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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLICLAZIDE AND SITAGLIPTIN PHOSPHATE MONOHYDRATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

The objective of this study was to develop a simple, specific, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM) in bulk and pharmaceutical dosage form. In the present work, SHIMADZU HPLC with UV detector LC 10AT VP with analytical column Phenomenex Luna (C18) A 100RP Column, 250mm x 4.6mm x 5µm, an injection volume of 20µl was injected and eluted with mobile phase Water: Acetonitrile (40:60) pumped at a flow rate of 1.0ml/min. Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM) were eluted at 3.268 and 2.260 min. The detection was carried out at a wavelength of 253nm. The method has shown good linearity in the concentration range of 5-25µg/ml for Gliclazide and 20-100µg/ml for Sitagliptin phosphate monohydrate with a correlation coefficient of (r^2) 0.9997 and 0.9940. The percentage assay values were found to be 100.01 and 99.3 for Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM). Limit of detection and limit of quantification were found to be 0.4364µg/ml and 1.3232 µg/ml for Gliclazide, 0.6 -µg/ml and 1.9 µg/ml for Sitagliptin Phosphate Monohydrate respectively. The method was validated for system suitability, linearity, accuracy, precision, robustness and ruggedness of the sample solution.

KEYWORDS: Gliclazide, Sitagliptin Phosphate Monohydrate, Reverse Phase - HPLC, Validation, Simultaneous estimation.

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INTRODUCTION

Gliclazide (GLZ) 1 - (1-azabicyclo - [3, 3, 0]-oct-3-yl (p-tolyl sulfonyl urea (Fig.1), is a potential oral hypoglycemic agent used in the treatment of Type-II diabetes mellitus. The drug shows good tolerability, low incidence of hypoglycemia and it has potential for slowing the progression of diabetic retinopathy.Sitagliptin Phosphate is a dipeptidyl - peptidase inhibitor (DPP-4 inhibitor) that has been approved for the therapy of Type-II diabetes mellitus. Sitagliptin Phosphate monohydrate (SPM) inhibits the enzyme DPP-4, this enzyme breaks down the incretins GLP-1 and GIP. The fixed dose combination of both drugs allows broad and complementary effect on antidiabetic actions. Both drugs have possible additive effect on glycemic control and reduced glycosylated hemoglobin (HbA1C) levels. The literature survey reveals that there is no RP-HPLC method was developed for simultaneous analysis of Gliclazide and Sitagliptin Phosphate monohydrate[1-9]. So, the main aim of the present analytical method is to develop simple, precise, accurate, rapid and economical RP-HPLC method for the assay of Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM) in bulk and tablet formulation.

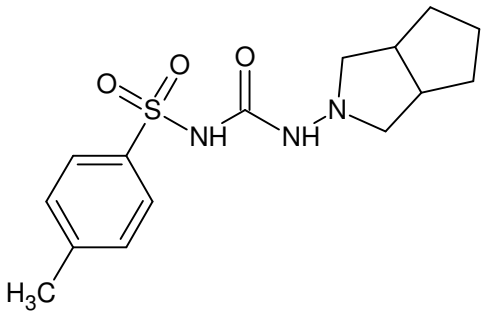

Figure 1
Chemical structure of Gliclazide

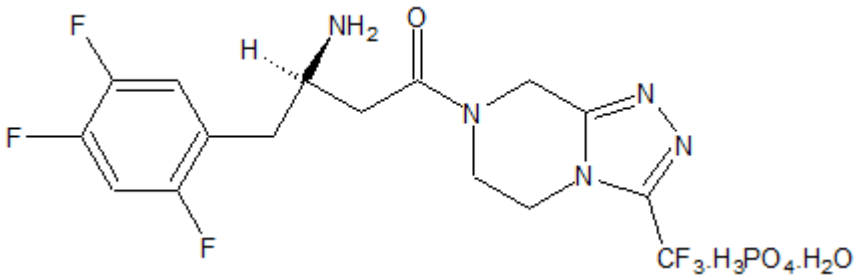

Figure 2
Chemical Structure of Sitagliptin Phosphate Monohydrate

MATERIALS AND METHODS

Gliclazide, Sitagliptin Phosphate Monohydrate gift samples were provided by MSV Labs, Hyderabad, India. All other chemicals were used of HPLC grade.

