

ORIGINAL RESEARCH ARTICLE

Simultaneous Estimation of Quercetin and Silymarin: Method Development and Validation

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ABSTRACT

A simple, rapid, accurate, precise, and economic spectrophotometric method for simultaneous estimation of quercetin and silymarin based on solving simultaneous equation in topical formulation has been developed. As quercetin and silymarin show absorbance maximum at 256 and 288 nm respectively, absorbance was measured at these wavelengths for estimation of quercetin and silymarin respectively. Both drugs, quercetin and silymarin obey the Beer- Lambert's law in the concentration ranges of 2-10 µg/ml and 8-16 µg/ml respectively. Method was developed and validated according to ICH guidelines and can be adopted for the routine estimation of quercetin and silymarin in topical formulation.

Key words: ICH guidelines, Quercetin, Silymarin, U.V. Spectrophotometer, Validation.

INTRODUCTION

Quercetin, chemically 2-(3,4-dihydroxy phenyl)-3,5,7- trihydroxy-4H-chromen-4-on, is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It is used as an ingredient of supplements, beverages and foods. Quercetin is frequently used therapeutically in allergic conditions, including asthma, hay-fever, diabetes, peptic ulcer, schizophrenia, cataract, eczema and hives. Additional clinical uses include treatment of gout, pancreatitis and prostatitis and other inflammatory conditions. It is also used for treating conditions of the heart and blood vessels including “hardening of the arteries” (atherosclerosis), high cholesterol, heart diseases, circulation problems, chronic fatigue syndrome (CFS), cancer and for treating chronic infections of the prostate. Quercetin is also used to increase endurance and improve athletic performance [1]. On the other hand silymarin is a group of flavonoid compounds obtained from *Silybummarianum*, as chemically defined mixture of three isomers, silibinin (major isomer), silycristin and silydianin. The drug is a unique hepatoprotective agent that has a positive effect on metabolism and physiology of liver cells, influencing their regenerative capacity due to two main actions: antioxidant and protein restoring activities. The drug also prevents toxic and foreign substances from penetrating liver cells by

stabilizing the outer membranes of liver cells [2]. The chemical structures of quercetin and silymarin are shown in [Fig 1(a,b)] respectively.

Literature survey revealed that several methods such as U.V. [3-5], HPLC [6,7], HPTLC [8,9], and electrochemical determination of quercetin [10], have been reported for estimation of quercetin. For silymarin, direct spectrometric assay, colorimetric estimation, in body fluids by TLC with fluorometric detection and HPLC with UV detection [11-14] estimation are reported. Not a single U.V., HPLC or HPTLC method is reported so far for simultaneous analysis of quercetin and silymarin in topical formulations. Due to wide range of therapeutic benefits and well established antioxidant potential, both quercetin and silymarin are few of the widely used compounds in a large number of herbal products specially topical preparations. But simultaneous estimation of these compounds still remains a challenge as no such analytical method is reported till date. 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 quercetin and silymarin in single formulation.

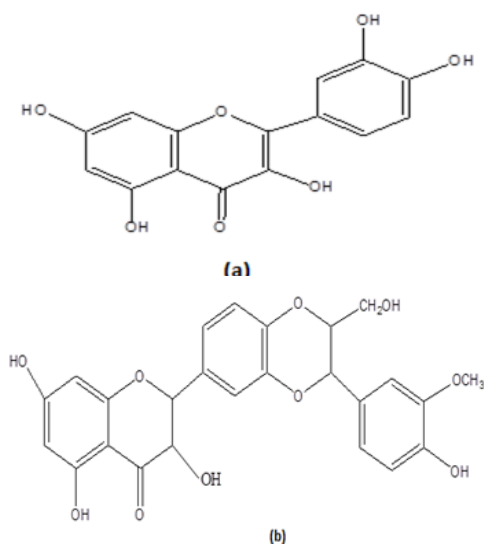


Fig 1: Chemical structure of (a) quercetin (b) silymarin

MATERIALS AND METHODS

Apparatus

A double beam UV-visible 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.

