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A NEW ANALYTICAL Q-ABSORBANCE RATIO METHOD DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF QUERCETIN AND MONOAMMONIUM GLYCYRRHIZINATE IN GEL FORMULATION

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***Abstract*:** A new simple, rapid and precise UV spectrophotometric method was developed and validated for the simultaneous estimation of Quercetin and MAG. The method involved estimation of Quercetin and MAG by simultaneous equation at 377nm and 248nm respectively in ethanol as a solvent. The Beer's law obeyed in the concentration range of 2-10 µg/ml and 10-60 µg/ml for Quercetin and MAG 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 Quercetin and Monoammonium glycyrrhizinate in gel formulation.

***Index Terms* - :** Quercetin, Monoammonium glycyrrhizinate, Simultaneous Method Development, UV Spectroscopy

1.Introduction

Quercetin is chemically, 2-(3,4-dihydroxy phenyl0-3,5,7- trihydroxy-4H-chromen-4-on.(figure 1 B) Quercetin, a flavonols, is a plant derived flavonoid found in fruits, vegetables, leavesand grains. It also may be used as an ingredient of supplements, beverages or foods. Quercetin is aflavonoid widely distributed in nature. Quercetin is found in vegetables and fruits, especially onions and apples, typically in the form of various glycosides [4]. Quercetin show poor water solubility, systematic bioavailability and therapeutic efficiencies. And also have high first pass metabolism and limited absorption Quercetin is frequenly used therapeutically in allergic conditions, including asthma, hay- fever, eczema and hives. Additional clinical uses include treatment of gout, pancreatitis and prostatitis; also used in inflammatory conditions. It is also used for diabetics, cataract, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, chronic fatigue syndrome and preventing cancer and for treating chronic infection of the prostate.[1-4]

Glycyrrhizic acid is a triterpene glycoside found in the roots of Glycyrrhiza glabra (Liquorice plant). MAG is the most important active ingredient in the Liquorice root, and Shows a wide range of pharmacological and biological activities. MAG possesses a wide range of pharmacological and biological activities.. It possesses anti-inflammatory activity, anti-ulcer, anti-bacterial, anti-allergic, antidote, antioxidant, antiviral, anti-tumour, anticonvulsant activity. Drug show anti-ulcer, anti-inflammatory, anti-bacterial, anti-viral which play major role in

curing mouth ulcer, but the major reason for section of this drug is its sweet test which mainly not seen in other drug, so this property increases patient compliance [5]

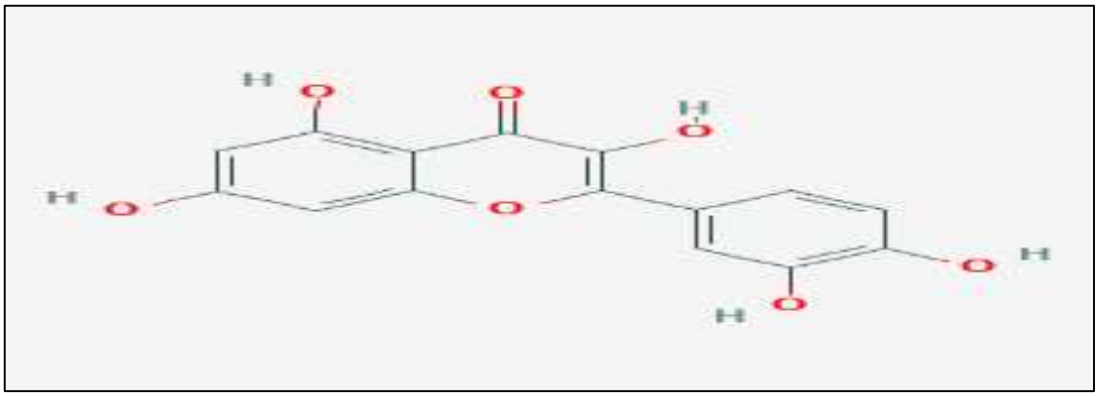

Figure 1: Quercetin [6]

