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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CAFFEINE AND QUERCETIN IN NIOSOME FORMULATION

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***Abstract*:** A new simple, rapid and precise UV spectrophotometric method was developed and validated for the simultaneous estimation of Caffeine and Quercetin. The method involved estimation of Caffeine and Quercetin by simultaneous equation at 273nm and 372nm respectively in their solution in methanol. The Beer's law obeyed in the concentration range of 2-10 µg/ml for both Caffeine and Quercetin respectively. This method was validated with respect to linearity, accuracy, precision, Limit of detection and quantification as per ICH norms. This method was found to be precise as %RSD was less than 2. Thus, the proposed method was found to be rapid, specific, precise and accurate for the routine analysis of Caffeine and Quercetin in niosomal formulation.

Index Terms: Caffeine, Quercetin, Simultaneous Method Development, UV Spectroscopy.

1. Introduction

Caffeine is a well-known central nervous system stimulant, and the most widely consumed psychoactive substance in the world **[1].** Caffeine is a methylxanthine derivative which has various pharmacological activities, such as blockade of adenosine receptors, inhibition of phosphodiesterase, inhibition of 5-nucleotidase, and modulation of intracellular calcium movement, which may affect neuronal functions **[2].** Caffeine seems to exert its effects directly on the central nervous system by blocking A1 and A2A adenosine receptors. It is a relatively nonspecific adenosine antagonist but seems to have the greatest affinity for A1 receptors **[3].** Quercetin is found in vegetables and fruits, especially onions and apples, typically in the form of various glycosides **[4].** The phenolic flavonoid quercetin has a high In vitro affinity for blocking same A1, and perhaps A2a, adenosine receptor as caffeine, so it has been suggested that it may have In vivo effects similar to those caffeine **[5].** Quercetin show poor water solubility, systematic bioavailability and therapeutic efficiencies. And also have high first pass metabolism and limited absorption **[6].**

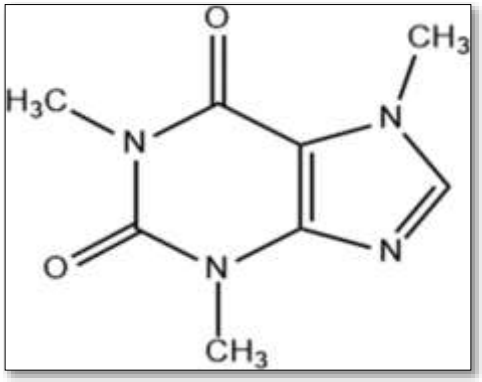

Figure 1: Caffeine **[7]**

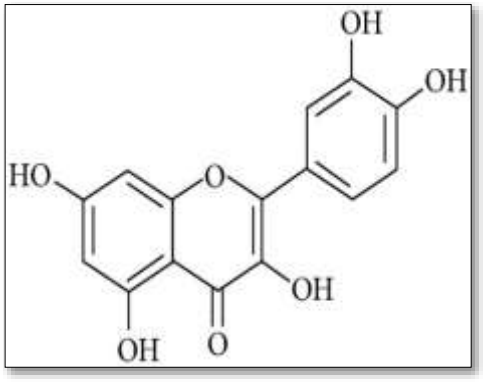

Figure 2: Quercetin **[8]**

Literature review revealed that there are various methods available for the estimation of caffeine, individually or with other drugs using UV-spectroscopy **[9; 10]**, Reverse phase High performance liquid chromatography **[11; 12],** High performance liquid chromatography **[13]** and LC-MS **[14]**. Similarly, quercetin was estimated by UV-spectroscopy **[15],** RP-HPLC **[16],** HPLC **[17]** and LC-MS **[18].** Hence from literature review it is clear that there is not a single UV method is reported so far for simultaneous analysis of Caffeine and Quercetin in niosomal formulations. Present research work is done to develop a precise, linear, simple, rapid, validated and cost effective UV-spectroscopic method for the estimation of caffeine and quercetin in a single formulation.

2. Materials and methods:

Apparatus:

A double beam UV-spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UVProbe2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, digital balance (Radwag.AS220/C/2 LC/GC), Ultrasonicator (Steryl 40050, Mumbai, India), volumetric flasks and pipettes of borosilicate glass were used for the development and validation of proposed analytical method.

Material and Reagents

Caffeine was purchased from Vijay Chemicals Pvt. Ltd (Pune) and Quercetin was purchased from Otto Chemie Pvt. Ltd (Mumbai, Maharashtra). All the reagents used in this assay were of analytical grade.

Selection of Solvent:

The solubility of drugs was determined in a variety of solvents as per Indian Pharmacopoeia standards for selection of common solvent. Solubility was carried out in polar to nonpolar solvents. The common solvent was found to be methanol which was used for the analysis of both Caffeine and Quercetin for the proposed method.