Reagents and chemicals

Quercetin and silymarin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals of pharmaceutical/analytical grades were used for the study. Mili Q water was used for all experiments.

Procedures

Preparation of standard stock solution and calibration curve

The standard stock solutions of quercetin and silymarin were prepared by dissolving 0.01 g of each drug in methanol, and the final volume was adjusted with the same solvent in 100 ml of a volumetric flask to get a solution containing 100 µg/ml of each drug. Working standard solutions of 10 µg/ml were 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 quercetin and silymarin respectively.

Simultaneous equation method

From the overlain spectra (Fig 2) of quercetin (10 µg/ml) and silymarin (10 µg/ml), two wavelengths

i.e. 256 nm as λ_{\max} of quercetin and 288 nm as λ_{\max} of silymarin were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 256 nm and 288 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 mixture were calculated using set of two simultaneous equations [15].

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{A_{x2} a_{y1} - a_{x1} a_{y2}} \quad (1)$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{A_{x2} a_{y1} - a_{x1} a_{y2}} \quad (2)$$

Where; C_x and C_y are concentrations of quercetin and silymarinin (µg/ml) respectively in known sample solution. A_1 and A_2 are absorbances of sample solutions at 256 nm and 288 nm respectively. a_{x1} and a_{x2} are absorptivity of quercetin at 256 nm and 288 nm, a_{y1} and a_{y2} are absorptivity of silymarin at 256 nm and 288 nm. The concentration of C_x and C_y in topical 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.

Analysis of the topical preparation

Accurately weighed quantity of lotion (50 mg) was transferred to 50 ml volumetric flask and dissolved in methanol, final volume was then made up with methanol. The sample solution was then filtered through Whatman filter paper. This solution was appropriately diluted to get approximate concentration of µg/ml of quercetin and silymarin, each, the absorbances of sample solution were measured at 256 nm and 288 nm against blank.

VALIDATION OF THE DEVELOPED METHOD [16,17]:

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 2-10 µg/ml for quercetin and 8-16 µg/ml for silymarin.

Limit of detection and limit of quantization

LOD and LOQ of the drug were derived by calculating the signal-to-noise ratio (S/N, 3.3 for

LOD and 10 for LOQ) using the following equation designated by ICH guidelines. The residual standard deviation of regression line or standard deviation of Y intercept of regression lines was used to calculate LOD and LOQ.

$$\text{LOD} = 3.3 \times (D/S) \dots\dots\dots(3)$$

$$\text{LOQ} = 10 \times (D/S) \dots\dots\dots(4)$$

Where; D=Standard deviation of y intercept on regression lines and S =Slop of calibration curve.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80%, 100% and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level.

Precision:

Interday and Intraday precision

The intraday precisions were determined by estimating the corresponding response 3 times on the same day for quercetin and silymarin; whereas the interday precision were determined by estimating the corresponding response on 3 different days over a period of 1 week. The results were reported in terms of relative standard deviation (RSD).

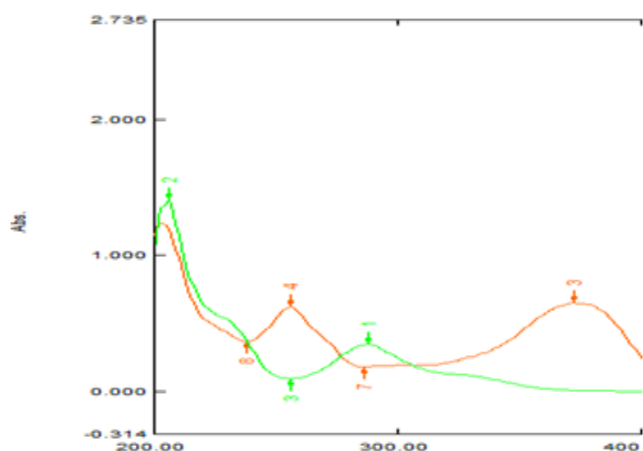


Fig 2: Overlay of maximum absorption of quercetin and silymarin on U.V. spectrophotometer

