

**SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF ALOGLIPTIN AND GLIBENCLAMIDE IN SYNTHETIC MIXTURE****\*Rana Anjali V.<sup>1</sup> and Patel Paresh U.<sup>2</sup>**

<sup>1</sup>Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India.

<sup>2</sup>Head of Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India.

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**\*Correspondence for  
Author****Rana Anjali V.**

Department of  
Pharmaceutical Quality  
Assurance, Shree S. K.  
Patel College of  
Pharmaceutical Education  
& Research, Ganpat  
University, Ganpat  
Vidyanagar – 384012,  
Mehsana, Gujarat, India.

**ABSTRACT**

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for the simultaneous determination of Alogliptin and Glibenclamide in synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Alogliptin has absorbance maxima at 275.7 nm and Glibenclamide has absorbance maxima at 229.7 nm in methanol. The linearity were obtained in the concentration range of 1-14 µg/ml for Alogliptin and 2-16µg/ml for Glibenclamide. The concentrations of the drugs were determined by using simultaneous equations method. The mean recovery was  $101.09 \pm 0.57$  and  $100.12 \pm 0.97$  for Alogliptin and Glibenclamide respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Alogliptin and Glibenclamide in synthetic mixture. The results of analysis have been validated statistically and by recovery studies.

**KEYWORDS:** Alogliptin, Glibenclamide, Simultaneous equations, Validation.

## INTRODUCTION

Glibenclamide (GLI) is chemically name N-p-[2-(5-Chloro-2-methoxybenzamido)ethyl] benzenesulfonyl-N'-cyclohexylurea<sup>[1]</sup> is a The drug labour via binding to and activating the ATP-sensitive potassium channels ( $K_{ATP}$ ) inhibitory regulatory subunit sulfonylurea receptor 1 (SUR1) in pancreatic beta cells, used in the treatment of Diabetes mellitus. It is approved in IP<sup>[2]</sup>, BP.<sup>[3]</sup> Literature review reveals UV spectrophotometry<sup>[4]</sup>, RP-HPLC<sup>[5]</sup>, HPTLC.<sup>[6]</sup> Method for estimation of Glibenclamide tablet, bulk and spectrophotometric method for simultaneous determination of GLI with other drug<sup>[7]</sup> and RP-HPLC method for simultaneous determination of GLI with other drug<sup>[8]</sup> are reported in literature for assement of GLI in pharmaceutical dosage forms, pure, as well as in biological fluid. Alogliptin (ALO) is chemically name 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydropyrimidin-1-yl} methyl) benzonitrile<sup>[9]</sup> is a dipeptidyl peptidase 4 inhibitor (DPP-4 Inhibitor). Used in the treating of Antidiabetic. Alogliptin is not approved in any pharmacopoeia. Various methods like LC<sup>[10]</sup>, spectrophotometric<sup>[11]</sup> and HPLC<sup>[12]</sup> method for simultaneous assement of ALO with other drug, RP-HPLC method for simultaneous assement of ALO with other drug<sup>[13]</sup> and stability-indicating RP-HPLC method<sup>[14]</sup> for the determination of ALO are reported in literature for assement of ALO in pharmaceutical dosage forms as well as in biological fluids. This combination is not available in any pharmacopoeia as well as not reported method available. The present parchment discuss simple, sensitive, accurate, precise, rapid and economic spectrophotometric method based on simultaneous equations for simultaneous estimation of ALO and GLI in synthetic mixture.

## MATERIALS AND METHODS

A shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

## REAGENTS AND MATERIALS

Alogliptin and Glibenclamide bulk powder was kindly gifted by Cadila Zyudus. Ahmadabad, India. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no. 41 (Millipore, USA) was also used in the study.

### Preparation of standard stock solutions

An accurately weighed quantity of ALO (10 mg) and GLI (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of ALO (100 µg/ml) and GLI (100 µg/ml). For Glibenclamide preparation amber colored volumetric flask were used.

### Preparation of synthetic mixture

Synthetic mixture (200 mg) was prepared by using ALO (25 mg) and GLI (5 mg) and excipients (50 mg) like Lactose, Talc and (70 mg) magnesium stearate.

