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RESEARCH ARTICLE

Development and Validation of Analytical Methods for Simultaneous Estimation of Sitagliptin Phosphate and Pioglitazone Hydrochloride

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ABSTRACT

Simple, rapid, accurate and precise UV spectrophotometric and RP-HPLC methods had been developed for simultaneous estimation of Sitagliptin Phosphate (STG) and Pioglitazone Hydrochloride (PIO). The method applied was Simultaneous Equation Method (Vierodt's Method), based on measurement of absorbance of Sitagliptin Phosphate and Pioglitazone Hydrochloride at λ_{max} 267nm and 270nm respectively. Linearity was found in the concentration range of 50-250µg/ml for Sitagliptin and 5-25µg/ml for Pioglitazone with regression coefficient r^2=09997 and r^2=0.9995 respectively. RP-HPLC method was carried on Phenomenex C-18 column (150 mm × 4.6 mm, 5 µ) by using a mobile phase acetonitrile: methanol: water (30:30:40) as a mobile phase at 1.0 ml/min flow rate at 270 nm. The linearity was found to be in the range of 10-50 µg/ml and 3-15µg/ml with regression coefficient of r^2= 0.9998, and r^2=0.9996 for Sitagliptin Phosphate and Pioglitazone HCl respectively. The peak obtained were sharp having clear baseline separation with a retention time 5.6 and 2.8 min for Sitagliptin Phosphate and Pioglitazone HCl. This method is accurate and precise and can be employed for routine analysis of Sitagliptin Phosphate and Pioglitazone hydrochloride in different pharmaceutical dosage forms.

KEYWORDS

Sitagliptin Phosphate, Pioglitazone HCl, UV- spectrophotometry, RP-HPLC

INTRODUCTION

[1]Sitagliptin Phosphate and Pioglitazone Hydrochloride are antidiabetic drugs. Sitagliptin belongs to the class of Dipeptidyl peptidase-4[DPP-4] inhibitors, a new class of anti-diabetic drugs which act by increasing glucose dependent insulin release. Therapeutically DPP-4 inhibitors are used to treat type 2 diabetes alone or combination with other drugs which increases the sensitivity of

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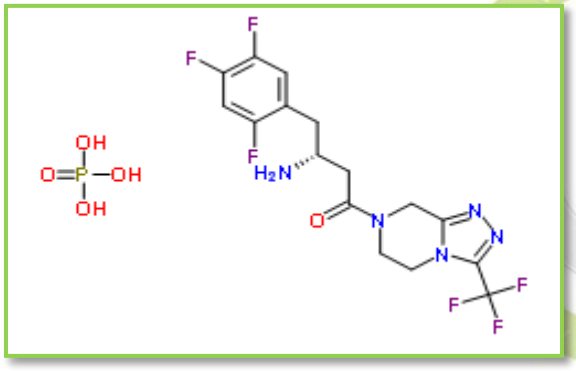
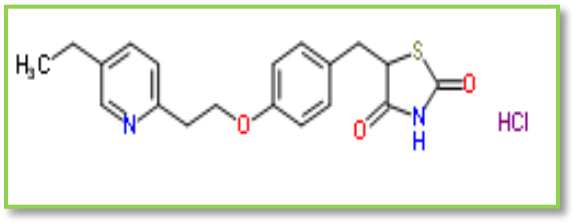
insulin at target site DPP-4 inhibitors act by inhibiting the inactivation of enteroendocrine incretins such as glucogon-like peptide-1(GLP-1) and glucose-dependent insulinotropic (GIP) polypeptide. [2]Pioglitazone is a drug belongs to the class of thiazolidinedione, which is used to decreases insulin resistance. It is an Antidiabetic agent to manage NIDDM [non-antihyperglycemic insulin-dependent diabetes mellitus, sugar diabetes) called type-2 diabetes.

There are various methods for analysis of the drugs like UV, HPLC or HPTLC. But there is no method reported for simultaneous estimation of STG and PIO in bulk and dosage form.

So, objective of the present study was to develop and validate simple, precise and accurate UV spectrophotometric and RP-HPLC method for simultaneous estimation of STG and PIO.