(i) Selection of Wavelength of Detection

The standard stock solution of 100µg/ml solution of Gliclazide and Sitagliptin Phosphate Monohydrate was scanned within the wavelength region of 200-400nm against methanol as blank. By observing the spectrum of standard solution, absorption maximizes at 226nm and 267nm for Gliclazide and Sitagliptin Phosphate Monohydrate. UV spectra of both drugs are shown in Figure.3 & Figure.4

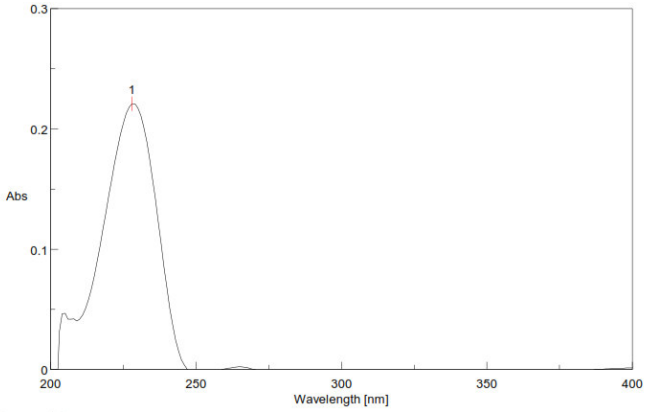

Figure 3
λmax of Gliclazide

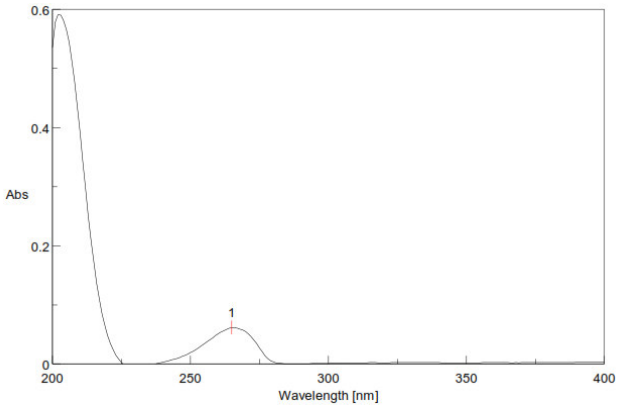

Figure 4
λmax of Sitagliptin Phosphate Monohydrate

(ii) Mobile Phase

Mix water and Acetonitrile in the ratio of 40:60 v/v.

(iii) Preparation of Diluent

Diligent into consideration, Water and Acetonitrile are selected as the solvent after the solubility studies of both the drugs.

(iv) Preparation of Standard solution

6mg of Gliclazide (GLZ) and 10mg of Sitagliptin Phosphate Monohydrate (SPM) were accurately weighed and dissolved with diluent in a 100ml volumetric flask and the solution was made up to 100ml to get the concentration of 100µg/ml. Then further dilution with the mobile phase to obtain the concentration of 10µg/ml for Gliclazide and 10µg/ml for Sitagliptin Phosphate Monohydrate.

(v) Preparation of Sample Solution[10]

Twenty tablets were weighed and powdered well. The quantity of powder equivalent to 60mg of Gliclazide and 100mg of Sitagliptin Phosphate Monohydrate was transformed to 100ml volumetric flask. The content was dissolved with a few drops of mobile phase and makes the required volume with water, sonicated for 20minutes and 10ml solution was further diluted with mobile phase to obtain 10µg/ml for Gliclazide and 10µg/ml for Sitagliptin Phosphate Monohydrate.