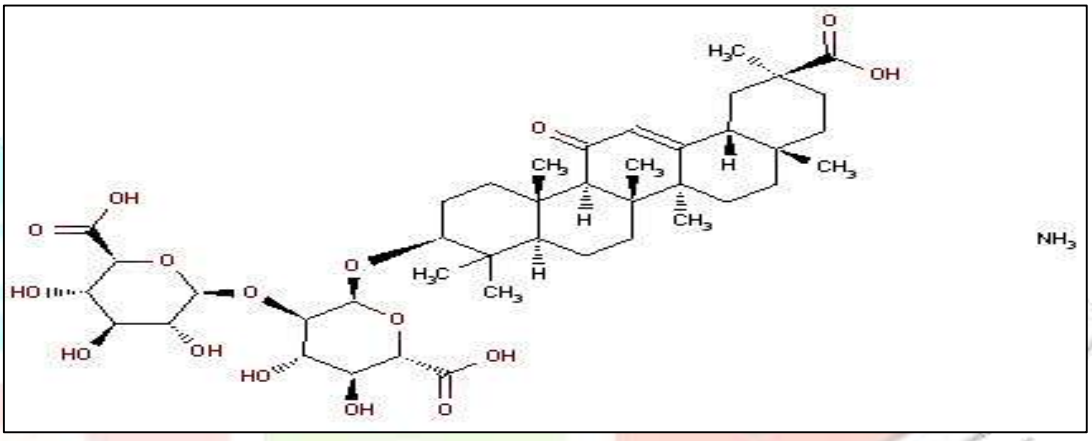

Figure 2: Monoammonium glycyrrhizinate

Literature review revealed that there are various methods available for the estimation of quercetin was estimated by UV-spectroscopy [7], RP-HPLC [8], HPLC [9] and LC-MS [10]. Similarly MAG, individually or with other drugs using UV-spectroscopy [11;12], High performance liquid chromatography [13] and LC-MS [14]. Hence from literature review it is clear that there is not a single UV method is reported so far for simultaneous analysis of Quercetin and MAG in gel formulations. Present research work is done to develop a precise, linear, simple, rapid, validated and cost effective UV-spectroscopic method for the estimation of quercetin and MAG in a single formulation.

2.MATERIALS AND METHODS

2.1.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.

2.2.Material and Reagents

MAG was purchased from SV Agro Pvt. Ltd (Mumbai) and Quercetin was purchased from Research lab Fine chem. industries (Islampur, Maharashtra). All the reagents used in this assay were of analytical grade.

2.3.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 Ethanol which was used for the analysis of both MAG and Quercetin for the proposed method.

2.4.DETERMINATION OF λ max

A] Preparation of stock solution

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

B] 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 ethanol to make 10µg/ml. Then the sample was scanned with UV-Vis Spectrophotometer in the range 200-400nm against ethanol as blank and the wavelength corresponding to maximum absorbance was noted which is its λ-max i.e. 377nm and 248nm for Quercetin and MAG respectively.

C] Preparation of calibration curve:

Preparation stock solution of Quercetin : (Ethanol AR)
10mg pure Quercetin was dissolved in 100 ml ethanol to get a 100ug/ml stock solution. Prepare concentration of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml respectively. Then measured absorbance of prepared dilutions at the respective wavelength.

Preparation stock solution of Monoammonium Glycyrrhizinate: (Ethanol AR)
10mg pure MAG was dissolved in 100 ml ethanol to get a 100ug/ml stock solution. Prepared concentration of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, and 60µg/ml respectively. Then measured absorbance of prepared dilutions at the respective wavelength.

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 and 10-60 µg/ml of Quercetin and MAG respectively.