### Method

The standard solutions of ALO (10 µg/ml) and GLI (10µg/ml) were scanned separately in the UV range of 200-400 nm to determine the  $\lambda_{max}$  of both the drugs. The of ALO and GLI were found to be 275 nm and 229 nm respectively. Six standard solutions having concentration 1,2, 4, 6, 8, 10, 12, 14 for Alogliptin and 2, 4, 6, 8, 10, 12, 14, 16 for Glibenclamide prepared in methanol using the standards solutions. The absorbance of resulting solutions was measured at 275.7nm and 229.7calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of GLI and ALO in sample solution was determined by solving the respective simultaneous equations<sup>[18]</sup> generated by using absorptivity coefficients and absorbance values of GLI and ALO at these wavelengths.

The concentration of two drugs in the mixture can be calculated using following equations

$$C_X = (A_2a_{Y1} - A_1a_{Y2}) / (a_{Y1}a_{X2} - a_{Y2}a_{X1}) \dots\dots\dots (1)$$

$$C_Y = (A_1a_{X2} - A_2a_{X1}) / (a_{Y1}a_{X2} - a_{Y2}a_{X1}) \dots\dots\dots (2)$$

Where,  $A_1$  and  $A_2$  are absorbance of mixture at 229.70 nm and 275.74nm; and  $a_{X1}$  and  $a_{Y1}$  are absorptivity of GLI and ALO at 229.70 nm;  $a_{X2}$  and  $a_{Y2}$  are absorptivities of GLI and ALO respectively at 275.7 nm.

### VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.<sup>[15]</sup>

**Linearity (Calibration curve)**

The calibration curves were plotted over a concentration range of 2-16 µg/ml for GLI and 1-14 µg/ml for ALO. Accurately measured standard solutions of GLI (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6ml) and ALO (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the solutions were measured at 229.70 and 275.74 nm against methanol as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

**Method precision (repeatability)**

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions ( $n = 6$ ) for GLI and ALO (10 µg/ml for GLI and 8 µg/ml for ALO) without changing the parameter of the proposed spectrophotometry method.

**Intermediate precision (reproducibility)**

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of GLI and ALO (8, 10, 12 µg/ml for both GLI and ALO).

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating recovery of GLI and ALO by the standard addition method. Known amounts of standard solutions of GLI and ALO were added at 80, 100 and 120% level to prequantified sample solutions of GLI and ALO 1 µg/ml and 5 µg/ml respectively. The amounts of GLI and ALO were estimated by applying obtained values to the respective regression line equations.

**Limit of detection and Limit of quantification**

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where,  $\sigma$  = the standard deviation of the response and S = slope of the calibration curve.

### Analysis of GLI and ALO in synthetic mixture

GLI (5 mg) and ALO (25 mg) standard drug powder were weighed and then mixed with commonly used formulation additives like lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 15 minutes. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. The above solution was suitably diluted with methanol to get a final concentration of 10 µg/ml of GLI and 10 µg/ml of ALO. The absorbances of the solution i.e.  $A_1$  and  $A_2$  were recorded at 229.7 nm and 275.7 nm and ratios of absorbance were calculated, i.e.  $A_2/A_1$ . Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equations generated by using absorptivity coefficients and absorbance values of GLI and ALO at these wavelengths.

### RESULTS AND DISCUSSION

In this method, two wavelengths were used for the analysis of the drugs. 229.7 nm ( $\lambda_{\max}$  of GLI) and 275.7 nm ( $\lambda_{\max}$  of ALO) are the wavelengths at which calibration curves were prepared for both the drugs. Once the absorptivity values are determined very little time is required for analysis, as would require determination of absorbances of the sample solution at two selected wavelengths and few simple calculations.

Maximum absorbance was obtained at 229.7 nm and 275.7 nm for GLI and ALO respectively. These two wavelengths were employed for the determination of GLI and ALO without any interference from the other drug in synthetic mixture.

