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Simultaneous Estimation of Gliclazide And Metformin In Bulk And Tablet Dosage Forms By Chemometric Assisted Spectrophotometric Method.

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ABSTRACT

To develop UV-Spectrophotometric method and to apply the chemometric designs to the developed method for the simultaneous estimation of Glicalzide(GLIC) amd Metformin(MET) in tablet dosage forms without further extraction. The UV-Spectrophotometric method was developed by using ethanol as solvent for both the drugs and the data generated from the absorption spectra was done by three chemometric designs which were based on the principles Linear regression analysis method (LRC), Cramer's matrix (CRM) and Method of least squares (MLS). The wavelength selected for all the above methods were 210 nm (wavelength of maximum absorption; λmax of GLIC), and 236 nm (wavelength of maximum absorption; λmax of MET).The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the two drugs and moreover they are effective in minimizing the errors in analysis, simple and economical.
**Keywords:** Chemometrics, UV-Visible, Simultaneous, Gliclazide, Metformin

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INTRODUCTION

Chemometric was coined in 1971 to describe the growing use of mathematical, statistical, and other logic- based methods in the field of chemistry and in particular in analytical chemistry. Chemometric is general used in three areas calibration, validation, and significance of analytical measurement, The optimization of chemical measurement and experimental procedure, The extraction of the maximum chemical information from analytical data. Chemometric has become a very powerful technique offering multivariate data reducution procedures that are now yielding chemical information previously not available to the analyst from data [1] .To overcome the significant problems in the analysis of intricate multi component formulations by conventional UV-spectroscopy [2-4], HPLC [5-13] methods Chemometric assisted analytical methods [18-21] are designed to perform analytical investigation of such complex formulations.

Gliclazide(GLC) is 1-(4-methylbenzenesulfonyl)-3-{octahydrocyclopenta[c]pyrrol-2-yl}urea which is a second generation sulphonylurea which acts as a hypoglycemic agent. It stimulates β cells of the islet of Langerhans in the pancreas to release insulin and also enhances peripheral insulin sensitivity.[2]
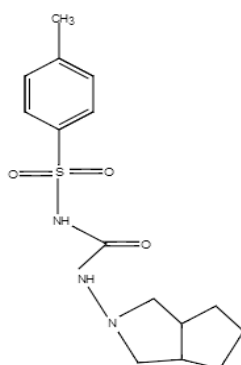
Metformin (MET) is chemically 1-carbamimidamido-N,N-dimethylmethanimidamide which is an oral anti-hyperglycemic agent that improves glucose tolerance in patients with NIDDM, which lowers both basal and postprandial plasma glucose. It decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. [1]
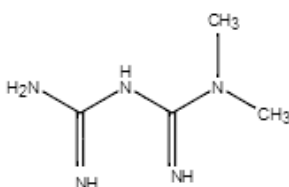
MATERIALS AND METHODS

Instruments used

- Analytical balance
- UV-Visible spectrophotometer (Lab India -3072)

Data handling systems:

- UV-win for the handling of spectrophotometer.
- Microsoft excel.

Materials used:

- Working standards of drugs were procured from Dr. Reddy s laboratory.
- Commercial formulation of drugs was purchased from local market.
- Ethanol was procured from Merck (India) ltd, Mumbai.

Preparation of Solutions:

Preparation of Glicalzide standard solutions:

10 mg of Glicalzide standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using ethanol and volume was made up to the mark with ethanol. Further dilutions were made with the ethanol to get required concentrations of 2, 4, 6, 8, 10μg/ml

Preparation of Metformin standard solutions:

10 mg of Metformin standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using ethanol and volume was made up to the mark with ethanol . Further dilutions were made with the ethanol to get required concentrations of 2, 4, 6, 8, 10μg/ml

Preparation of Glicazide and Metformin

The marketed formulation equivalent to 250mg of Gliclazide and Metformin was taken in a 250ml volumetric flask. Dissolved in ethanol and make up with aethanol. From the above standard solution pipette out 1ml of solution in a 10 ml of volumetric flask and make up with aethanol. From the above stock solution pipette out 0.4ml of a solution in a 10 ml of volumetric flask and make up with aethanol to get 4μg/ml.

Design of chemometric models:

Chemometric models were designed for the developed spectrophotometric methods for the simultaneous estimation of Gliclazide(GLIC) and Metformin(MET)

Linear regression analysis (LRC):

For linear regression method two wavelengths are consider for the analysis of component mixture [GLIC (X), and MET(Y)].The two linear regression equations were obtained by using the absorbance measured at two wavelengths against concentrations of standard solutions for each component. The slope values obtained from the linear regression analysis for each component were used for the formation of matrix set. The wavelengths selected for analysis were 210nm (λ_{max} of GLIC), 236nm and (λ_{max} of MET).

