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Development and Validation by UV Spectrophotometric Method for Simultaneous Estimation of Gliclazide and Quercetin in Formulation

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Abstract: ***A simple, quick, accurate, precise, and cost-effective spectrophotometric approach for simultaneous estimation of Gliclazide and Quercetin has been developed by Simultaneous equation method. Gliclazide and Quercetin shows absorbance maximum at 226 and 255 nm respectively, as a result absorbance was measured at these wavelengths for the estimation of Gliclazide and Quercetin. In concentration ranges from 2-18 g/ml, and 2-22 g/ml for Gliclazide and Quercetin respectively followed Beer-Lambert's law. The method has been developed and validated in accordance with ICH recommendations and can be used for estimation of Gliclazide and Quercetin in formulations.***
Keywords: Gliclazide, Quercetin, Simultaneous equation, U.V. Spectrophotometer, Method Development and Validation.

I. INTRODUCTION

Gliclazide (1-(3-azabicyclo [3.3.0] oct- 3- yl) - 3- ptolylsulfonylurea is an oral hypoglycemic drug used in the treatment of type-II diabetes mellitus.

It comes under category of sulfonylurea class, which works by activating pancreatic cells to release insulin. It lowers blood glucose levels by dealing with both inadequate insulin secretion and peripheral insulin resistance, improving ß-cell glucose sensitivity, lowering hepatic glucose synthesis, and enhancing glucose clearance. Additionally, it has anti-platelet adhesive activity and lowers free radical levels, reducing vascular problems.

It has also been shown to lower plasma cholesterol and triglyceride levels following repeated administration [1]. Quercetin, a bioflavonoid an has vast therapeutic potential [2-3] such has antidiabetic [4], anti-obesity [5], anti-inflammatory [6] and antiviral properties against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) [7], antibacterial [8], and Influenza virus [9]. It acts on multiple targets and regulates key signaling pathways which improve the symptoms as well as the complications of Type 2 diabetes [2].

It indicates that, Herb i.e. a phytoconstituent and Synthetic drug when given in combination not only controls blood glucose levels but also reduces the dosage of of synthetic drug by 25–50 %. Also from the literature search, herbal extract supplementation reduces dose of synthetic drug and improves the conditions in diabetic complications from lipid peroxidation and antioxidant systems when experiments were carried in type-2 diabetic rats [10-12].

Literature survey revealed that methods such as UV [1, 13-14], HPTLC [15], determination of quercetin and gliclazide, have been reported for estimation of quercetin and gliclazide separately. Not a single UV, HPLC or HPTLC method are reported for simultaneous estimation of Gliclazide 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.

II. MATERIALS AND METHODS

A. *Materials*

Gliclazide was procured from Ideal cures (Mumbai, Maharashtra), Quercetin was procured from Ozone Labs. All the chemicals and reagents that were used were of analytical grade.

B. Procedures

1) Preparation of standard stock solution and calibration curve

The standard stock solution of gliclazide and quercetin were prepared by dissolving 10 mg of each drug in 10 ml methanol, and the final volume was adjusted using water 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 λmax. Calibration curves were constructed to obtain regression equations for gliclazide and quercetin respectively.

C. Simultaneous equation method

From the overlay spectra of gliclazie (10 µg/ml) and quercetin (10 µg/ml), two wavelengths i.e. 226 nm as λ max of gliclazide and 256 nm as λ max of quercetin were selected as the working wavelength, at which both drugs showed absorbance. The absorptivity of these two drugs was determined at 226 nm and 256 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 formulation were calculated using set of two simultaneous equations [16-17]. The equations are:

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \qquad (1)$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \qquad (2)$$

Where;

Cx and Cy are concentrations of gliclazide and quercetin (µg/ml) respectively in known sample solution.

A1 and A2 represent absorbance of sample solutions at 226 nm and 256 nm respectively.

ax1 and ax2 are absorptivity of gliclazide at 226 nm and 256 nm, ay1 and ay2 are absorptivity of quercetin at 226 nm and 256 nm.

