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Research Article

SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND PIOGLITAZONE BY UV-SPECTROSCOPIC METHOD AND STUDY OF INTERFERENCE OF VARIOUS EXCIPIENTS ON THIS COMBINATION OF DRUGS

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ABSTRACT

New spectrophotometric method has been developed for the simultaneous estimation of Sitagliptin phosphate and Pioglitazone hydrochloride in bulk. This method was based on the application of Vierodt's method which involves the formation and solving of simultaneous equations at 267nm and 269nm, as absorbance maxima of Sitagliptin and Pioglitazone respectively. The Calibration curves were linear with correlation coefficient of 0.9999 over the concentration range of 20-120µg/ml for Sitagliptin and 2.5-25µg/ml for Pioglitazone respectively. The mean percent recovery was found to be 101.3±0.88 and 94.5±3.47for Sitagliptin and Pioglitazone respectively. The results of analysis were validated statistically. The proposed simple UV method was rapid, accurate, precise and economical and can be used in the quality control of pharmaceutical formulations and routine laboratory analysis. The drug excipient interference study was carriedout on various commonly used excipients.

Keywords: Sitagliptin, Pioglitazone, Spectroscopy, Excipients.

INTRODUCTION

Sitagliptin phosphate (SIT) is chemically((R)-4-oxo-4-[3(trifluoromethyl)-5,6 dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine) is an oral antihyperglycemic (ant diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class.[1]It works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4).

Pioglitazone hydrochloride (PIO) is chemically [(±)-5-[[4-[2-(5-ethyl-2pyridinylethoxy] phenyl]methyl] -2, 4-] thiazolidinedione monohydrochloride. It is a potent agonist for peroxisome proliferator-activated receptor gamma (PPARγ).[2]A potential novel combination in development brings together the dipeptidyl peptidase-4 (DPP-4) inhibitor Sitagliptin with the thiazolidinedione Pioglitazone into a fixed-dose single-tablet combination. The former component acts mainly to increase prandial insulin secretion; the latter improves insulin sensitivity.[3,4,5]

There are various methods like UV[6], HPTLC[7], andHPLC[8,9] for estimation of SIT and PIO alone or in combination with other drugs in various dosage forms But there is no method reported for simultaneous estimation of SIT and PIO in bulk.

MATERIALS AND METHODS

Instrument

A Perkin Elmer UV-VISIBLE spectrophotometer model no: Lamda-35 with spectral bandwidth of 1nm, scanning speed of 120nm/sec, wavelength accuracy of ±0.1nm and a pair of 1cm quartz were used in the present study.

Materials

Sitagliptin phosphate (SIT) and Pioglitazone hydrochloride (PIO) were procured as a gift samples from Dr.Reddys Labs Ltd. Hyderabad India.

Solvents used

Double distilled water, 0.1N Hydrochloricacid and Methanol.

Selection of solvent

A little amount of both the drugs were taken individually and their solubility was tested in various solvents.

Table 1: Solubility Profile

Solvent	Sitagliptin	Pioglitazone
Water	Soluble	Insoluble
Methanol	Sparingly soluble	Soluble
Water: Methanol (1:1v/v)	Soluble	Soluble but precipitates
0.1N HCl	Soluble	Soluble

Preparation of 0.1N HCl

8.5ml of 35% hydrochloric acid was accurately measured and transferred into a 1000ml volumetric flask and the volume was made upto the mark with distilled water.

Preparation of standard stock solution

Standard stock solutions of Sitagliptin and Pioglitazone were prepared by dissolving 10mg of each drug in 0.1N HCl and making up the volume to 10ml in two different volumetric flasks to get 1mg/ml.

Determination of λmax

From the stock solutions, a working standard was prepared. The absorption spectrum for Sitagliptin was recorded and the maximum absorption was found to be 267nm. Similarly the stock solution of Pioglitazone was diluted to get 10µg/ml and it was observed that it shows two absorption maxima at 269 nm.