Equations for the formation of matrix are:

$$A_{mix1} = b_{x1}C_x + b_{y1}C_y + b_{z1}C_z + a_{xyz1}$$
$$A_{mix2} = b_{x2}C_x + b_{y2}C_y + b_{z2}C_z + a_{xyz2}$$

Where, A_{mix1}, A_{mix2}, are the absorbance of the mixture of   X, Y, analytes at three wavelengths set. a_{xy1}, a_{xy2}, are the sum of intercepts of the linear regression equation at the three wavelengths.C_X and $C_Y$are the concentration of Gliclazide and Metforminb_{x1} ,b_{x2} ,b_{y1}, and b_{y2} are slopes of Gliclazide and Metformin 210nm and 236 nm

Conversion of equation into matrix form:

$$\begin{bmatrix} Amix1 - & axy1 \\ Amix2 - & axy2 \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx2 & by2 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

Cramer's Matrix Method

Molar absorptivity (ε) values were calculated by using the absorbance measured at 210nm, and 236 nm for each compound in the binary mixture. The selected wavelength values were λ $_{max}$ of GLIC and MET respectively. By using absorptivity (ε) values, a system of equations with two unknowns in the double mixture has been written as follows:

$$A_{m, 210} = \varepsilon_{GLIC, 210}\, C_{GLIC} + \varepsilon_{MET, 210}\, C_{MET}$$
$$A_{m, 236} = \varepsilon_{GLIC, 236}\, C_{GLIC} + \varepsilon_{MET, 236}\, C_{MET}$$

Where A_m denotes the absorbance of the binary mixture and ε represents the values of molar absorptivity for the calculated GLIC nad MET respectively at 210 and. C is the molar concentration of GLIC nad MET.

The matrix simplifies and solves the system of equations with three unknown as follows

$$\begin{bmatrix} A_m, 210 \\ A_m, 236 \end{bmatrix} = \begin{bmatrix} \varepsilon\, GLIC, 210 & \varepsilon\, MET, 210 \\ \varepsilon\, GLIC, 236 & \varepsilon\, MET, 236 \end{bmatrix} \times \begin{bmatrix} C\, GLIC \\ C\, MET \end{bmatrix}$$

Method of Least Squares:

The standard stock solutions of GLIC (4µg/ml), and MET (4µg/ml) were measured at 210nm, 214nm, 218nm, 222nm, 226 nm, 230nm, 234nm, 238nm,242nm, 248 nm, 252nm and their absorbances were recorded (acts as calibration set) and tabulated in MS- Excel. The individual drug absorbances of known concentrations of GLIC nadMET were added and synthetic mixture (as validation set) was created and absorbances were recorded. Similarly the test sample was also measured at same wavelengths and absorbances were recorded and tabulated. By applying method of least squares using Solver add-in in MS-Excel, the actual concentration of GLIC and MET were predicted in test samples.

Validation of spectrophotometric method:

Linearity and range:

The linearity of analytical method is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

The range of analytical procedure is the interval between the upper and lower concentrations of the sample for which the analytical procedure has a suitable level of Precision, Accuracy and Linearity.

Precision:

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Accuracy:

The accuracy of analytical procedure express the closeness or agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by adding known quantities of analyte (pure drug) to the drug product and applying the developed methods to determine the quantity of the drug present in the spiked sample.

Samples were spiked with 50,100,150% level solutions of the standards and analysed. The experiment was performed triplicate (n=3). Percent recovery values were reported.

$$Accuracy = \frac{Amount\ of\ Sample\ Conc.\ found\ -\ Amount\ of\ Test\ Conc.\ taken}{Amount\ of\ Standard\ Conc.\ added} \times 100$$

Assay:

The commercial marketed formulation containing 250mg of Gliclazide, and 250mg Metformin. The sample solution was treated same as standard solution. The resulting solution scanned under UV using ethanol as blank.

$$Percent\ Assay = \frac{Calculated\ qty\ of\ test\ sample(mg)}{Weight\ of\ test\ sample(mg)} \times 100$$

RESULTS AND DISCUSSION:

BILINEAR REGRESSION ANALYSIS:

Table No 1: Absorbance of Gliclazide at 210 nm, and 236 nm.