The concentration of Cx and Cy in the formulation can be obtained by solving equations (1) and (2). T

he validity of the above-mentioned equations was examined by using a mixed standard of pure drugs and measuring their absorbance at respective wavelengths, and determining the concentration of the two components.

D. Validation of the developed method [18-22]

1) Linearity and Range

Appropriate dilutions of the standard stock solution where done separately to construct Beer lambert's law plot, and the calibration curve was established for gliclazide in the concentration range 2-18 µg/mL (2, 4, 6, 8, 10, 12, 14, 16 and 18 µg/mL) and quercetin in the concentration range 2-22 µg/mL (2, 4, 6, 8, 10, 12, 14, 16 , 18, 20, and 22 µg/mL).The linearity data for method is presented in Table 1.

2) Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% for the test concentration (I.e formulation which was formulated in lab) as per ICH guidelines. The recovery study was performed three times at each level. The result of the recovery studies are reported in Table 2.

3) Precision

Interday and Intraday precision

The Interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively (six replicates). The results of the same are presented in Table 3.

4) Ruggedness

It expresses the precision within laboratories by varying the analysts. The results of the same are presented in Table 3.

5) Limit of detection (LOD) and Limit of quantitation (LOQ)

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.

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
The LOD and LOQ were determined based on the standard deviation of the response and the slope.

$$LOD = \frac{3.3\,\sigma}{S} \quad \text{and} \quad LOQ = \frac{10\,\sigma}{S}$$

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

6) Assay:
Assay was done for the formulation prepared in the laboratory. Six capsules were weighed accurately and ground into a fine powder. Powder equivalent to 100 mg of Gliclazide and Quercetin was weighed accurately and transferred into a 100 mL volumetric flask with 10 mL methanol. The content was shaken for 15-20 min, and later make-up the volume with distilled water.

III. RESULTS AND DISCUSSION

A. Linearity and Range
Linearity range for Gliclazide and Quercetin are 2-18 µg/ml and 2-22 µg/ml at selected wavelengths respectively. The coefficient of correlation for gliclazide at 226 nm and for quercetin at 256 nm is 0.998 and 0.9985 respectively. Both drugs shows good regression values at their respective wavelengths.

