLC technique is precise, specific and accurate and can be used for the routine quality control analysis and simultaneous quantitative determination of rutin and isoquercitrin from the dried leaf powder of *Jasminum sambac* Ait.

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Table 1: Method validation data for simultaneous quantitation of rutin and isoquercitrin

Parameters	Results	
	Rutin	Isoquercitrin
Linear range (n=3) (µg/mL)	0.1-100	0.05-250.0
Correlation coefficient (r)	0.999	0.999
LOD (µg/mL)	0.050	0.015
LOQ (µg/mL)	0.10	0.50
System suitability (n=6)	Less than 2	Less than 2
% R.S.D. for repeatability (n=3) (on the same day)	1.22	1.16
% R.S.D. for intermediate precision (n=9) (for three successive days)	1.18	1.10

Table 2: Results of recovery study for simultaneous HPLC quantitation of rutin and isoquercitrin from methanolic extract of dried leaf powder of *Jasminum sambac* Ait.

Level	Amount of sample (g)	Amount of standard added to sample (mg)	*Mean amount of standard found (mg)	Percent recovery
Rutin				
0	1.002	0.0000	0.4957±0.00541	98.62
1	1.001	0.1000	0.6113±0.00689	
2	1.002	0.2000	0.7071±0.00697	
3	1.001	0.3000	0.7925±0.00474	
Isoquercitrin				
0	1.002	0.0000	0.6430± 0.00673	98.80
1	1.001	0.1000	0.7448± 0.00608	
2	1.002	0.2000	0.8421± 0.00811	
3	1.001	0.3000	0.9399± 0.00618	

*Average ± S.D. (n=7)

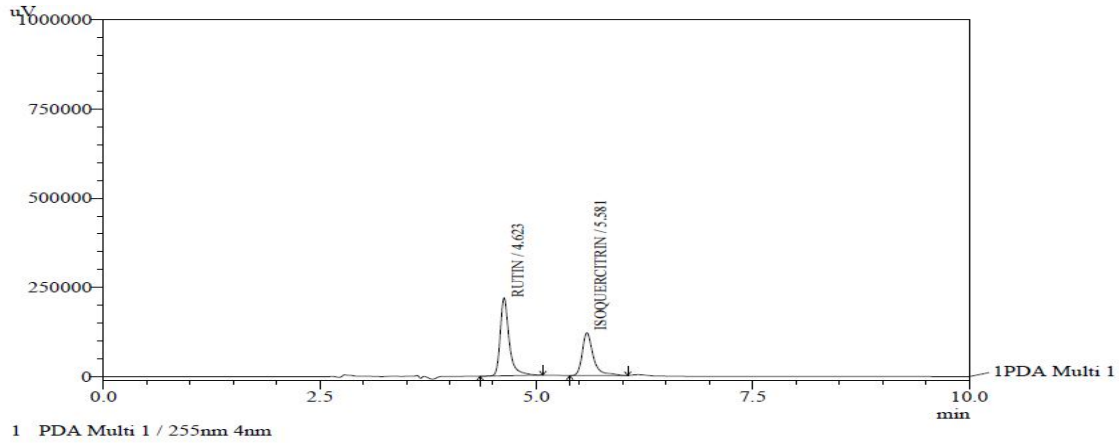

Fig. 1: Typical HPLC chromatogram of standard rutin and isoquercitrin

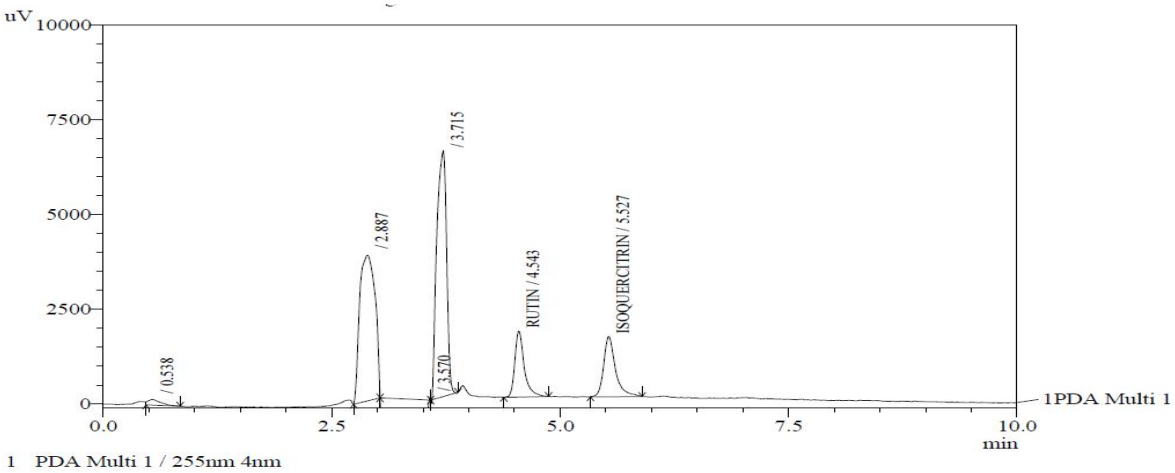

Fig. 2: Typical HPLC chromatogram of rutin and isoquercitrin in methanolic extract of dried leaf powder of *Jasminum sambac* Ait

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