Construction of calibration curve

The calibration curves were constructed for Sitagliptin and Pioglitazone in the concentration range of 20-120µg/ml and 2.5-25 µg/ml at 267 and 269nm by diluting aliquot portions of stock solution of each drug. The correlation coefficient was found to be 0.9999 and 1.0000 for Sitagliptin and Pioglitazone. The results are shown in the Table 3 and Fig. 2 and 3.

Preparation of mixed standards

Sitagliptin and Pioglitazone combination is available in the dose ratio of 100:30 mg,considering this, serial dilutions of mixed standards were prepared and linearity was observed.

Application of method (simultaneous equation method)[10]

From the overlain zero order spectra of Sitagliptin and Pioglitazone, 267nm and 269nm were selected as the wavelengths for this estimation. A set of two simultaneous equations were formed using absorptivity coefficients A(1cm, 1%)at selected wavelengths.

The molar absorptivity values are shown in the Table 3, are substituted in the following equations:

At $\lambda_1 A_1 = ax_1 bc_x + ay_1 bc_y$---------- (1)

$A_1 = 0.03757Cx + 0.2150Cy$---- (2)

At λ_2 $A_2 = ax_2 bc_x + ay_2 bc_y$---------- (1)

$A_2 = 0.0335Cx + 0.2215Cy$ ----- (2)

Cx and Cy are the concentrations of x and y.

A_1 is the absorbance of mixture at λ_1

A_2 is the absorbance of mixture at λ_2

ax_1 is the absorptive value of x at λ_1

ax_2 is the absorptive value of x at λ_2

ay_1 is the absorptive value of x at λ_1

ay_2 is the absorptive value of x at λ_2

The concentration of two drugs in the mixture was calculated using the two simultaneous equations. Statistical parameters like slope, intercept, correlation coefficient, standard deviation and relative standard deviation were calculated.

Concentration of drugs in synthetic mixture:

Sitagliptin and Pioglitazone combination is effective in the dose ratioof 100mg: 30mg. based on this synthetic mixture of Sitagliptin and Pioglitazone were prepared by taking 10mg and 3mg respectively and dissolving it in 50ml and various dilutions are made to apply this method. The results are shown in Table 2.

Validation of the Method[11]

The developed method was validated in accordance with ICH guidelines with respect to Linearity, LOD, LOQ, Accuracy, and Precision. The results are indicated in Table 3,4,5,6,7.

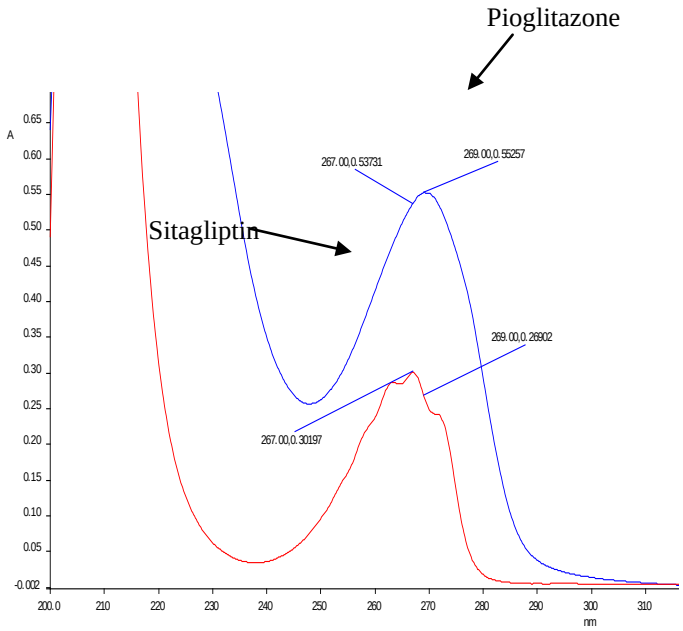


Fig. 1: Overlain spectra of SIT and PIO

Table 2: Concentration of two drugs in mixture

Mixture	C_XSIT	C_YPIO	Recovery %	
			Sit	Pio
Syn Mix. 1	49.5 mg	14.7 mg	99	98
Syn Mix. 2	49.1 mg	14.9 mg	98.2	99.3
Syn Mix. 3	49.03mg	14.4 mg	98.6	96

