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Development and validation of UV spectrophotometric method for simultaneous estimation of rutin and quercetin in niosome formulation

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ABSTRACT

A simple, rapid, accurate, precise, and economic spectrophotometric method for simultaneous estimation of Rutin and Quercetin based on solving simultaneous equation in niosome formulation has been developed. As Rutin and Quercetin show absorbance maximum at 257 and 372 nm respectively, absorbance was measured at these wavelengths for estimation of Rutin and Quercetin respectively. Both drugs, Rutin and Quercetin obey the Beer-Lambert's law in the concentration ranges of 2-20 µg/ml. Method was developed and validated according to ICH guidelines and can be adopted for the routine estimation of Rutin and Quercetin niosome formulation.

Key words: Rutin, Quercetin, Simultaneous equation, U.V. Spectrophotometer, Validation.

INTRODUCTION

Numerous epidemiological studies suggest a protective role of dietary flavonoids against Cerebral ischemic disease. Flavonoids possess a wide variety of potential cerebroprotectivebeneficial effects like antioxidant, anti-inflammatory, anti-platelet aggregatory activities and theycan also restore endothelial function, prevent neutrophil accumulation and LDL oxidation. Many of them proved to be antithrombogenic. They also have been shown regulatory activity on certain Hormones and enzymes [1] .Quercetin and Rutin are members of the class of flavonoids termed asflavonols. They are widely distributed in the plant kingdom. Quercetin is found abundantly in redwine, green tea, onions, berries, citrus fruits, parsley, apples, and garlic. Rutin is abundantly found in black wheat and also in apple peels, garlic, tomatoes, and black tea. Commercially, quercetin is derived from blue-green algae (VITAMIN Retailer).Bioflavonoid quercetin and rutin were evaluated for their cerebroprotective role in experimental ischemia reperfusion induced cerebral infarction in rats. They show the good cerebroprotective activity [2].Literature survey revealed that methods such as U.V. [3-4], HPTLC [5], determination of quercetin, have been reported for estimation of quercetin. Forrutin, U.V.[6],HPLC and TLC [7]method reported, for simultaneous estimation of rutin and quercetin HPTLC[8-10],HPLC[11] ,RP-UFLC[12] methods are reported .not a single U.V. method reported for simultaneous estimation of Rutin and Quercetin in formulation. Due to wide range of therapeutic benefits it is necessary to develop the method for estimation of both drugs in formulation. Present research work is done to develop and validate a simple UV spectroscopy based methods using simultaneous equation for rapid, accurate and precise estimation of Rutin and Quercetin in single formulation.

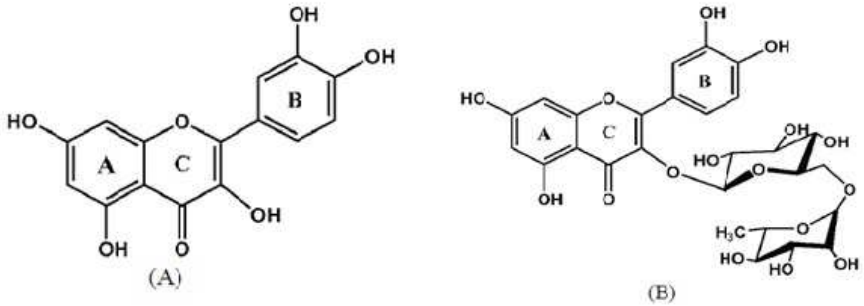

Figure 1Structure of (A) Quercetin and (B) Rutin

MATERIALS AND METHODS

2.1 Apparatus

A double beam UV-spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV Probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, digital balance (Mettler Toledo, AB265-S/FACT, Switzerland), ultrasonicator (Steryl 40050, Mumbai, India), volumetric flasks and pipettes of borosilicate glass were used for the development and validation of proposed analytical method.

2.2 Material

Rutin was purchased from Otto Chemie Pvt.Ltd (Mumbai, Maharashtra), Quercetin (dihydrate) purchased from sigma Aldrich Pvt.Ltd. All the chemicals and reagents were of analytical grade and were purchased from S.D fine, Mumbai.

2.3 Procedures

2.3.1 Preparation of standard stock solution and calibration curve

The standard stock solution of Rutin and quercetin were prepared by dissolving 10mg of each drug in methanol, and the final volume was adjusted with the same solvent in 100 ml of volumetric flask to get a solution containing 100µg/ml of each drug. Working standard solution of 10 µg/ml was scanned in the entire UV range of 400–200 nm to determine the λ_{max}. Calibration curves as concentration vs. Absorbance were constructed to study the Beer-Lambert's Law and regression equations for Rutin and quercetin respectively.