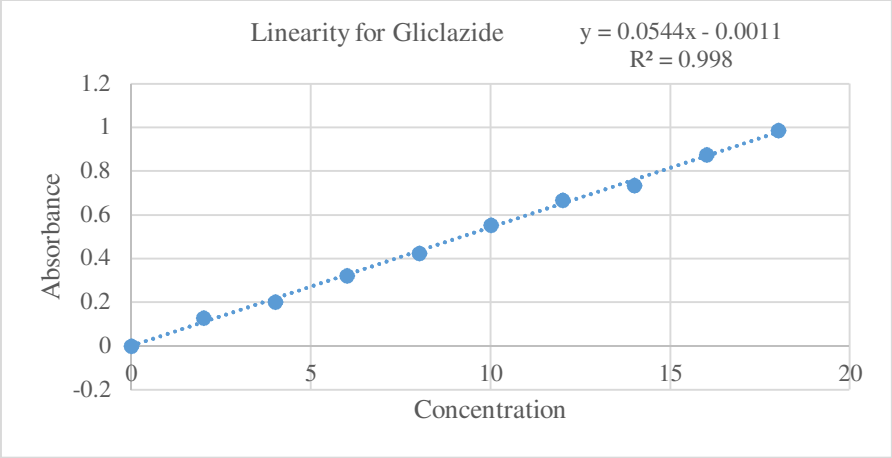

Fig. 1 Linearity for Gliclazide

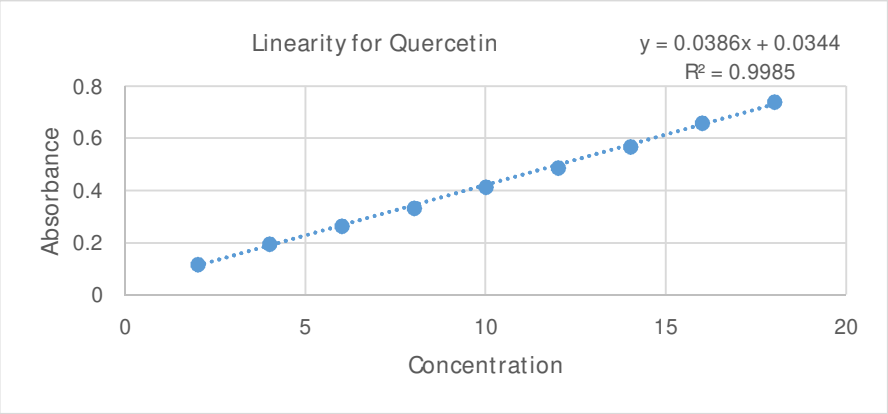

Fig. 2 Linearity for Quercetin

TABLE 1: RESULT OF VALIDATION PARAMETERS

Parameters	Gliclazide	Quercetin
λmax	226	256
Linearity	2-18 µg/mL	2-22 µg/mL
Regression equation	Y= 0.0544x - 0.0011	y = 0.0386x + 0.0344
Standard Deviation	0.00079	0.00395
%RSD	0.3	0.885
Correlation coefficient	0.998	0.9985
LOD	0.63	0.66
LOQ	1.921	2.01

B. Accuracy and % Recovery

The results of recovery studies were found between 80 to 120 % (98.23 to 106.2%), This has shown that the approach is accurate and that regularly used excipients and additives used in pharmaceutical formulations did not interfere with the suggested procedure. Recovery experiments verified the method's accuracy, which was determined to be considerable and within specification limitations. (i.e. within the acceptable range 98-120 % recovery).

TABLE 2: RESULT FOR % RECOVERY

Drug	Concentration of Drug added µg/mL	%Level	% Recovery ±SD	% Recovery ±SD
Gliclazide	8	80	99.04± 0.07	98.25± 0.3
	10	100	103.046± 0.5	102.5± 0.48
	12	120	105.37± 0.19	104.1± 0.21
Quercetin	8	80	98.63± 0.255	98.23± 0.41
	10	100	105.04± 0.14	104.3± 0.02
	12	120	106.2± 0.02	106.13± 0.5

C. Precision

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).

D. Ruggedness

The ruggedness of developed method was checked by analyzing gliclazide by different analysts at similar operational and environmental conditions. The % RSD values were found to be less than 2% (Table 2).

TABLE 3: Result For Repeatability, Precision And Ruggedness

Drugs	Parameters				
	Repeatability	Precision		Ruggedness	
		Intra-day	Inter-day	Analyst 1	Analyst 2
Gliclazide	0.557	0.554	0.542	0.559	0.554
Quercetin	0.415	0.415	0.418	0.409	0.421

E. Robustness

Robustness of the proposed method was determined by estimating a drug at slightly different wavelength from the selected wavelength. No significant difference was found in the absorbance of samples. Therefore, the proposed method was considered as robust

F. Specificity

The proposed method was found to be specific as there is no interference with other excipients.

G. *Assay for Formulation*

TABLE 4: RESULT FOR ASSAY

Drugs	Label claim (mg)	Concentration found (mg)
Gliclazide	80	80.7
Quercetin	50	48.8

IV. CONCLUSION

The developed method was found to be sensitive, accurate, precise, reproducible and linear over the concentration range studied. Spectrophotometric method of analysis is more economical and easier, compared to methods such as chromatography and electrophoresis. The proposed method can be used for the routine quality control analysis of Gliclazide and Quercetin pharmaceutical dosage forms.

V. ACKNOWLEDGEMENT

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VI. CONFLICT OF INTEREST

The authors report no conflicts of interest.