(vi) Optimized Chromatographic Conditions

Mode of operation: isocratic
Stationary Phase: Phenomenex Luna C18A 100 C18 column (250mm x 4.6mm x 5µm)
Mobile Phase: Water: Acetonitrile
Ratio: 40:60
Diluent: Water and Acetonitrile
Flow rate: 1.0ml/min
Temperature: 25°C

Sample volume: 20µl

METHOD VALIDATION[11, 12]

The method was validated in terms of the following parameters, system suitability, linearity, LOD, LOQ, specificity, accuracy, precision, robustness and ruggedness as per the ICH guidelines.

(i) System Suitability

The chromatographic conditions were set as per the optimized parameters and the mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Six replicates of working standard solution are injected and the Chromatograms are recorded. The % relative standard deviation (%RSD) of retention time, asymmetry, theoretical plate count and peak areas were determined and the results were shown in Table 1.

(ii) Linearity

Accurately measured volume of the standard stock solution was diluted with diluents to get the final concentrations of Gliclazide standard as 5-25µg/ml and Sitagliptin phosphate standard as 20-100µg/ml respectively. Six different concentrations of the mixed standard drugs of Gliclazide and Sitagliptin Phosphate were prepared for linearity studies and injected into the system (n=6). The response was measured as peak areas. Each concentration was prepared from individual stock solution. The peak areas were plotted versus concentrations to get the calibration curve.

(iii) Detection limit and quantification limit [LOD and LOQ]

The sensitivity of the simultaneous method of Gliclazide and Sitagliptin Phosphate Monohydrate is estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated using the formula.

LOD = 3.3 x σ/S

LOQ = 10 x σ/S

σ = Standard Deviation of Response

S = Slope of the calibration curve

The results were shown in Table.1

(iv) Specificity

The specificity of the method was performed by injecting a blank solution (without any sample) and then a drug solution of 10µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Gliclazide and Sitagliptin Phosphate Monohydrate.

(v) Accuracy

The accuracy was carried out by adding known amounts of each analyte corresponding to three concentration levels (80%, 100%, 120%) of the label claim to the excipients. At each level, six determinations were determined and the results are expressed as a percentage. Analyte recovered by the proposed method. The results are given in the Table.3

(vi) Precision

The precision of the method was assessed by studying intra-day and inter-day variation. In the intra-day studies, standard and sample solutions were analyzed in triplicate on the same day and % RSD was calculated. In case of inter-day studies, standard and sample solutions were analyzed in triplicate on three consecutive days and % RSD were calculated. The results are shown in the table.4

(vii) Robustness

Robustness is a measure of capacity of analytical methods to remain unaffected by small deliberate variation of the operating conditions. It was tested by changing the flow rate, temperature and wavelength by ±2 nm. The results are shown in the Table.5

(viii) Ruggedness

The ruggedness of the method was analyzed in different days and different chemists to check for any changes in the chromatograph, % RSD for the retention time and the area was calculated. The results are shown in the Table.6

RESULTS AND DISCUSSION

Method Validation

Chromatographic separation was achieved on C18 column, mobile phase consisting of a

mixture of water: acetonitrile with detection of 253nm. The retention time was found to be 3.284 min and 2.260 min for Gliclazide and Sitagliptin phosphate monohydrate. Optical chromatogram was shown in figure 5.

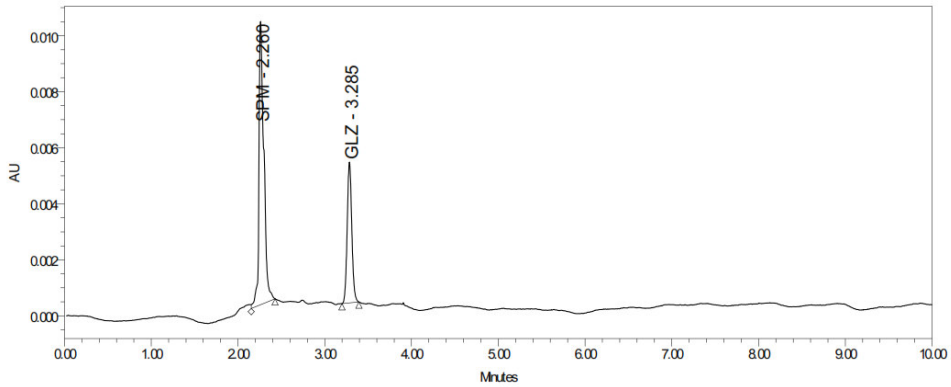

Figure 5
Optimized Chromatogram of Gliclazide and Sitagliptin Phosphate Monohydrate