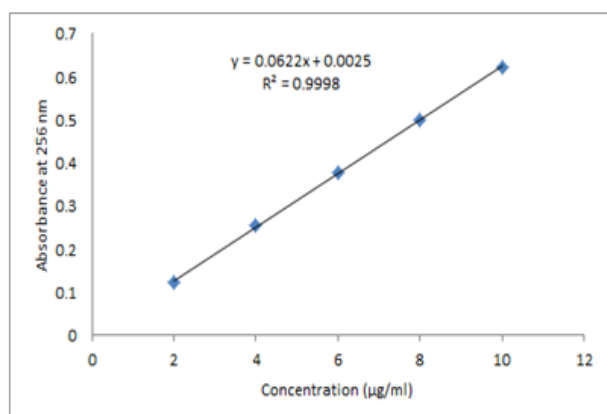


Fig 3: Calibration curve of quercetin

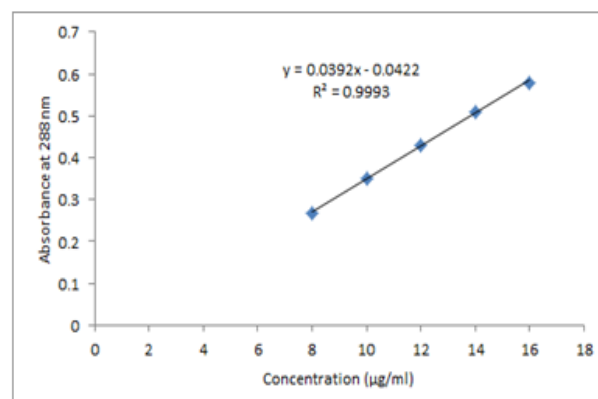


Fig 4: Calibration curve of silymarin

Table 1: Validation parameter for quercetin and silymarin estimation

Parameter	Quercetin	Silymarin
λ_{max}	256.0 nm	288.0 nm
Linearity equation	$y = 0.0622x + 0.0025$	$y = 0.0392x + 0.0422$
Slope	0.0622	0.0392
R^2	0.9998	0.9993
LOD	0.0535 $\mu\text{g/ml}$	0.0397 $\mu\text{g/ml}$
LOQ	0.1621 $\mu\text{g/ml}$	0.0397 $\mu\text{g/ml}$
Intraday precision (%RSD)	0.877 \pm 0.0034	0.878 \pm 0.0043
Interday precision (%RSD)	0.993 \pm 0.0054	1.156 \pm 0.0068

Table 2: Recovery study of quercetin and silymarin

Concentration variation	Concentration prepared ($\mu\text{g/ml}$)		% Recovery (w/w)	
	Quercetin	Silymarin	Quercetin	Silymarin
80%	8	8	98.13	97.89
100%	10	10	96.10	97.82
120%	12	12	98.99	97.51
	Mean recovery		97.32	97.69
	\pm S.D		1.47	0.197

RESULTS AND DISCUSSION

Linearity range for quercetin and silymarin were 2-10 $\mu\text{g/ml}$ and 8-16 $\mu\text{g/ml}$ at respective selected wavelengths i.e 256 and 288 nm. The coefficients of correlation for quercetin at 256 nm and for silymarin at 288 nm were 0.9998 and 0.9993 respectively (Fig 3 & 4). Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method. Percentage estimation of quercetin and silymarin in topical formulation was found to be 99.08 \pm 0.13 and 99.52 \pm 0.47 with standard deviation <2. The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the compounds was in good agreement, which suggested no interference of other extracted content in estimations. Precision was determined by studying the interday and intraday precision. In both intra and inter day precision study for both the drugs % RSD were not more than 2.0%, indicates good repeatability and intermediate precision (Table 1 & 2).

CONCLUSION

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated as per ICH guidelines in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for simultaneous estimation of quercetin and silymarin in topical formulation.

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