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Research Article

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Simultaneous estimation of Gliclazide and Sitagliptin Phosphate Monohydrate by Q-analysis method

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ABSTRACT

The aim of the present study is to develop and validate simultaneous estimation of Gliclazide (GLZ) and Sitagliptin Phosphate Monohydrate (SPM) by Q-Analysis method. The method was simple, precise, accurate, reproducible and economical. Linearity was observed in concentration range of 5 - 25 µg/ml for Gliclazide and 20 - 100 µg/ml for Sitagliptin Phosphate monohydrate. Validation was performed as per ICH guidelines.

Keywords: Gliclazide, Sitagliptin Phosphate Monohydrate, λmax, Q-analysis method.

INTRODUCTION

Gliclazide (GLZ), a sulfonyl urea derivative is used as an oral hypoglycemic agent. Chemically it is (1-3-azabicyclo [3.3.0] oct-3-yl) – 3- p-tolylsulfonylurea or 1-(hexahydrocyclopenta [c] pyrrol-2 (1H)-y1) - 3- (p-tolylsulfonyl) urea. Sitagliptin phosphate monohydrate (SPM) is an orally active Dipeptidyl peptidase 4 (DPP-4) inhibitor. Chemically, it is (3R) – 3 amino-1- [3-(trifluoromethyl)-5,6 dihydro [1,2,4] triazolo [4,3-a] pyrazin-7 (8H)-yl] – 4- (2,4,5, triflurophenyl) butan – 1- one phosphate hydrate.Both the drugs allows broad and additive effect on glycemic control. Literature survey revealed that, the combination of both the drugs in Q-Analysis method is not developed so for. Hence an attempt has been made to develop new Q-Analysis UV method for GLZ and SPM with good accuracy and simplicity.[1-7]

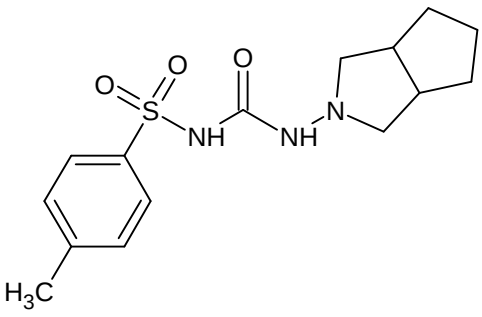

Fig .1: Chemical structure of Gliclazide

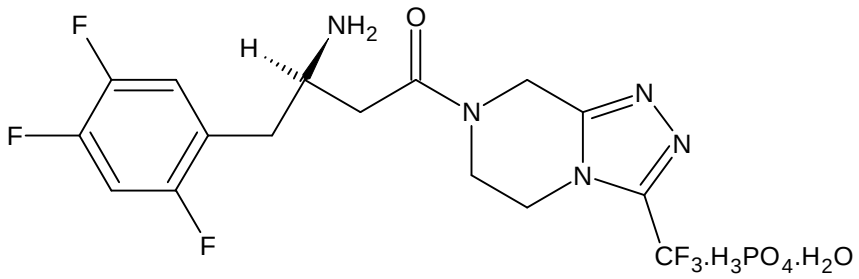

Fig .2: Chemical Structure of Sitagliptin Phosphate

EXPERIMENTAL SECTION

Materials
All the reagent and chemicals used were analytical grade.

Absorbance ratio (Q-Analysis) UV spectrophotometric method.

Apparatus
A shimadzu UV – 1700 UV/VIS spectrophotometer was used with 1cm matches quarts cell.

Selection of Solvent
Sitagliptin phosphate monohydrate is dissolved in water. Gliclazide is dissolved in methanol. So water and methanol were used as the solvent.

Preparation of standard solution
10mg of Gliclazide (GLZ) and 10mg of Sitagliptin phosphate monohydrate (SPM) were accurately weighed in 100ml volumetric flask, dissolved in few drops of methanol and make up 100ml with water. Standard solution was prepared by further diluting 1ml stock solution with 10ml water to obtain appropriate concentration of Gliclazide (GLZ) and Sitagliptin phosphate monohydrate (SPM).