Conc. (µg/ml)	210 nm	236 nm
2	0.305	0.034
4	0.447	0.100
6	0.575	0.172
8	0.740	0.253
10	0.853	0.313
Linear Equation	y = 0.0695x+0.1673	y = 0.0356x-0.038
R^2	0.997	0.998

Table No 2: Absorbance of Metformin at 210 nm, and 236 nm.

Conc. (µg/ml)	210 nm	236nm
2	0.281	0.096
4	0.435	0.105
6	0.578	0.106
8	0.750	0.125
10	0.915	0.139
Linear Equation	y = 0.0792x-0.1169	y = 0.0352x+0.1854
R^2	0.998	0.998

$$\begin{bmatrix} Amix1 - & axy1 \\ Amix2 - & axy2 \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx2 & by2 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} 0.879 - & 0.2842 \\ 0.430 - & 0.1465 \end{bmatrix} = \begin{bmatrix} 0.0695 & 0.0792 \\ 0.0356 & 0.0352 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} 0.594 \\ 0.284 \end{bmatrix} = \begin{bmatrix} 0.0695 & 0.0792 \\ 0.0356 & 0.0352 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \end{bmatrix} = \begin{bmatrix} 4.151 \\ 3.87 \end{bmatrix}$$

The concentration of Gliclazide (C_x), and Metformin (C_y) present in the given formulation sample were found to be 4.151 µg/ml, and 3.87 µg/mlrespectively.

Cramer's matrix method:

$$A_{mix1} = b_{x1}C_x + b_{y1}C_y + a_{xy1}$$
$$A_{mix2} = b_{x2}C_x + b_{y2}C_y + a_{xy2}$$

$$\begin{bmatrix} Am, 210 \\ Am, 236 \end{bmatrix} = \begin{bmatrix} \varepsilon\ GLIC, 210 & \varepsilon\ MET, 210 \\ \varepsilon\ GLIC, 236 & \varepsilon\ MET, 236 \end{bmatrix} \times \begin{bmatrix} C\ GLIC \\ C\ MET \end{bmatrix}$$

By substituting the values in matrix and it was solved and each compound was determined by solving the following operations (Δ = Determinant value of matrix).

$$\Delta = \begin{bmatrix} 111750 & 108750 \\ 25000 & 81750 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} 0.595 & 108750 \\ 0.284 & 81750 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} 111750 & 0.595 \\ 25000 & 0.284 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration of GLIC and MET were found as follows
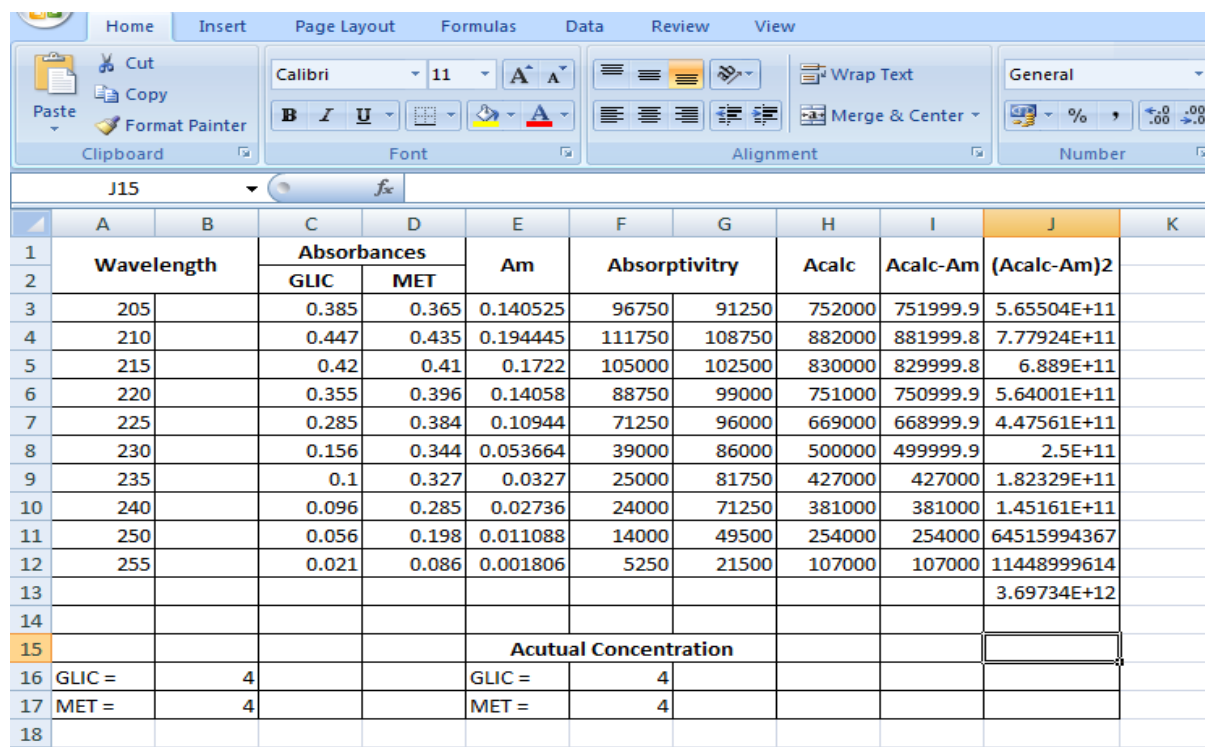
$C_{GLIC} = \Delta_1 / \Delta$
    = 3.89µg/mL
$C_{MET} = \Delta_2 / \Delta$
    = 4.08µg/mL