2.3.2 Simultaneous equation method

From the overlain spectra (**Fig 2**) of rutin (10 µg/ml) and quercetin (10 µg/ml), two wavelengths i.e. 257 nm as λ_{max} of rutin and 372 nm as λ_{max} of quercetin were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 257 nm and 372 nm. A set of two simultaneous equations were formed using absorptivity values as given in equation (1) and (2), at selected wavelengths. The concentrations of two drugs in niosome formulation were calculated using set of two simultaneous equations. [13-14]

$$Cx = \frac{A_2\,ay_1 - A_1 ay_2}{ax_2\,ay_1 - ax_1\,ay_2} \qquad (1)$$

$$Cy = \frac{A_1\,ax_2 - A_2\,ax_1}{ax_2\,ay_1 - ax_1\,ay_2} \qquad (2)$$

Where; Cx and Cy are concentrations of rutin and quercetin (µg/ml) respectively in known sample solution. A_1 and A_2 are absorbance of sample solutions at 257 nm and 372 nm respectively. ax_1 and ax_2 are absorptivity of rutin at 257 nm and 372 nm, ay_1 and ay_2 are absorptivity of quercetin at 257 nm and 372 nm. The concentration of Cx and Cy in niosome formulation can be obtained by solving equation (1) and (2). Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components

2.3.3 Analysis of the niosome formulation

Niosome solution (1 ml) containing equivalent to 10 mg of both drugs was transferred to 50 ml volumetric flask and excipient extracted with chloroform. Then drug dissolved in methanol. The sample solution was then filtered through Whatman filter paper. This solution was appropriately diluted to get approximate concentration of 20 µg/ml of rutin and quercetin, each, the absorbance of sample solution were measured at 257 nm and 372 nm against blank.

2.4 VALIDATION OF THE DEVELOPED METHOD [15-19]

2.4.1 Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range was found to be 2-20 µg/ml for Rutin and 2-20 µg/ml for quercetin. The linearity data for method is presented in **Table1.**

2.4.2 Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the testconcentration as per ICH guidelines. The recovery study was performed three times at each level. The result ofthe recovery studies are reported in **Table 3.**

2.4.3 Precision:

Interday and Intraday precision

The Interday and intraday precision was determined by assay of the sample solution on the same day and ondifferent days at different time intervals respectively (six replicates). **The results of the same are presented inTable 2.**

2.4.4 Ruggedness study:

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by spiking the standard 3 times with different analyst by using same equipment. The results of the same are presented **in Table 5.**

2.4.5 Limit of detection

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$\mathbf{DL} = \frac{3.3\sigma}{S}$$

Where σ =the standard deviation of the response
S =the slope of the calibration curve

2.4.6 Limit of quantitation

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyteand by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

$$\mathbf{QL} = \frac{10\sigma}{S}$$

Where σ =the standard deviation of the response
S = the slope of the calibration curve

RESULTS AND DISCUSSION

Linearity range for rutin and quercetin are2-20 µg/ml and 2-20 µg/ml at respective selected wavelengths. The coefficient of correlation for Rutin at 257 nm and for quercetin at 372 nm is 0.997 and 0.992 respectively. Both drugs shows good regression values at their respective wavelengths and the results of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation of Rutin and quercetin in niosomal formulation was found by method is 14.21±1.156and 15.50±1.360 ± standard deviation with standard deviation <2.