Determination of λ max
Both the standard solutions were scanned separately between 200 to 400 nm. The overlay spectrum of both drugs was reordered. It showed that, both the drugs are observed at 226nm (λmax of Gliclazide) and 267nm (λ max of Sitagliptin phosphate monohydrate) and were selected as the wavelength for the estimation of drugs in absorbance ratio method. Isobestic point for the combination was found to be 248nm.

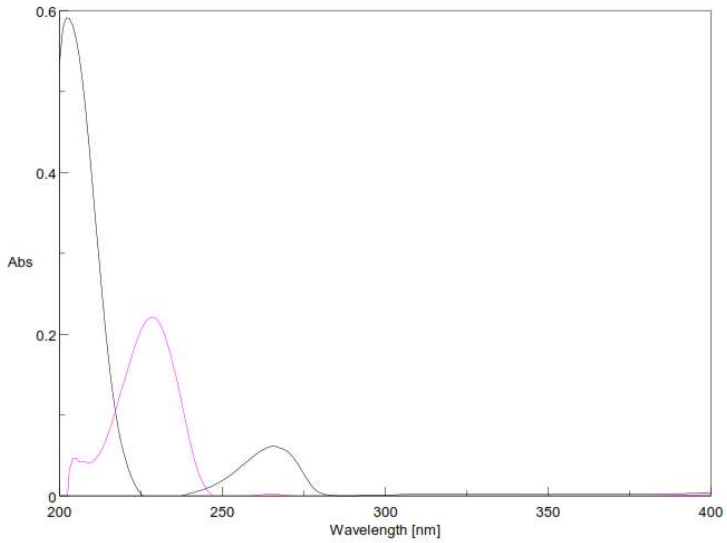

Fig. 3: Overlay spectrum of GLZ and SPM

Study of Bee Lambert's Law

Aliquots of standard stock solution of Gliclazide (GLZ) and the Sitagliptin phosphate monohydrate (SPM) were diluted in a series of 100ml flask with water to get concentration in range of 5-25 µg/ml for Gliclazide (GLZ) and 20-100µg/ml for Sitagliptin phosphate monohydrate (SPM). Similarly aliquots of mixed standard stock solution were diluted in a sereis of 100ml flasks with water to get appropriate concentration range. Absorbances of each of the resulting solutions were measured at 226nm and 267nm in 1cm cell using solvent blank.

Determination of Absorptivity value at selected wavelength:

For Gliclazide

An accurately weighed quantity of Gliclazide (GLZ) (6mg) was transferred in 100ml volumetric flask, dissolved in methanol, then dilute to the required volume with water. The above stock solution is further diluted with water to form final standard solutions.

For Sitagliptin Phosphate Monohydrate

An accurately weighed quantity of Sitagliptin Phosphate Monohydrate (SPM) (10mg) was transferred in 100ml volumetirc flask, dissolved in water, and then dilutes up to the mark with water. The above stock solution is further diluted with water to form final standard solutions. The absorbance of each of the above solutions was measure in triplicate against blank at 226nm, 267nm.

Application of proposed method for estimation in standard laboratory Mixture

An accurately weighed quantity of Gliclazide (10mg) and Sitagliptin Phosphate monohydrate (10 mg) were transferred to a 100ml volumetric flask. Few drops of methanol were added and make up the required volume with water to obtain the standard stock solution. After further adequate dilution, the absorbance was measured at 226nm and 267nm with blank. The contents were calculated by using the following formula. [8-10]

For estimation of GLZ For estimation of Sitagliptin Phosphate Monohydrate

$$Cx = \frac{Qm-Qy}{Qx-Qy} \text{ x } \frac{A}{ax} \qquad Cy = \frac{Qm-Qx}{Qy-Qx} \text{ x } \frac{A}{ay}$$

Where Cx = Concentration of Gliclazide in gm/100ml
Cy = Concentration of Sitagliptin Phosphate Monohydrate in gm/100ml
Qm = Ratio of absorbance of Laboratory mixture at 248nm and 267nm
Qx = Ratio of absorptivity of Sitagliptin phosphate monohydrate at 248nm and 267nm
ax = Absorptivity of Gliclazide
ay = Absorptivity of Sitagliptin Phosphate Monohydrate
A = Absorbance of mixture at Isoabsorpive point

Amount of drug estimated = C x D x V

Where
C = Cx or Cy = Concentration of Gliclazide or Sitagliptin phosphate Monohydrate (mg/ml)
D = Dilution factor =10
V = Volume of stock solution = 100ml.