DETERMINATION of λmax

Preparation of Stock Solution

The solution was prepared by dissolving 10 mg Caffeine and Quercetin in 100ml methanol which gives 100µg/ml respectively. The UV spectrum was recorded using UV visible double beam spectrophotometer in value range of 400-200nm using methanol as blank.

Preparation of Working Solution

From the above stock solution 1ml of each drug solution was transferred into 10ml volumetric flask and volume was made up to the mark with methanol to make 10µg/ml. Then the sample was scanned with UV-Vis Spectrophotometer in the range 200-400nm against methanol as blank and the wavelength corresponding to maximum absorbance was noted which is its λ-max i.e. at 273nm and 372nm for Caffeine and Quercetin respectively.

Preparation of Calibration Curve:

The standard stock solution of Caffeine and Quercetin were prepared by dissolving 10mg of each drug in methanol, and the final volume was adjusted with the same solvent in 100ml of volumetric flask to get a solution containing 100µg/ml of each drug. Working standard solution (2, 4, 6, 8 and 10 µg/ml for each drug) were prepared and scanned in the entire UV range of 400-200nm. Calibration curve as concentration v/s absorbance were constructed taking concentration on x-axis and absorbance on y-axis which showed a straight line. This straight line obeyed linearity in the concentration range of 2-10µg/ml of both drugs. The correlation coefficient was found to be 0.993 for Caffeine and 0.9964 for Quercetin.

Simultaneous Equation Method:

Simultaneous estimation of drug is very important method as the new combined formulation approved in market. The main aim behind the quantitative estimation is to ensure that whether a particular drug contains the same amount of drug as mentioned because if the dose given will be high then it will cause over dosage side effects and if it is less, then the patient will not get the required dose **[19].** When combination of drug contains two drug or more than two drugs in combined dosage form then the simultaneous equation or Vierordt's method were applied to that formulation **[20].**

From the overlain spectra (**Figure 3**) of Caffeine (10µg/ml) and Quercetin (10µg/ml), two wavelengths i.e. 273nm as λmax of Caffeine and 372nm as λmax 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 273nm and 372nm. A set of two simultaneous equation 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 following two simultaneous equations.

$$C_x = (A_2ay_1 - A_1ay_2)/ (ax_2ay_1 - ax_1ay_2) \quad \text{------(1) [20]}$$

$$C_y = (A_1ax_2 - A_2ax_1)/ (ax_2ay_1 - ax_1ay_2) \quad \text{------(2) [20]}$$

Where, C_x and C_y are concentrations of Caffeine and Quercetin (µg/ml) respectively in known sample solution. A_1 and A_2 are absorbance of sample solutions at 273nm and 372nm respectively. ax_1 and ax_2 are absorptivity of Caffeine at 273nm and 372nm, ay_1 and ay_2 are absorptivity of Quercetin at 273nm and 372nm. The concentration of C_x and C_y in niosomal formulation can be obtained by solving equation (1) and (2). Validity of the 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 Niosome Formulation

Niosome solution (1ml) containing equivalent to 10 mg of both drugs was weighed and transferred to volumetric flask and excipient extracted with chloroform. Then drug dissolved in methanol. The sample solution was then filtered through what man filter paper. This solution was appropriately diluted to get approximate concentration of 20 µg/ml of Caffeine and Quercetin each, the absorbance of sample solution was measured at 273 nm and 372 nm against blank. **[21]**

VALIDATION OF THE DEVELOPED METHOD

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range for both the drugs was found to be 2- 10µg/ml. The linearity data for method is presented in **Table 1.**

Accuracy:

Accuracy means test output match with true value. To study the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Here to a pre-analyzed sample solution, standard drug solution was added and then percentage drug content were calculated. The % recovery of the added pure drug was calculated as % recovery= $[(C_t - C_s)/C_a] \times 100$, where C_t is the total drug concentration measured after standard addition; C_s, drug concentration in the formulation sample; C_a ,drug concentration added to formulation [23]. The result of recovery studies are reported in **Table 2.**

Precision:

Inter-day and Intra-day precision

The repeatability of the method was confirmed by the formulation analysis, repeated for six times with the same concentration. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of the formulation was repeated three times in the same day at an interval of one hour and on three successive days, respectively. The amount of drug was determined and % RSD was also calculated [23]. The results of both inter and intraday precision studies are reported in **Table 3.**

Ruggedness Study

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by for the standard 3 times with diff. analyst by using same equipment [23]. The result was indicated as %RSD & given in **Table 4**.