The concentration of Gliclazide (C_x), andMetformin (C_y) present in the given formulation sample were found to be 3.89 µg/ml, and 4.08 µg/ml respectively.

Method of least squares:

The standard stock solutions of GLIC (4µg/mL), MET (4µg/mL), were measured at 210-255 nm with 5 nm interval. Molar absorptivity's are calculated and tabulated. Further calculations are done as shown below

Wavelength		Absorbances		Am	Absorptivitry		Acalc	Acalc-Am	(Acalc-Am)2	
		GLIC	MET							
205		0.385	0.365	0.140525	96750	91250	752000	751999.9	5.65504E+11	
210		0.447	0.435	0.194445	111750	108750	882000	881999.8	7.77924E+11	
215		0.42	0.41	0.1722	105000	102500	830000	829999.8	6.889E+11	
220		0.355	0.396	0.14058	88750	99000	751000	750999.9	5.64001E+11	
225		0.285	0.384	0.10944	71250	96000	669000	668999.9	4.47561E+11	
230		0.156	0.344	0.053664	39000	86000	500000	499999.9	2.5E+11	
235		0.1	0.327	0.0327	25000	81750	427000	427000	1.82329E+11	
240		0.096	0.285	0.02736	24000	71250	381000	381000	1.45161E+11	
250		0.056	0.198	0.011088	14000	49500	254000	254000	64515994367	
255		0.021	0.086	0.001806	5250	21500	107000	107000	11448999614	
									3.69734E+12	
				Acutal Concentration						
GLIC =	4			GLIC =	4					
MET =	4			MET =	4					

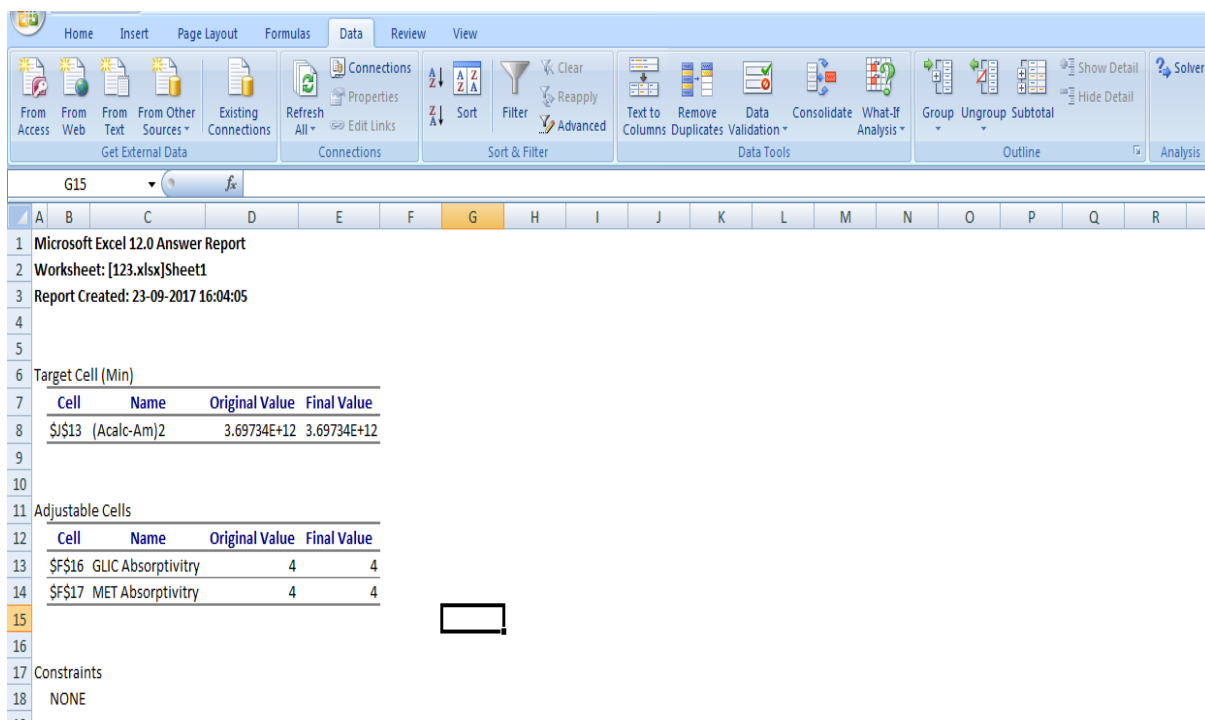**Fig No 3: Screen shot of arranging data into excel sheet**