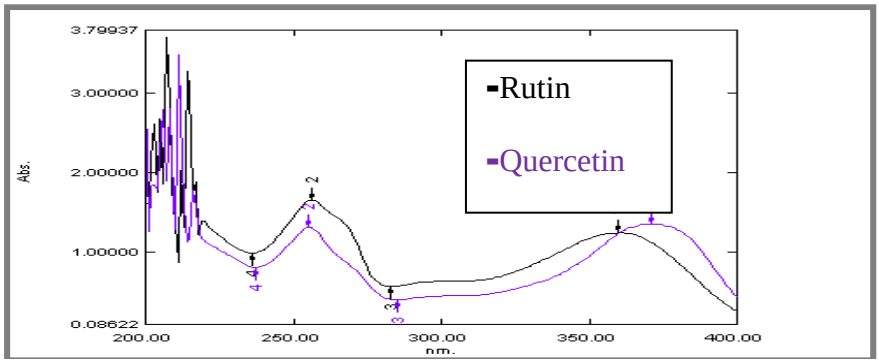

Figure 2Overlay of maximum absorption of Rutin and Quercetin

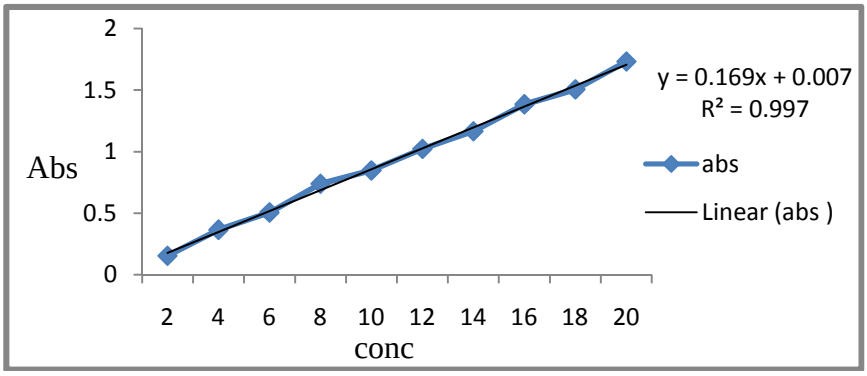

Figure 3calibration curve of Rutin

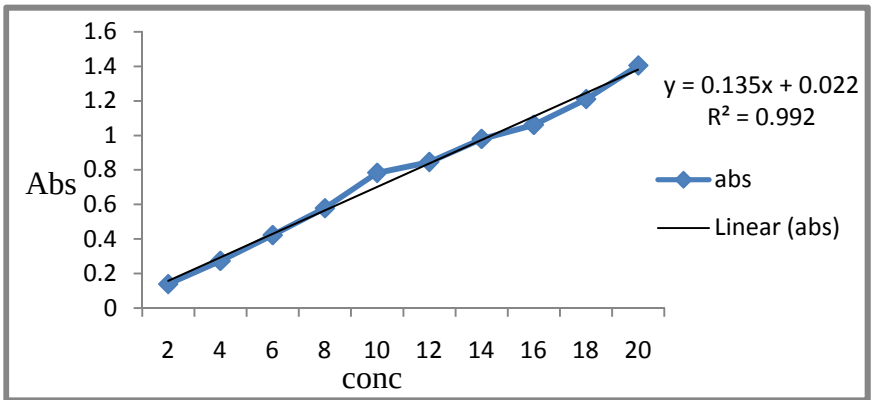

Figure 4Calibration curve of Quercetin

Table 1Result of validation parameters

Parameters	Rutin	Quercetin
λmax	257.0 nm	372.0 nm
Linearity range	2-20 µg/ml	2-20 µg/ml
Linearity equation	Y=0.169x+0.007	Y=0.135x+0.022
R^2	0.997	0.992
Slope	0.169	0.135
LOD	0.0274 µg/ml	0.1435 µg/ml
LOQ	0.0832 µg/ml	0.4349 µg/ml

Precision is determined by studying the Interday and intraday precision. In both intra and inter day Precision study for both the methods % RSD are not more than 2.0% indicates good repeatability and Intermediate precision (Table 2).

Table 2 Interday and Intraday precision

	Interday precision		Intraday precision	
	%Amount found±SD*	**% RSD**	**%Amount found± SD***	**% RSD**
RUTIN	97.12±0.0088	0.0090	93.45±0.0162	0.0174
QUERCETIN	106.68±0.0079	0.0068	102.45±0.018	0.016

**Average of six determinations*

The validity and reliability of proposed methods are assessed by recovery studies , analysis of formulation and ruggedness study. Sample recovery for both the methods is in good agreement, which suggest noninterference of other content in estimations (Table 3).Also reproducibility of results from formulation and its ruggedness was found to be in range(Table 4 and 5).

Table 3 Recovery studies

Concentration of the drug added to the formulation	RUTIN % Recovery ±SD*	%RSD	Quercetin % Recovery ± SD*	%RSD
80%	99.07±0.005	0.116	108.88±0.03	0.050
100%	107.7±0.069	0.117	106.31±0.083	0.143
120%	104.02±0.055	0.107	103.24±0.630	0.496

**Average of three determinations*

Table 4 Result of analysis of formulation

Formulation	Drug	Amount Found µg/ml ± S.D*
Niosome formulation	Rutin	14.21±1.156
	Quercetin	15.50±1.360

**Average of three determinations*

Table 5 Ruggedness study

Niosome formulation	Drug	%Amount found ± S.D*
Analyst 1	Rutin	98.33±0.024
	Quercetin	107.4±0.038
Analyst 2	Rutin	93.03±0.023
	Quercetin	104.63±0.045

CONCLUSION

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for simultaneous estimation of Rutin and Quercetin in niosome formulation.

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