From the amount estimated by above method percentage estimation was calculated by

% Estimation = Amount of drug estimated / weight of drug estimated X 100

The laboratory mixture study was shown in Table 1.

Accuracy study

The most widely used recovery study is performed by spiking analyte in blank matrices. spiked samples are prepared in triplicate at three levels over a range of 80%,100% and 120% of the target concentration.% Recovery was determined using the formula.

$$\text{Percentage recovery} = \frac{\text{Amount found}}{\text{Actual amound added}} \text{ x } 100$$

$$\text{Actual amount added (mg)} = \frac{\text{Amount added x Potency}}{100}$$

Results are shown in Table 2.

Method validation [11]

Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies. Results are shown in Table 2.

Precision

Precision of any analytical method is expressed as SD and RSD of series of measurements. Precision of the method was studied as intra-day and inter-day variations. A sample powder containing equivalent to 60mg of Gliclazide and 100mg of Sitagliptin phosphate monohydrate was transferred to 100ml volumetric flask, sufficient quantity of methanol was added, and sonicated for 15 minutes and diluted upto mark with water. Filter, 2ml of filtrate was further diluted to 100ml with water to get final concentration of about 6µg/ml of Gliclazide and 10µg/ml of Sitagliptin phosphate monohydrate .The absorbance of the final solution was measured after 0hr, 3hr and 6hr at 248nm and 267nm.Similarly the absorbance of the same solution was measured on day 1st, 3rd and 5th and the present assay was calculated.

The results are shown in Table 3.

Table 1: Analysis of GLZ and SPM in standard laboratory mixture

Analysis of Standard Laboratory Mixture				
Sl.No	GLZ Estimated	SPM Estimated	% Estimation GLZ	% Estimation SPM
1	10.04	10.03	100.4	100.3
2	10.16	10.15	101.6	101.5
3	10.05	10.08	100.5	100.8
4	9.96	10.14	99.6	101.4
5	10.07	10.16	100.7	101.6
	Mean±	SD	100.56±	101.12±
	%RSD		0.16455	0.10081

Table 2: Standard Addition Technique for Determination of GLZ and SPM using Absorption ratio method (n=3)

Compound (mg)	Amount added (%)	Amount Found (mg)	Recovery (%)	Average (%)
Gliclazide (80)	80	79.64mg	99.55	99.9
	100	80.10mg	100.12	
	120	80.04mg	100.05	
Sitagliptin phosphate monohydrate (100)	80	99.85mg	99.85	99.94
	100	100.05mg	100.05	
	120	99.92mg	99.92	

Table 3: Precision studies of proposed absorption ratio method

Drug	Concentration mg/ml	Intraday found concentration ± SD%	Average %	Interday found concentration ±SD%	Average %
Gliclazide	6	100.26	100.9	100.15	100.06
		100.04		100.02	
		99.98		99.86	
Sitagliptin phosphate monohydrate	10	99.61	99.89	99.6	99.96
		100.05		100.05	
		100.02		100.07	

Linearity and Range

A series of working standard solutions of GLZ and SPM were prepared in the concentration range from 5-25µg/ml and 20-100µg/ml.The absorbance of resulting solution was measured at 226nm and 267nm using solvent blank.The graphs were constructed as concentration versus absorbance and found to be linear as depicted in fig 4 and 5.