Linear correlation was obtained between absorbances and concentrations of GLI and ALO in the concentration ranges of 2-16 µg/ml and 1-14 µg/ml for drugs respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. Relative standard deviation was less than 2%, which indicates that proposed method is repeatable. The low RSD values of interday (0.07-0.31% and 0.44-1.54% for GLI at 229.7 and 275.7 nm respectively and 0.62-0.45% and 0.39-1.91% for ALO at 229.7 and 275.7 nm respectively) and intraday (0.27% and 0.087 and 0.46-1.58% for GLI at 229.7 and 275.7 nm respectively and 0.76-0.087% and 0.22 -1.46% for ALO at 229.7 and 275.7 nm respectively) variation for GLI and ALO reveal that the proposed method is precise. LOD and LOQ values for GLI were found to be 0.10 and 0.32 µg/ml and 0.16 and 0.50 µg/ml at 229.7 and 275.7 nm respectively. LOD and LOQ values for ALO were found to be 0.13 and 0.42 µg/ml and 0.24 and 0.74 µg/ml at 229.7 and 275.7 nm respectively. These data show that method is sensitive

for the determination of GLI and ALO. All the regression analysis data and summary of validation parameters for the proposed method is reported in Table 3.

The recovery experiment was performed by the standard addition method. The mean recoveries were  $100.1 \pm 0.97$  and  $101.0 \pm 0.57$  for GLI and ALO respectively indicates accuracy of the proposed method (Table 1). The proposed validated method was successfully applied to determine GLI and ALO in synthetic mixture. The results obtained for GLI and ALO were comparable with the corresponding labeled amounts (Table 2). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of GLI and ALO in pharmaceutical tablet dosage forms.

**Table 1: Recovery Data for Proposed Method.**

Drug	Level	Amount taken ( $\mu\text{g/ml}$ )	Amount added (%)	% Mean recovery $\pm$ S.D. (n = 3)
GLI	I	1	80	$99.9 \pm 1.25$
	II	1	100	$101.3 \pm 1.15$
	III	1	120	$100.5 \pm 0.92$
ALO	I	5	80	$101.9 \pm 0.40$
	II	5	100	$101.2 \pm 0.46$
	III	5	120	$101.8 \pm 0.28$

**Table 2: Analysis of GLI And ALO By Proposed Method.**

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim $\pm$ S. D. (n = 5)	
	GLI	ALO	GLI	ALO	GLI	ALO
I	5	25	5.025	25.025	$100.1 \pm 0.97$	$100.0 \pm 0.57$

**Table 3: Regression Analysis Data and Summary of Validation Parameters for the Proposed Method.**

PARAMETERS	GLI		ALO	
Wavelength range (nm)	229.7	275.7	229.7	275.7
Beer's law limit ( $\mu\text{g/ml}$ )	2-16	1-14	2-16	1-14
Regression equation ( $y = mx \pm c$ )	$y = 0.0538x + 0.0064$	$y = 0.0542x + 0.0225$	$y = 0.0028x + 0.0033$	$y = 0.0306x + 0.0081$
slope(b)	0.0538	0.0542	0.0028	0.0306
Intercept (a)	0.0064	0.0225	0.0033	0.0081
Correlation Coefficient ( $r^2$ )	0.9992	0.995	0.9976	0.9994
Method precision (Repeatability) (%RSD, n = 6)	1.90	1.68	0.66	0.48
Intraday (n = 3) (%RSD)	0.54-0.55	0.03-1.27	0.56-0.88	0.31-1.06
Interday (n = 3) (%RSD)	2.91-0.15	0.03-1.25	0.56-0.53	0.31-1.04

LOD( $\mu\text{g/ml}$ )	0.10	0.13	0.16	0.24
LOQ ( $\mu\text{g/ml}$ )	0.32	0.42	0.50	0.74
Accuracy (Mean % Recovery $\pm$ S.D) (n = 3)	100 $\pm$ 0.97		101.09 $\pm$ 0.49	
% Assay $\pm$ S.D. (n = 5)	100.1 $\pm$ 0.97		100.0 $\pm$ 0.57	

LOD = Limit of Detection. LOQ = Limit of Quantification. S.D = Standard Deviation. RSD = Relative Standard Deviation.

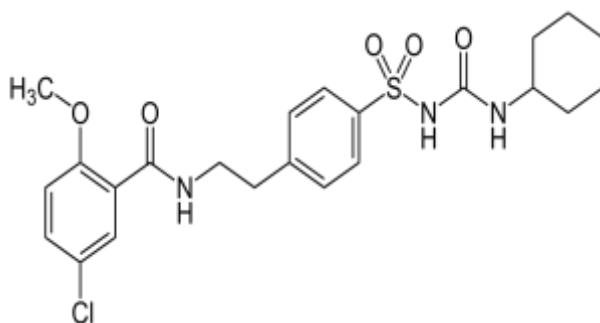


Figure 1: Chemical structure of Glibenclamide (GLI).

