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Development Of Analytical Method And Validation For Estimation Of Quercetin And Monoammonium Glycyrrhizinate In Their Formulation

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ABSTRACT

A unique, rapid, straightforward, and precise UV spectrophotometric approach for the simultaneous quantification of quercetin and Monoammonium glycyrrhizinate was developed and validated. Ethanol served as a solvent while simultaneous equations were employed to quantify quercetin and Monoammonium glycyrrhizinate at wavelengths of 377 nm and 248 nm, respectively. The concentration ranges for quercetin and Monoammonium glycyrrhizinate were 2 to10 µg/ml and 10 to 60 µg/ml, respectively, adhering to Beer's law. This methodology was validated in accordance with ICH requirements on accuracy, linearity, precision, LOD, and LOQ. Given that the %RSD was below 2, this method was deemed accurate. The proposed approach for the routine analysis of quercetin and monoammonium glycyrrhizinate in gel formulation has demonstrated efficiency, specificity, accuracy, and precision.

KEYWORDS: Quercetin, Monoammonium glycyrrhizinate, Estimation, Development and Validation.

INTRODUCTION

A key aspect of developing analytical methods is the simultaneous estimate of bioactive chemicals in pharmaceutical formulations, especially when working with complex mixtures [1]. Quercetin and monoammonium glycyrrhizinate are two such compounds of significant interest due to their numerous therapeutic properties [2]. Quercetin, a flavonoid, is known for its potent antioxidant, anti-inflammatory, and anticancer effects, while monoammonium glycyrrhizinate, derived from licorice root, has been shown to possess anti-inflammatory, hepatoprotective, and immune-boosting properties [3, 4]. Both compounds are often included in various pharmaceutical preparations, including tablets, creams, and syrups, aimed at managing conditions such as inflammation, allergies, and liver diseases [5].

The estimation of these compounds in their pharmaceutical formulations requires a robust and reliable analytical method that ensures accurate, precise, and reproducible results. Method development and validation are crucial in ensuring that the analytical procedure meets regulatory standards and provides consistent, trustworthy data for quality control and product safety [6, 7].

In this context, the analytical method development process for quercetin and monoammonium glycyrrhizinate typically involves choosing an appropriate technique that can resolve these compounds from the formulation matrix and other excipients [8]. High-performance liquid chromatography (HPLC) is often favored due to its sensitivity, precision, and ability to separate complex mixtures. A thorough validation of the developed method, including parameters such as accuracy, linearity, specificity, precision, and LODand LOQ, is essential to confirm the reliability and robustness of the method for routine analysis [9, 10].

In this work, a precise, accurate, and repeatable analytical approach for the simultaneous measurement of quercetin and monoammonium glycyrrhizinate in pharmaceutical formulations is developed and validated. In order to ensure that the active ingredients are present in the proper concentrations and that the products satisfy the relevant pharmacopoeial standards, the goal is to develop a validated method that can be used for quality control in the production of these formulations [11-13].

MATERIALSANDMETHODS:

Instruments and Equipments:

A double beam UV spectrophotometer with a wavelength precision of 0.5 nm and a spectral width of 2 nm, two 1 cm matched quartz cells, a digital balance, and a software program called UVProbe2.0. The proposed analytical technique was formulated and validated utilising an ultrasonicator, volumetric flasks, and borosilicate glass pipettes [14, 15].

Material and Reagents

Quercetin was bought from Merck Chemicals, while Monoammonium glycyrrhizinate was bought from a nearby source. Every reagent utilised in this test was of analytical quality [16].

Selection of solvent:

In accordance with Indian Pharmacopoeia requirements for selecting a universal solvent, the solubility of pharmaceuticals was evaluated across several solvents. Polar and nonpolar solvents were utilised to ascertain solubility. Ethanol was shown to be the universal solvent in the analysis of monoammonium glycyrrhizinate and quercetin using the suggested approach [17, 18].

DETERMINATIONOFWAVELENGTH MAXIMA

Stock Solution Preparation:

To make the solution, 10 mg of quercetin and 10 mg of monoammonium glycyrrhizinate were dissolved in 100 millilitres of ethanol, yielding a concentration of 100 µg/ml. Using ethanol as a blank and a UV-visible double beam spectrophotometer, the UV spectrum was captured in the 200–400 nm region [19, 20].