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

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ. The LOD and LOQ were calculated by using the average of slope and standard deviation of response (Intercept). The LOD and LOQ of Caffeine and Quercetin by proposed methods were determined using calibration standards. LOD= 3.3σ/S and LOQ= 10σ/S

Where, S is the slope of the calibration curve and σ is the standard deviation of response (intercept) [23]. The results of LOD and LOQ are shown in **Table 1.**

3. RESULTS AND DISCUSSION

The solutions of 10μg/ml for both Caffeine and Quercetin were analyzed and the λmax was found to be 273 nm and 372 nm, respectively. The calibration curve of Caffeine and Quercetin at 273nm and 372nm were plotted (**Figures 4, 5**). The relationship between the absorbance and the concentration of Caffeine and Quercetin was found to be linear in the range of 2-10 μg/mL at both wavelengths 273 nm and 372 nm. The regression coefficient for Caffeine and Quercetin was found to be 0.9993 and 0.9964 respectively which indicates good correlation between concentration and absorbance within the concentration range tested. The limit of detection of Caffeine and Quercetin was found to be 2.5437μg/ml and 3.3μg/ml respectively. Where, the limit of quantification for Caffeine and Quercetin was found to be 7.7083μg/ml and 10μg/ml. Evaluation Interday and Intraday precision was found to be less than two (<2), indicating good precision. Good percent recovery indicates good accuracy of the proposed method in standard addition method. It ranged between 98.88% to 100.2% for Caffeine and 100.3 to 100.7% for Quercetin. Percentage estimation of Caffeine and Quercetin in niosomal formulation was found to be 20.02 ± 1.156 and 29.56 ± 1.360 respectively.

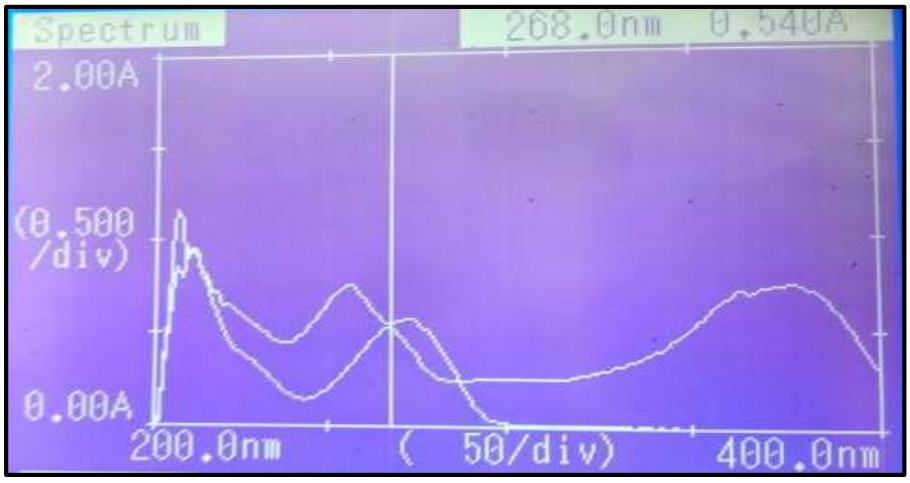

Figure 3: Overlay of maximum absorption of Caffeine and Quercetin.