2.5.Determination of Isoabsorptive Point and Wavelength :

Solutions of 10 µg/mL of both drugs were prepared from working stock solution and scanned in

the range of 200nm to 400nm against phosphate buffer (pH 7.4) as blank. The overlaying

spectrum was also obtained to determine isoabsorptive point. [15]

2.6.Application of the proposed method for estimation of mixture:

The absorptivity coefficient of both drugs was determined and the individual concentration MAG

and Quercetin was determined using the following equation:

$C_x = (Q_M - Q_Y/Q_X - Q_Y) \times A_1/ ax_1$ ----------(1)

$C_y = (Q_M - Q_X/Q_Y - Q_X) \times A_2/ ay_1$ ----------(1)

Where, $Q_M = A_2/A_1$, $Q_X = ax_2/ax_1$ and $Q_Y = ay_2/ay_1$; C_X and C_Y are the concentrations of MAG and Quercetin (µg/ml) respectively in known sample solution. A_1 and A_2 are absorbance mixture at 248nm and 268nm respectively. ax_1 and ax_2 are absorptivities of MAG and Quercetin at 248nm respectively; ay_1 and ay_2 are absorptivities of MAG and Quercetin at 268nm respectively. [15,16]

2.7.Analysis of Gel formulation

Sample preparation

For the estimation of drugs from the gel formulation, quantity of gel equivalent to 10mg of Quercetin and 10 mg of MAG was transferred to 100 ml volumetric flask, dissolved in sufficient quantity of ethanol, sonicated and the volume was adjusted up to the mark with ethanol to obtain a stock solution of 100µg/ml of Quercetin and MAG. The solution then filtered through whatman filter paper no. 41 and the filtrate was appropriately diluted for final concentrations 10 µg/ml of Quercetin and MAG. Absorbance of this solution was measured at appropriate wavelengths, and values were substituted in the respective formulae to obtain concentrations. The result of this method is presented in **Table 5.** [17]

2.8.VALIDATION OF THE DEVELOPED METHOD

A] Linearity:

For both drugs dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range for Quercetin and MAG was found to be 2- 10 µg/ml and 10-60 µg/ml respectiuvely. The linearity data for method is presented in **Table 1.**

B] 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 Percentage recovery of the added pure drug was calculated as follows

$\%$ recovery = $[(C_t - C_s)/C_a] \times 100$

Where,

C_t = Total drug concentration measured after standard addition

C_s = Drug concentration in the formulation sample

C_a = Drug concentration added to formulation [18]

The result of recovery studies are reported in **Table 2.**

C] 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 [18]. The results of both inter and intraday precision studies are reported in **Table 3.**

D] Ruggedness Study

It It shows that the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by for the standard 3 times with different analyst by using same equipment [18]. The result was indicated as %RSD and given in **Table 4**.

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

The LOD and LOQ were separately determined with the help of 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 Quercetin and MAG 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) [18]. The results of LOD and LOQ are shown in **Table 1.**

3.RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 268 nm(iso-absorptive point) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of Quercetin (377 nm) and MAG (248 nm) showing iso-absorptive point (268 nm) in ethanol is shown in Figure 3. The validation parameters were studied at all the wavelengths for the proposed method. The relationship between the absorbance and the concentration of Quercetin and MAG was found to be linear in the range of 2-10 μg/mL and 10-60 μg/mL at both wavelengths 377nm and 248nm respectively. The regression coefficient for Quercetin and MAG was found to be 0.9969 and 0.9974respectively which indicates good correlation between concentration and absorbance within the concentration range tested. The limit of detection of Quercetin and MAG was found to be 2.86μg/ml and 3.49μg/ml respectively. Where, the limit of quantification for Quercetin and MAG was found to be 6.66μg/ml and 10.59μg/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 100.3% to 100.66% for Quercetin and 98.33% to 100.18% for MAG. Percentage estimation of Quercetin and MAG in Gel formulation was found to be 99.8 % and 99.6 % respectively.