Preparation of working solution

Considering the previously recommended stock solution Onemillilitre of each drug solution was introduced into a 10-milliliter volumetric flask, and the volume was supplemented with ethanol to achieve a concentration of 10 µg/ml. The material was analysed with a UV-Vis spectrophotometer in the 200–400 nm range, with ethanol as a blank. The wavelength corresponding to the maximum absorbance, or λ-max, was determined to be 377 nm for quercetin and 248 nm for monoammonium glycyrrhizinate [21, 22].

Calibration Curve Preparation:

Preparation stock solution of Quercetin:

To prepare a 100 µg/ml stock solution, 10 mg of pure quercetin was dissolved in 100 ml of ethanol. Concentrations of 2µg/mlto 10µg/ml must be generated. The absorbance of the produced dilutions at the designated wavelength was then measured [23-25].

Preparation stock solution of Monoammonium Glycyrrhizinate:

To prepare a 100 µg/ml stock solution, 10 mg of pure Monoammonium glycyrrhizinate was dissolved in 100 ml of ethanol. The concentrations prepared were 10µg/mlto 60µg/ml. The absorbance of the produced dilutions at the designated wavelength was then measured.

A calibration curve for concentration vs. absorbance was generated, with concentration plotted on the x-axis and absorbance on the y-axis, resulting in a linear relationship. The straight line demonstrated linearity within the concentration ranges of 2 to 10 µg/ml for quercetin and 10 to 60 µg/ml for monoammonium glycyrrhizinate [26-31].

Determination of Isoabsorptive Point and Wavelength:

Solutions of both drugs at a concentration of 10 µg/mL were produced from the working stock solution and analysed by scanning from 200 to 400 nm, utilising phosphate buffer (pH 7.4) as a blank. The overlaid spectrum was also acquired to ascertain the isoabsorptive point [27, 32-38].

Analysis of Gel formulationSamplepreparation

A quantity of gel equivalent to 10 mg of quercetin and 10 mg of monoammonium glycyrrhizinate was transferred to a 100 ml volumetric flask, dissolved in adequate ethanol, sonicated, and the volume was adjusted with ethanol to obtain a stock solution of 100 µg/ml of quercetin and monoammonium glycyrrhizinate. This process was used to quantify the pharmaceuticals in the gel formulation. After filtering the solution through Whatman filter paper number 41, the filtrate was suitably diluted to attain final concentrations of 10 µg/ml for both monoammonium glycyrrhizinate and quercetin. We quantified the absorbance of this solution at the designated wavelengths and used the results to the pertinent equations to ascertain the concentrations. Table 5 displays the outcomes of this strategy [28, 39-44].

VALIDATIONOFTHEDEVELOPEDMETHOD

Linearity:

The established techniques evaluated the standard stock solution dilutions of both medicines. The Beer-Lambert concentration range for quercetin and monoammonium glycyrrhizinate was determined to be 2 to 10 µg/ml and 10 µg/ml to 60 µg/ml, respectively. Table 1 displays the linearity data for the procedure [45-53].

Accuracy:

When test findings correspond with the true value, they are deemed accurate. Recovery studies employing the conventional addition method were performed at three specific levels (80%, 100%, and 120%) to assess the validity of the proposed methodology. A standard drug solution was included into a previously analysed sample solution, and the drug content % was subsequently ascertained. The subsequent formula was employed to ascertain the % recovery of the added pure drug:

$$\% \text{ recovery} = [(Ct-Cs)/Ca] \times 100$$

Where,

Ct = Total drug concentration measured after standard addition

Cs = Drug concentration in the formulation sample

Ca = Drug concentration added to formulation

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

Precision:

Inter-dayandIntra-dayprecision

The formulation analysis, conducted six times at the same concentration, confirmed the method's repeatability. We calculated the RSD as a percentage. The intraday and interday analyses, which involved doing the formulation analysis three times in one day, every hour, and over three days in a row, confirmed that the method was a good middle ground. We calculated the quantity of medication and the percentage relative standard deviation (RSD). Table 3 displays the results of the intraday and inter-day precision analyses [54-64].

Ruggedness Study

It illustrates the variability of accuracy between laboratories, including across analysts. The method's robustness was assessed thrice with identical equipment and varying analysts. The results were presented in Table 4 as a percentage RSD [65-74].