Fig No 4: Screen shot of solver report

The concentration of Gliclazide (C_x), andMteformin (C_y) present in the given formulation sample were found to be 4µg/ml and 4 µg/ml respectively.

Table No 3: Percentage assay for the three methods

DRUG	LRC			CRM		MLS	
	Actual concentration (µg/ml)	Predicted concentration (µg/ml)	Assay %	Predicted concentration (µg/ml)	Assay %	Predicted concentration (µg/ml	Assay %
GLIC	4	4.151	103.775	3.89	97.25	4	100
MET	4	3.87	96.75	4.08	102.00	4	100

Acceptance criteria: 85-105 %

METHOD VALIDATION:

Accuracy:

Table No. 4: Percentage recovery for all the methods

DRUG	% RECOVERY			
	PERCENTAGE LEVEL	LNR	CRM	MLS
GLIC	75 %	100.50	97.20	99.25
	100 %	99.95	101.90	98.52
	125 %	101.20	98.30	101.36
MET	75 %	98.98	98.65	99.852
	100 %	99.02	100.36	97.31
	125 %	101.52	99.54	98.65

Linearity and range:

Table No 5: Linear equation parameters

DRUG	Wavelength	For LRC Method			For Cramer's method (CRM)		
		Linear equation	R^2	Range µg/ml	Linear equation	R^2	Range µg/ml
GLIC	210	y=0.0695x+0.1673	0.997		y=0.0695x+0.1673	0.997	
	236	y=0.0356x-0.0389	0.998		y=0.0356x-0.0389	0.998	
MET	210	y=0.0792x+0.1169	0.998	2-10	y=0.0792x+0.119	0.998	2-10
	236	y=0.0352x+0.1854	0.998		y=0.0352x+0.184	0.998	

Precision:

Table No 6: Percentage RSD for all the methods

DRUG	Intraday precision (%RSD)				Interday precision (%RSD)			
	CON	LRC	CRM	MLS	CON	LRC	CRM	MLS
GLIC	4	0.58	1.98	0.22	4	1.21	1.32	0.96
	6	1.02	1.69	1.25	6	1.08	1.45	0.85
	8	1.14	1.26	1.69	8	1.37	1.08	1.80
MET	4	1.45	0.74	0.963	4	1.48	1.06	1.08
	6	1.28	1.85	1.59	6	1.54	1.14	1.25
	8	1.34	1.25	1.26	8	1.35	1.61	0.95

The proposed spectrophotometric method was found to be linear and the data is presented in the Table No 5. The intra-day and inter-day precision values for both the chemometric designs were presented in Table No 6. Accuracy was performed in terms of the Percent recovery values and the values for Gliclazidenad Metformin by all the chemometric designs were presented in Table No 4. The assay of the commercial formulation of the drugs was performed and their percentage assay values were presented in Table No 3.

Abbrevations:

GLIC : Glicazide

MET : Metformin

RESULTS AND DISCUSSION

The methods hold good linearity for GLIC from 2-10 µg/ml and MET from 2-10 µg/ml with regression coefficient values of 0.982, and 0.991 respectively. The intraday and inter-day precision was found to be less than 2% RSD. The percentage recovery and percentage assay was in the range of **85-105%** for Glicazide (GLIC) and Metformin (MET) by all the methods.

CONCLUSION

The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the three drugs and moreover they are effective in minimizing the errors in analysis, simple and economical. Finally it is concluded that the developed methods were simple and accurate can be used in routine analysis.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

This is a non-funding research work. There were no conflicts of interest.

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