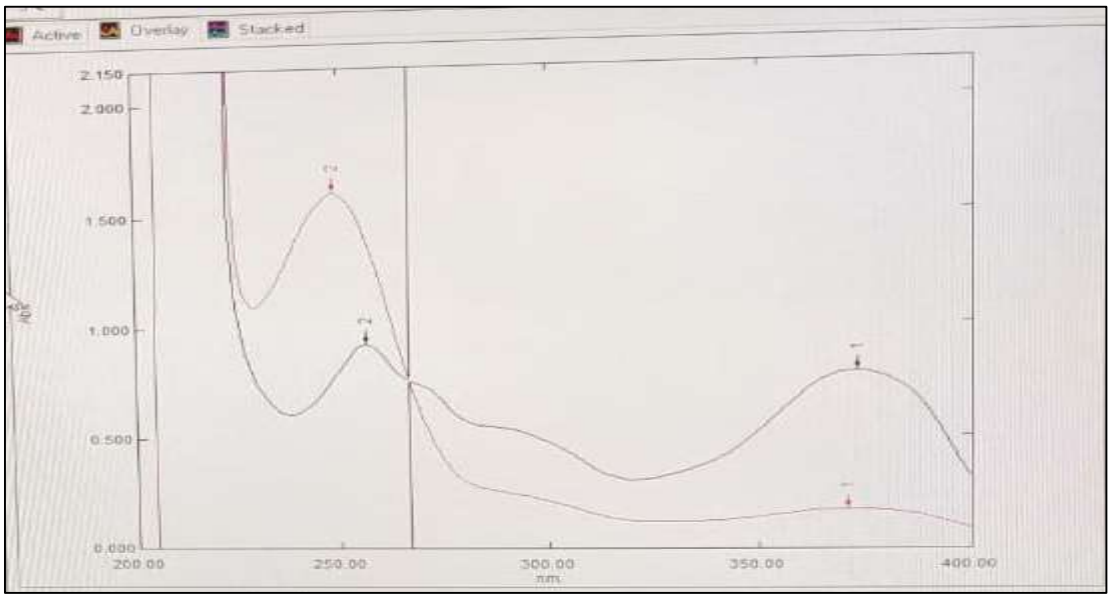

Figure 3: Overlay of maximum absorption of Quercetin and MAG

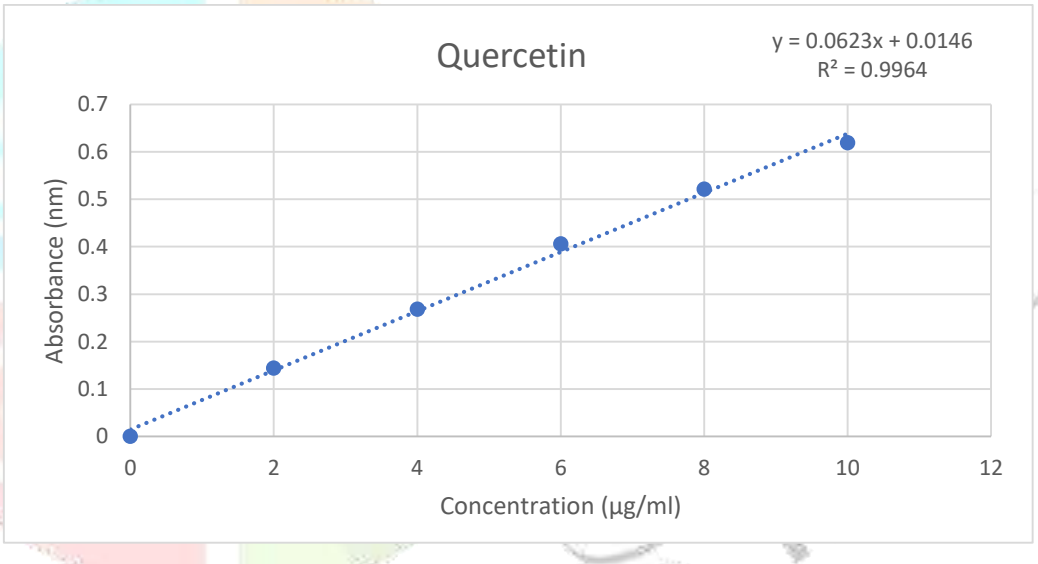

Figure 4: Calibration curve of Quercetin

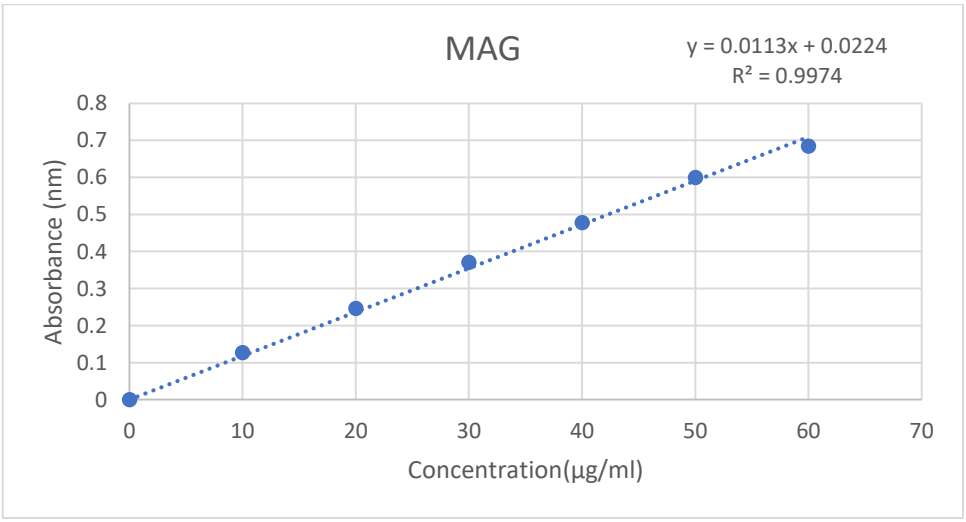

Figure 5: Calibration curve of MAG

Table 1: Result of validation parameter

Parameter	Quercetin	MAG
Λmax (nm)	377nm	248nm
Linearity range(µg/ml)	2-10µg/ml	10-60µg/ml
Linearity equation	y = 0.0623x + 0.0146	y = 0.0113+0.0224
Correlation coefficient	0.9964	0.9974
Slope (b)	0.0623	0.0113
Intercept (a)	0.0146	0.0224
LOD	2.86	3.49
LOQ	6.66	10.59

Table 2: Drug recovery

Drug concentration(µg/ml)	%recovery	Amount added(µg/ml)	Total amount recovered(µg/ml)	%Recovered
Quercetin (40µg/ml)	80	32	72.22	100.3
	100	40	80.63	100.78
	120	48	88.5	100.66
MAG (10µg/ml)	80	8	17.70	98.33
	100	10	19.96	99.8
	120	12	22.04	100.18

Table 3: Precision study

Conc(µg/ml)	Interday precision		Intraday precision	
	SD	%RSD	SD	%RSD
Quercetin	0.0010	0.524	0.0015	0.658
MAG	0.0013	0.96	0.0014	0.718

Table 4: Ruggedness study

Drug	SD	%RSD
Quercetin	0.0035	0.602
MAG	0.001	0.524

Table 5: Analysis of Gel formulation

Drug	Labeled claim (%w/w)	% Estimated	% RSD	% Recovery
Quercetin	1%	98.49	0.48	99.8
MAG	1%	98.32	0.96	99.6

4.Conclusion

The proposed method absorbance ratio method (Q-analysis) was successfully applied to the simultaneous determination of Quercetin and MAG from bulk and pharmaceutical gel formulation. The presented method was found to be simple, accurate, precise and rugged. It can be directly and easily applied to the analysis of the combined pharmaceutical gel formulation of Quercetin and MAG. Moreover, the present method is quick and cost effective as compared to chromatographic techniques. Therefore, it can be concluded that the proposed method provides an alternative procedure for the quality control of Quercetin and MAG in pharmaceutical gel formulations

5.Acknowledgement

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