Limit of Detection and Limit of Quantitation:

The calibration curve was utilised to ascertain the LOD and the LOQ independently. The LOD and LOQ were established utilising the RSD of a regression line or the standard deviation of the y-intercepts of regression lines. The mean slope and response standard deviation (intercept) were utilised to calculate the LOD and LOQ. The LOD and LOQ and monoammonium glycyrrhizinate were determined using calibration standards [75-84]. The results of LOD and LOQ are shown in Table 1.

$$LOD = 3.3\sigma/S \text{ and } LOQ = 10\sigma/S$$

Where, S is the slope of the calibration curve and

σ is the standard deviation of response (intercept).

RESULTS AND DISCUSSION:

The primary need for developing an analytical method using the absorbance ratio method (Q-analysis) is that the entire spectrum must comply with Beer's law at all wavelengths, a condition satisfied by both drugs in question. Two wavelengths were utilised to evaluate the pharmaceuticals: the calibration curves for both compounds were established at the iso-absorptive point of 268 nm. Figure 3 illustrates the superimposed UV absorption spectra of Monoammonium glycyrrhizinate (248 nm) and quercetin (377 nm), highlighting the iso-absorptive point (268 nm) in ethanol. The validation parameters were analysed at each wavelength for the proposed method. At both wavelengths (377 nm and 248 nm), the absorbance and concentration of quercetin and Monoammonium glycyrrhizinate exhibited linearity within the ranges of 2 to 10 μg/mL and 10 to 60 μg/mL, respectively. In the examined concentration range, a strong correlation between concentration and absorbance was observed, as evidenced by the regression coefficients for quercetin and Monoammonium glycyrrhizinate, which were 0.9969 and 0.9974, respectively. The detection limits for Monoammonium glycyrrhizinate and quercetin were determined to be 3.49 g/ml and 2.86 g/ml, respectively. The quantification limits for Monoammonium glycyrrhizinate and quercetin were determined to be 10.59 and 6.66 g/ml, respectively. The precision

was assessed as satisfactory, with both intraday and interday precision being below two (<2). A high recovery rate indicates that the proposed method is precise when employing the typical addition technique. The range for Quercetin was 100.3% to 100.66%, whereas Monoammonium glycyrrhizinate range was 98.33% to 100.18%. The calculated concentrations of quercetin and Monoammonium glycyrrhizinate in the gel formulation were 99.8% and 99.6%, respectively.