(i) System Suitability

The RSD values of peak area and retention time for both drugs are with 2%, which is shown in Table.1

Table 1
System Suitability of GLZ & SPM

Parameters	GLZ	SPM
Area	983.62	1910.23
± SD	13.09	29.82
± RSD	0.13	0.14
Retention Time (Min)	3.268	2.260
Theoretical Plate Count	3950.09	2410.14
Asymmetry	1.23	1.12
LOD	0.4364	0.6
LOQ	1.3232	1.9

(ii) Linearity

The calibration was linear in the concentration range of 5-25µg/ml for Gliclazide and 20-100µg/ml for Sitagliptin phosphate monohydrate respectively and the regression coefficient (r^2) 0.9997 and 0.9940. The results of validation parameters were shown in Table- 2.

Table 2
Validation Parameters

S. No	Parameters	Results	
		GLZ	SPM
1	Linearity (µg/ml)	5-25	20 – 120
2	Correlation Coefficient (r^2)	0.997	0.994
3	Slope	80.371	98.623
4	Y –intercept	16.429	489.54

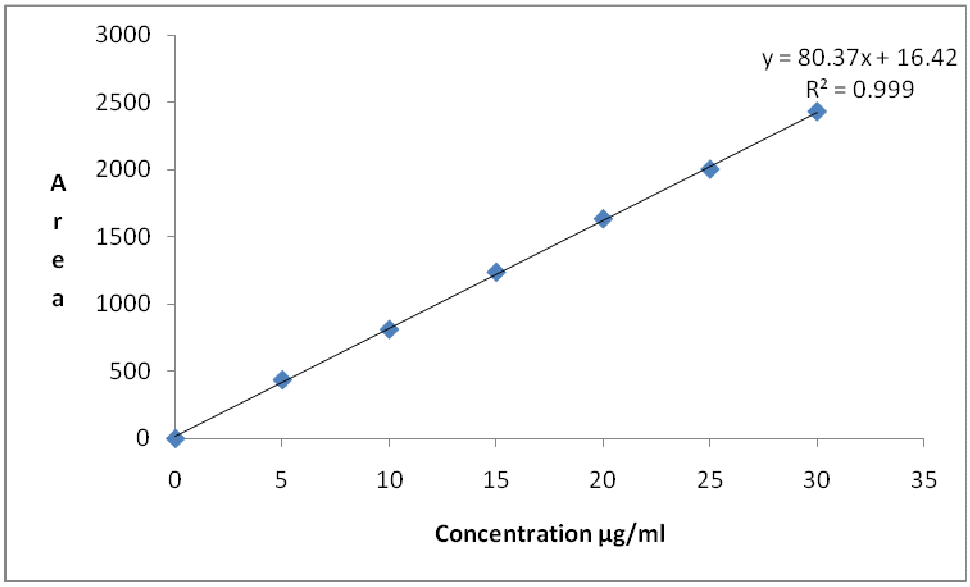

Figure 6
Linearity of Gliclazide

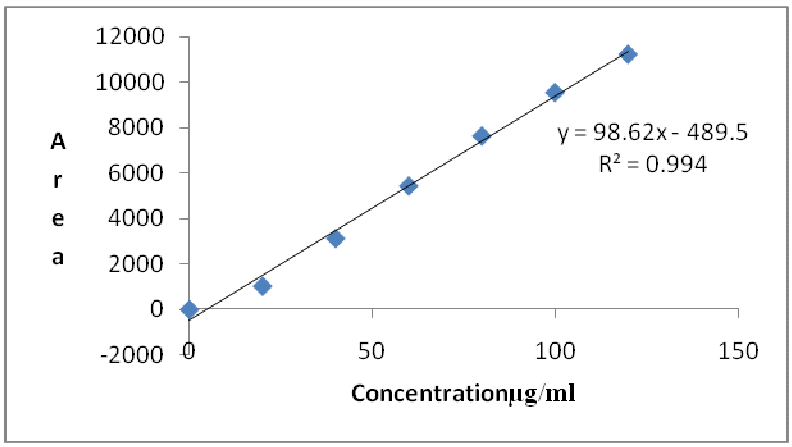

Figure 7
Linearity of Sitagliptin Phosphate Monohydrate

(iii) Detection limit and quantification limit (LOD and LOQ)