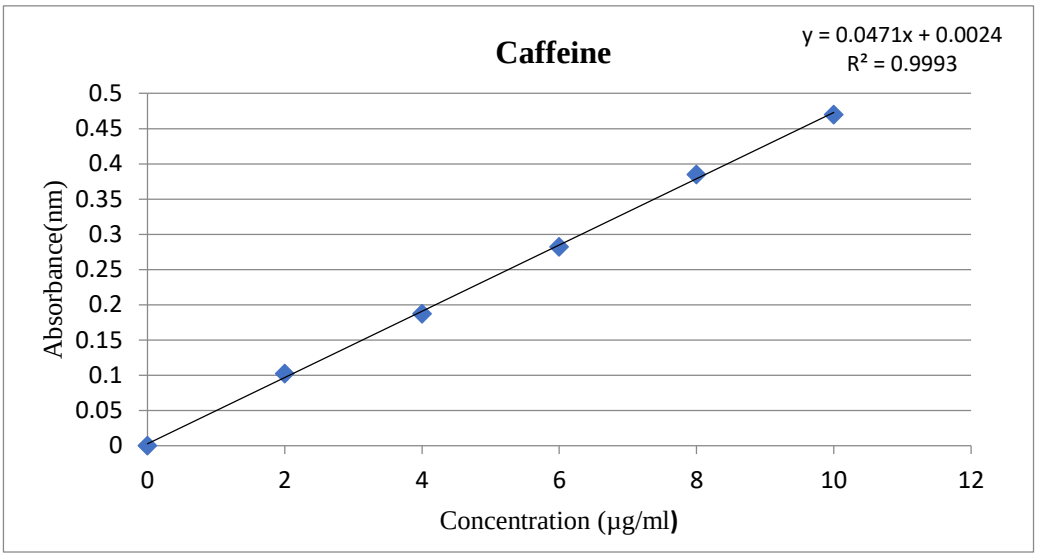

Figure 4: Calibration curve of caffeine

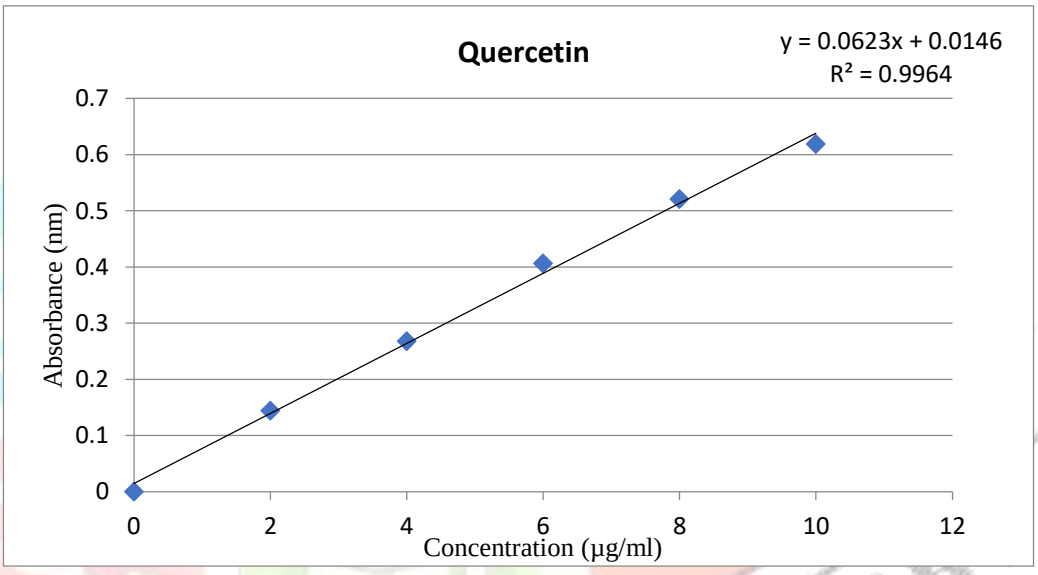

Figure 5: Calibration Curve of Quercetin

Table 1: Result of validation parameter

Parameter	Caffeine	Quercetin
Λmax (nm)	273nm	372nm
Linearity range(µg/ml)	2-10µg/ml	2-10µg/ml
Linearity equation	y = 0.0471x + 0.0024	y = 0.0623x + 0.0146
Correlation coefficient	0.9993	0.9964
Slope (b)	0.0471	0.0623
Intercept (a)	0.0024	0.0146
LOD	2.5437	3.3
LOQ	7.7083	10

Table 2: Drug recovery

Drug concentration(µg/ml)	%recovery	Amount added(µg/ml)	Total amount recovered(µg/ml)	%Recovered
Caffeine (10µg/ml)	80	8	17.80	98.88
	100	10	20.04	100.20
	120	12	21.78	99.00
Quercetin (50µg/ml)	80	40	90.27	100.3
	100	50	100.78	100.78
	120	60	110.73	100.66

Analysis of niosomal formulation:

Drug	Labeled amount(mg/ml)	Amount found (mg/ml) ± SD	% Label claim
Caffeine	20	20.02 ± 1.156	100.1
Quercetin	30	29.56 ± 1.360	98.5

Table 3: Precision study

Precision	%Estimation of caffeine ± SD	%RSD	% Estimation of quercetin ± SD	%RSD
Interday precision	99.79± 0.053	0.531	99.82± 0.86	0.861
Intraday precision	99.88± 0.056	0.562	99.92± 0.84	0.844

Table 4: Ruggedness study

Ruggedness	Caffeine		Quercetin	
	Mean ± SD	%RSD	Mean ± SD	%RSD
Analyst 1	0.452 ± 0.0015	0.33	0.598 ± 0.049	0.82
Analyst 2	0.453 ± 0.0005	0.12	0.601 ± 0.0005	0.09

4. CONCLUSION

The evaluation of obtained values suggests that the proposed UV Spectrophotometry method provide simple, precise, rapid and accurate analytical method for simultaneous estimation of Caffeine and Quercetin in niosomal formulation. Correlating the obtained results with the standard values, the method is found to be valid and hence the method can be easily and conveniently adopted for routine analysis in quality control laboratories.

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