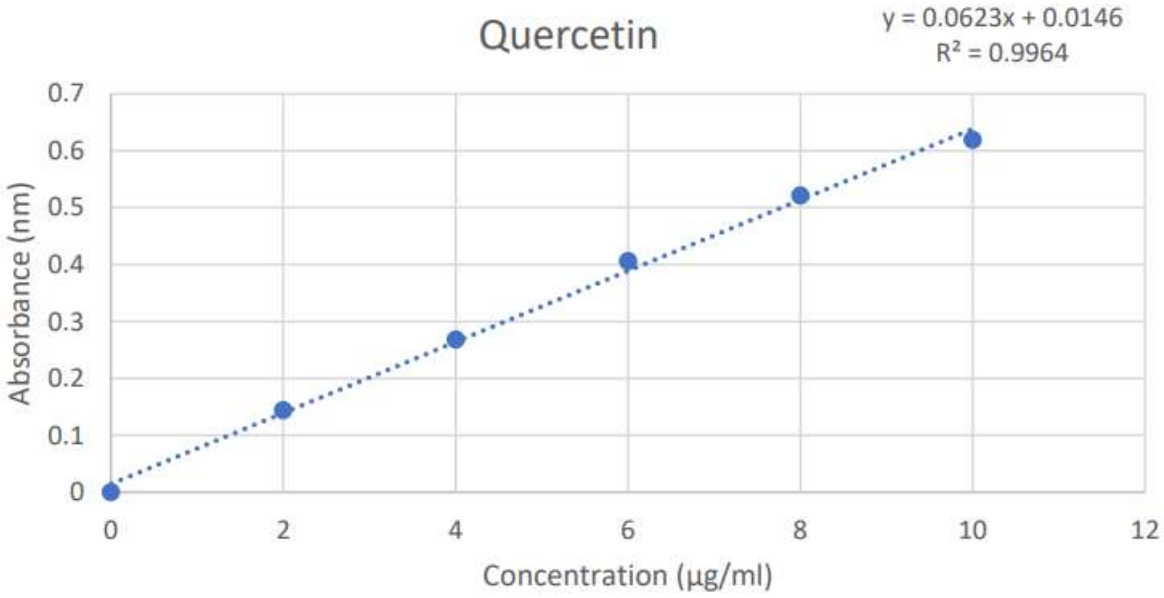

Figure1: Calibration curve of Quercetin

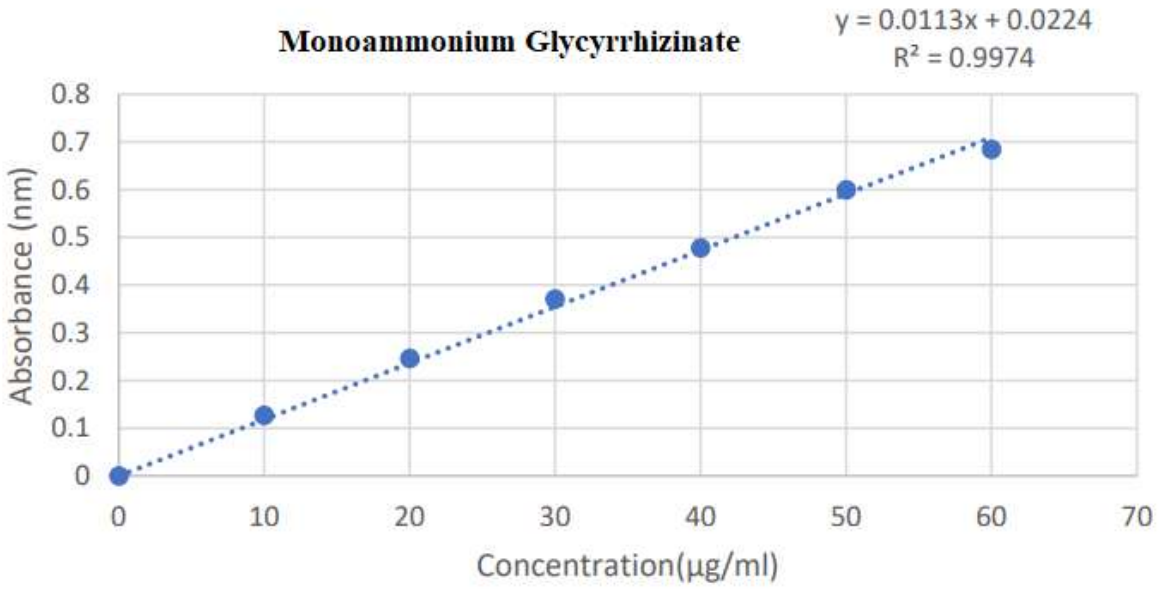

Figure2: Calibration curve of Monoammonium glycyrrhizinate

Table1: Interpretation of validation parameter

Parameter	Quercetin	Monoammonium Glycyrrhizinate

Wavelength maxima(nm)	377nm	248nm
Range ofLinearity(µg/ml)	2 to 10µg/ml	10 to 60µg/ml
Equation ofLinearity	y=0.0623x+0.0146	y=0.0113+0.0224
RSD	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

Table2:% Recovery Sample Drug

Drug concentration (µg/ml)	**% Drug Recovery**	**Concentration added(µg/ml)**	**TotalConc.recovered (µg/ml)**	**%Drug Recovered**
Quercetin (40µg/ml)	80	32	72.22	100.3
	100	40	80.63	100.78
	120	48	88.5	100.66
Monoammoniumglycyrrhizinate (10µg/ml)	80	8	17.70	98.33
	100	10	19.96	99.8
	120	12	22.04	100.18

Table3:Precision study

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

Table4: Ruggedness study

Drug	**SD**	**%RSD**
Quercetin	0.0035	0.602
Monoammonium glycyrrhizinate	0.001	0.524

Table5: Analysis of Gel formulation

Drug	**Labeled claim (%w/w)**	**% Estimated**	**% RSD**	**% Recovery**

Quercetin	1%	98.49	0.48	99.8
Monoammonium glycyrrhizinate	1%	98.32	0.96	99.6

Conclusion

Quercetin and Monoammonium glycyrrhizinate were effectively quantified concurrently in bulk and medicinal gel formulations utilising the proposed absorbance ratio method (Q-analysis). The process was found to be simple, accurate, exact, and reliable. It is immediately and straightforwardly applicable to the analysis of the Quercetin and Monoammonium glycyrrhizinate combination medical gel formulation. Moreover, the present methodology is more rapid and cost-effective than chromatographic techniques. Thus, the proposed method provides an alternative procedure for assessing the quality of Monoammonium glycyrrhizinate and quercetin in pharmaceutical gel formulations.

DECLARATIONS:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

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Not applicable.

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