LOD and LOQ for Gliclazide (GLZ) were 0.4364µg/ml and 1.3232µg/ml and for Sitagliptin Phosphate Monohydrate (SPM) were 0.6µg/ml and 1.9 µg/ml respectively. The results were shown in Table-1

(iv) Specificity

The specificity of the RP-HPLC method was determined by the complete separation of Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM) as shown in Figure 5. The peaks obtained for GLZ and SPM were sharp and have a clear baseline separation.

(v) Accuracy

The average percentage recoveries obtained are 100.4 and 100.2 for Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM) indicates this method is accurate. And the results were shown in Table-3.

Table 3
Results of accuracy studies for GLZ and SPM

Spiked level of drug %	Amount of drug added (µg/ml)		Amount of drug found (µg/ml)		% of the recovery	
	GLZ	SPM	GLZ	SPM	GLZ	SPM
20	5	20	10.02	18.23	99.4	100.2
40	10	40	10.09	39.37	99.8	100.1
60	15	60	14.04	79.10	99.7	100.3
80	20	80	20.27	80.10	100.2	100.04
100	25	100	25.08	100.4	100.4	100.02

(vi) Precision

The precision results obtained with the method, shown in Table-4 indicating good precision.

Table 4
Results of precision for GLZ and SPM

Sample	Assay of GLZ		% Label claim	Assay of SPM		% Label Claim
	Mean area	mg/ml		Mean area	mg/ml	
1	984.12	60.01	100.01	1910.25	98.73	98.73
2	985.42	60.02	100.02	1913.17	99.30	99.30
3	983.27	59.99	99.98	1914.73	98.10	98.10
4	983.63	60.03	100.03	1922.28	98.72	98.72
5	983.21	59.06	98.43	1929.34	98.01	98.01
	Mean		99.69			98.56
	%RSD		0.40			0.004

(vii) Robustness

Minor changes in the experimental conditions, is important to demonstrate robustness of the method, deliberate changes in the flow rate, pH, Temperature showed significant change.The results were shown in Table-5

Table 5
Results of Robustness

Experiment	Tailing Factor		Theoretical plates	
	GLZ	SPM	GLZ	SPM
Control	1.35	1.14	983.64	1910.23
Flowminus	1.32	1.12	983.23	1893.21
FlowPlus	1.34	1.13	978.12	1896.21
Temperature Plus	1.36	1.15	963.22	1910.21
pH Plus	1.36	1.15	972.73	1912.42
pH Minus	1.32	1.15	980.15	1810.23

(viii) Ruggedness

Ruggedness was established by charging the analysts and not much change in all the parameters are observed within the limits. The results (Table-6) indicate that the method was sensitive.

Table 6
Results of Ruggedness of GLZ and SPM

Sample	Assay % Label Claim GLZ 60mg		Assay % Label Claim SPM 100mg	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
1	99.8	99.82	98.99	97.59
2	99.3	99.9	99.00	97.38
3	100.02	100.00	99.00	99.98
4	99.9	100.0	100.00	99.99
5	100.01	100.01	100.02	100.00
Mean	99.80	99.94	99.40	98.98
%RSD	1.33	0.58	0.39	0.98

CONCLUSION

The proposed method was validated in accordance with ICH guidelines and all the results obtained with this method are all within the limits. The retention time of both drugs was under 5 min and this method was not time consuming. This method can be suitable for routine analysis in laboratories. Therefore, this method is simple, accurate, rapid, reliable method for simultaneous estimation of Gliclazide and Sitagliptin Phosphate Monohydrate.

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