Table 3: Linear regression analysis of calibration curves with their absorptivity values

Concentration		267 nm				269 nm			
		Absorbance		Absorptivity		Absorbance		Absorptivity	
SIT	PIO	SIT	PIO	SIT	PIO	SIT	PIO	SIT	PIO
20	2.5	0.07594	0.05396	0.03797	0.21584	0.06771	0.05567	0.033855	0.22268
40	5	0.15046	0.1059	0.037615	0.2118	0.13433	0.10913	0.033583	0.21826
60	10	0.2278	0.21577	0.037967	0.21577	0.20305	0.22208	0.033842	0.22208
80	15	0.30197	0.32368	0.037746	0.215787	0.26903	0.33277	0.033629	0.221847
100	20	0.37031	0.43257	0.037031	0.216285	0.33127	0.44507	0.033127	0.222535
120	25	0.44514	0.53731	0.037095	0.214924	0.39593	0.555257	0.032994	0.222103
Average				0.037571	0.215068			0.033505	0.221584
Standard Deviation				0.000416	0.001661			0.000364	0.001658
% RSD				1.108077	0.7721			1.085195	0.748084

Table 4: Recovery study of synthetic mixtures of SIT and PIO

Percentage Level	Absorbance		SIT		PIO		% Recovery	
	267	269	Amount found	Amount added	Amount found	Amount added	SIT	PIO
80%	0.3839	0.3745	40.34	40.1	10.86	11.85	100.5	91.6
100%	0.4896	0.4775	51.3	50.125	13.86	14.81	102.3	93.5
120%	0.58	0.57	60.9	60.15	17.485	17.77	101.2	98.3
Mean Recovery							101.3±0.88	94.5±3.47

Table 5: Results of precision study

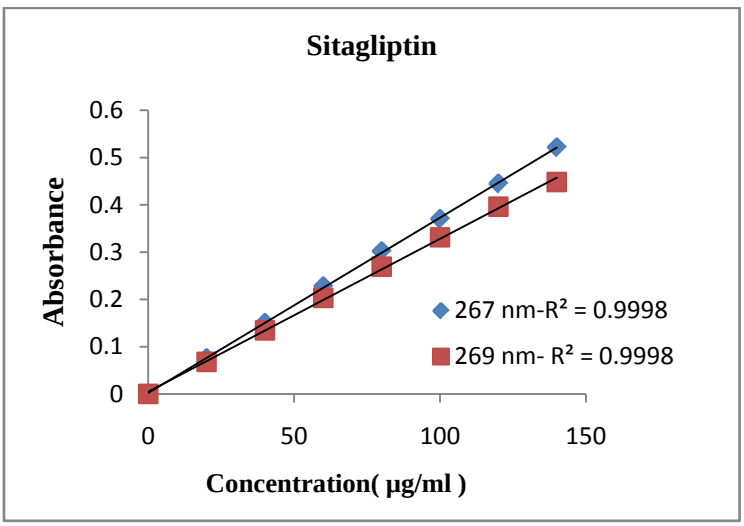
S. No	Intermediate Precision		Measurement Precision
	Intra day	Inter day	
1	0.5022	0.5022	0.4986
2	0.5013	0.5050	0.4997
3	0.5052	0.4929	0.5022
Average	0.5029	0.5000	0.5002
Std Dev	0.0020	0.006	0.001
% RSD	0.4042	1.26	0.36