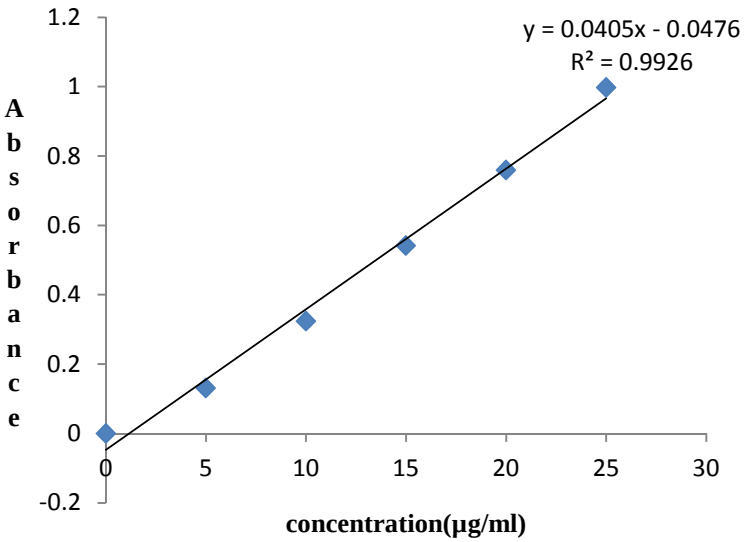

Fig 4: Calibration curve of Gliclazide

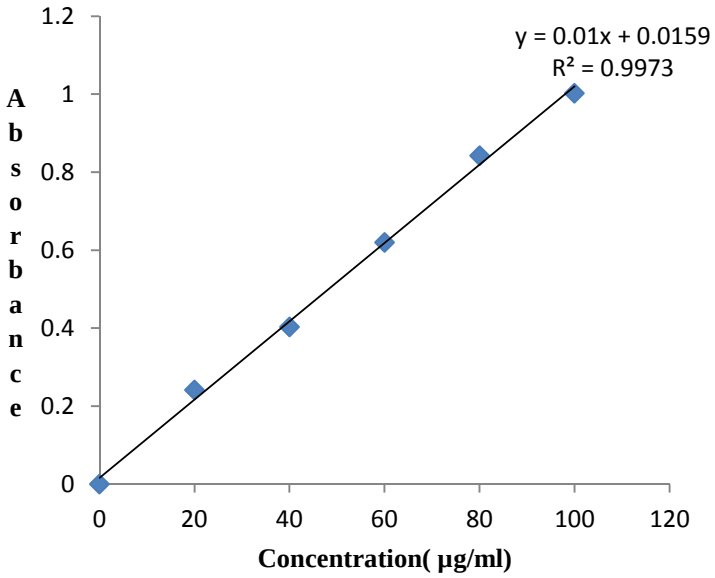

Fig 5: Calibration curve of Sitagliptin phosphate monohydrate

Ruggedness

Ruggedness of the proposed method was determined by analyzing aliquots from homogeneous slot by two analyst using same operational and environmental conditions. The results are shown in Table 4.

Table 4: Ruggedness study

Study by Analyst1

Analyst 1	GLZ	SPM
Mean (% Assay) ± SD	100.07 ±	99.87 ±
% RSD	0.1632	0.0995

Study by Analyst 2

Analyst 2	GLZ	SPM
Mean (% Assay) ± SD	100.07 ±	99.87 ±
% RSD	0.1632	0.0995

RESULTS AND DISCUSSION

From the Literature review, there is no absorbance ratio UV spectrophotometric method was developed for GLZ and SPM.Fig.3 shows overlay spectrum of GLZ and SPM. In this method, the maximum absorbance was measured at 226nm, 267nm for GLZ and SPM respectively. The isobestic point was found at 248nm.The intra-day precision was performed by relative standard deviation of repeated assays of samples at the three concentration levels. Inter-day precision by analyzing the same set of samples of three different days, the results indicates good precision. The recovery study results are showed in Table 2 and it indicates the method is accurate. The linearity of the method was statistically confirmed and the correlation coefficient (r^2) is not less than 0.99 for both drugs. Ruggedness was established by changing the analysis and all parameters were observed within the limits .The Absorbance ratio UV spectrophotometric method was validated.

CONCLUSION

Absorptive ratio method was developed and validated as per ICH guidelines for GLZ and SPM. The results attained in the study indicate that, the prepared method was found to be accurate, simple, rapid and economical for routine analysis.

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