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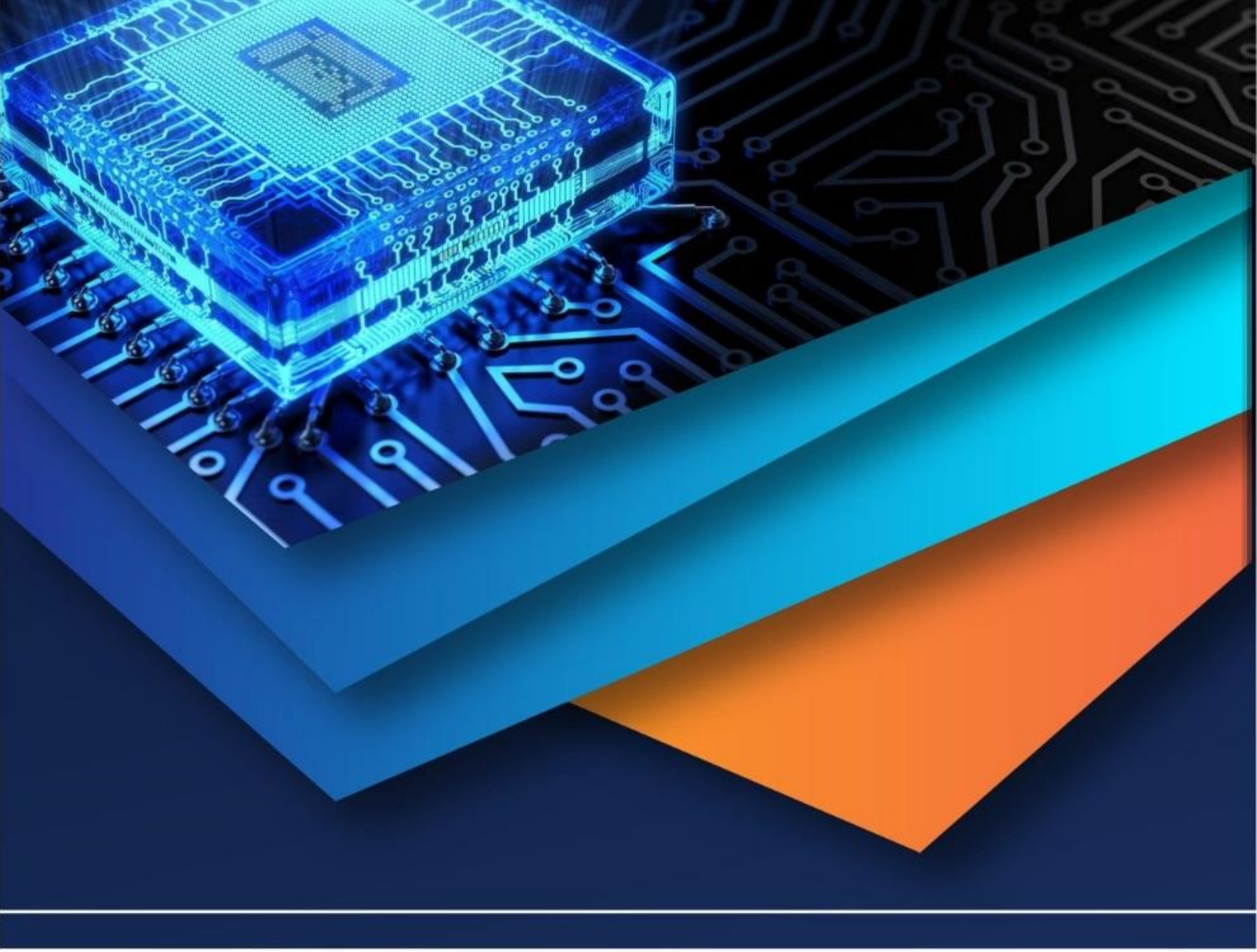

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