Table 6: Excipient Interaction Study

S. No.	Excipient	Amount Added	Percentage Recovery	
			SIT (%)	PIO (%)
1	Calcium Hydrogen Phosphate	50 mg	99.9	100.1
2	Sod. Stearate	50 mg	97.9	98.8
3	Cellulose	50 mg	81.1	87.5
4	Mg. Stearate	50 mg	95.2	101.4
5	PVP	50 mg	88.5	93.1
6	Crosscarmellose	50 mg	98.0	98.7
7	Talc	50 mg	98.5	100.5
			94.1±6.8	97.1±5.0

Table 7: Optical Characteristics of SIT & PIO

Parameter		Vierodts Method	
		SIT	PIO
1	λmax	267	269
2	Linearity Range (µg/ml)	10-200	2.5-37.5
3	Regression equation y=mx+C	y = 0.0037x + 0.0046	y = 0.022x + 0.0011
4	Slope (m)	0.0037	0.022
5	Intercept (C)	0.0046	0.0011
6	Correlation coefficient (r^2)	0.9997	0.9999
7	Sand ell's sensitivity (µg/cm^2/0.001A.U)	0.268361	0.04512961
8	LOD (µg/ml)	0.002749	0.009163
9	LOQ (µg/ml)	0.007192	0.0232975



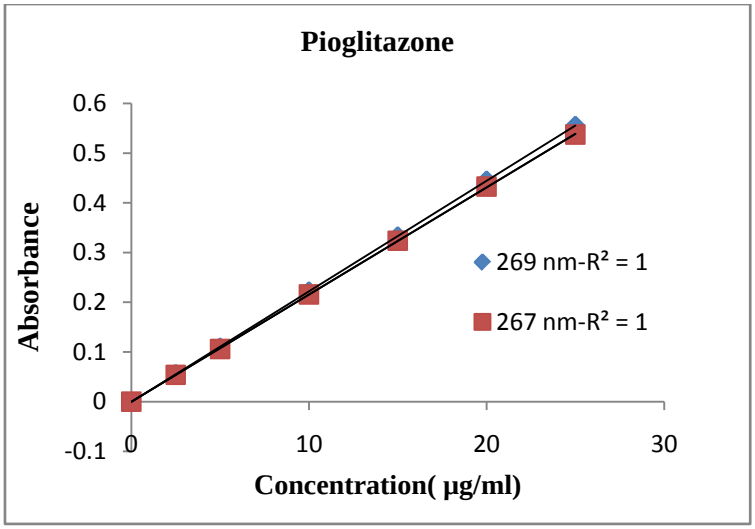


Fig. 2-3: Calibration plots

RESULTS AND DISCUSSION

The proposed method was based on the spectrophotometric simultaneous estimation of new anti-diabetic combination of Sitagliptin and Pioglitazone in UV region using 0.1N HCl. Even though the combination was sparingly soluble in methanol, 0.1N HCl was selected because it is eco-friendly and cost effective.

Sitagliptin and Pioglitazone are basic drugs with pKa values of 7.7 and 12.06 respectively. Hence both are soluble in 0.1 N HCl.

The UV spectra show the maximum absorbance at 267nm for SIT and 269nm for PIO. The above method is based on vierodt's method which involves generation and solving of simultaneous equations using absorptivity coefficient values and absorbance at 267 and 269 nm is chosen for the estimation.

Beer's law obeyed in the concentration range of 40-140 µg/ml and 5-25 µg/ml for both the drugs.

The correlation coefficient was found to be in between 0.9998 and 1.000 which shows the good linear relationship for both components.

Interference from tablet excipients was not observed in this method. The results of recovery studies were found to be 94.1±6.8 and 97.1±5.0 respectively.

CONCLUSION

The results indicate that the proposed UV spectrophotometric method is simple, rapid, precise, and accurate. The method can be easily and conveniently adopted for routine quality control analysis.

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List of Non-Standard Abbreviations used

SIT: Sitagliptin
PIO: Pioglitazone
Std Dev: Standard Deviation
RSD: Relative Standard Deviation
Exp: Excipient