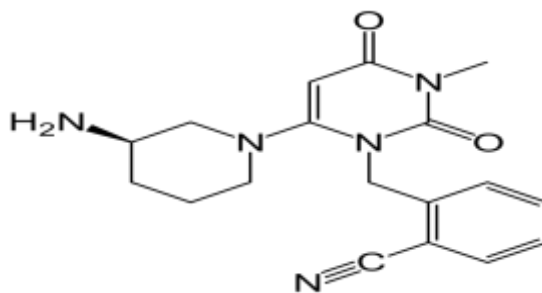
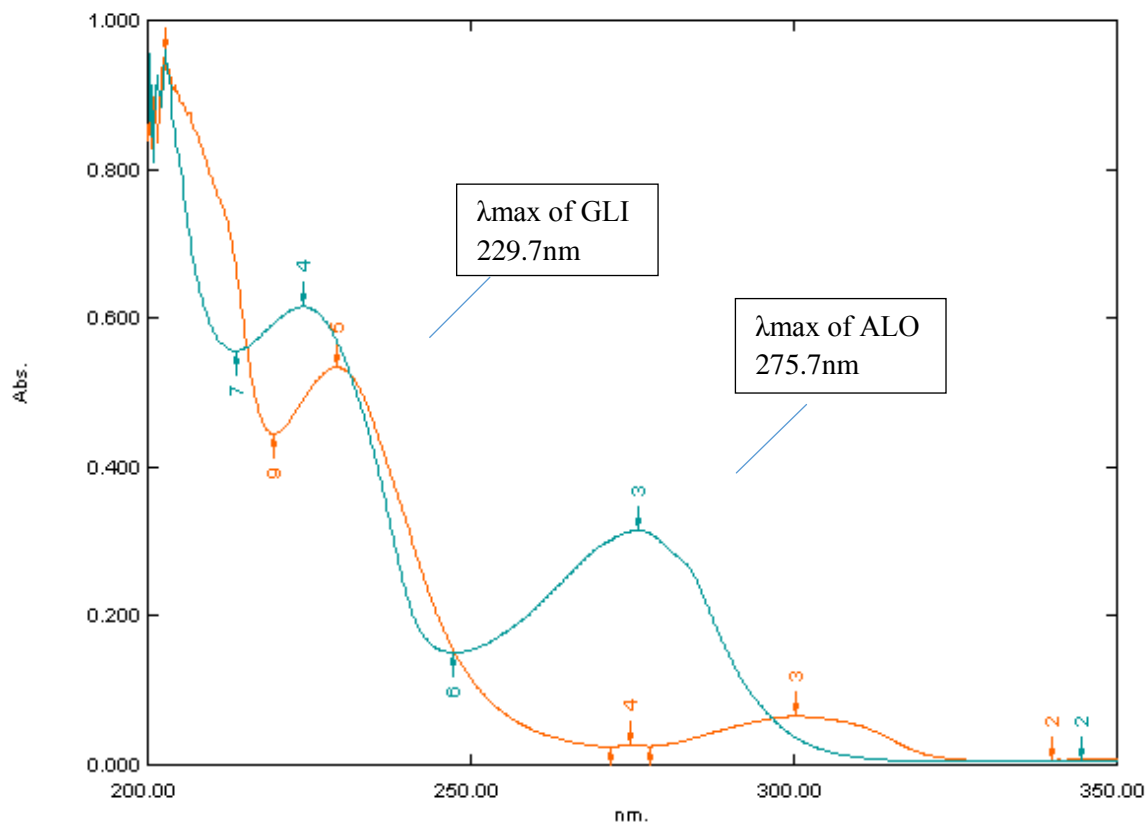


Figure 2: Chemical structure of Alogliptin (ALO).



**Figure 3: Overlay spectra of GLI (229.7 nm) and ALO (275.7 nm) showing in methanol.**

## CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for simultaneous determination of GLI and ALO in synthetic mixture. The method utilizes easily available and low cost solvent like methanol for analysis of GLI and ALO hence the method was also found to be economic for estimation of GLI and ALO from synthetic mixture. The method has linear response in the range of 2-16  $\mu\text{g/ml}$  for GLI and 1-14  $\mu\text{g/ml}$  ALO in methanol.

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