[1]Figure 1: Structure of STG



[2]Figure 2: Structure of PIO

MATERIAL & METHODS

Instruments

UV- VIS double beam spectrophotometer of Systronic 2201, Mumbai, with spectral bandwidth of 2nm and a pair of matched quartz cells of length 1cm were used for analytical work. Younglin Acme 9000 HPLC was used for identification and separation. All the weighing was carried out on the Electronic Balance AY220, Shimadzu, Japan. Sonication was carried out by Microclean-103.

Materials and Reagents

Pioglitazone Hydrochloride was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad. Sitagliptin Phosphate was supplied as a Gift sample by Torrent Pharmaceutical Ltd, Ahmadabad, Gujarat. The reagents used were Hydrochloric acid and Distilled water for UV-spectrophotometric method and for RP-HPLC method, Acetonitrile LiChrosolv®, Methanol LiChrosolv® and water LiChrosolv® which were procured from Merck specialities Pvt. Ltd., Mumbai.

Methods

UV- Spectrophotometric Method

Preparation of 0.1N HCl

0.85ml of concentrated Hydrochloric acid was diluted with Distilled water to make the volume of 100ml to obtain 0.1N HCl.

Preparation of Standard Stock Solution

10 mg each of STG and PIO was weighed separately and transferred to two different 10 ml volumetric flasks. Both the drugs were dissolved in 5 ml of 0.1N HCl separately in volumetric flasks and volume made up to mark with 0.1N HCl to make the concentration of 1000µg/ml. From these solutions 1ml was pipetted out separately from both the volumetric flasks and transferred to two another 10ml volumetric flasks. Made up the volume up to the mark with the same solvent to obtain the final concentration of 100µg/ml for both the drugs.

Assay of Tablet

The fixed dose combination of these drugs is 100mg of STG with 30mg of PIO. Due to unavailability of the dosage form of this combination, standard STG drug 100mg is added to the tablets of PIO of 15mg (PIOZ 15) to simulate the condition of actual product. The required number of tablets were taken, weighed

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and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 30mg of PIO was weighed and to this weighed powder, 100mg of standard STG drug was added to get 100mg:30mg combination. Now all this weighed powder was transferred to 100ml volumetric flask containing 50ml of 0.1N HCl, dissolved, made the volume up to 100ml to get the concentration of 300µg/ml and 1000µg/ml of PIO and STG respectively filtered through whatman filter paper. Now from this solution 1ml was diluted to 10 ml in other volumetric flask with same solvent to get the concentration of 30µg/ml and 100µg/ml. From above solution 5ml was diluted to 10 ml with the same solvent to get final concentration of 15µg/ml of PIO and 50µg/ml of STG.

Simultaneous Equation Method (Vierodt's Method)

If sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of other. It may be possible to determine both the drugs by the technique of simultaneous equations if criteria below are met;

i. The λ_{max} of two components is reasonably dissimilar.

ii. The two components do not interact chemically.

iii. The two absorbing drugs (X and Y), each of which should absorb at the λ_{max} of other.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

$C_x = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$ and

$C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$

Where, A_1 and A_2 are absorbances of diluted mixture at λ_1 and λ_2 respectively.

C_x and C_y are the concentrations of x and y respectively.

ax_1 and ax_2 are absorptivities of x at λ_1 and λ_2 respectively.

ay_1 and ay_2 are absorptivities of y at λ_1 and λ_2 respectively.

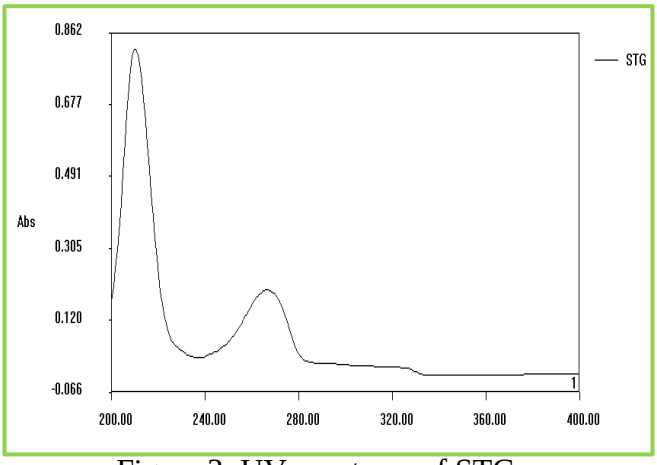

Figure 3: UV spectrum of STG

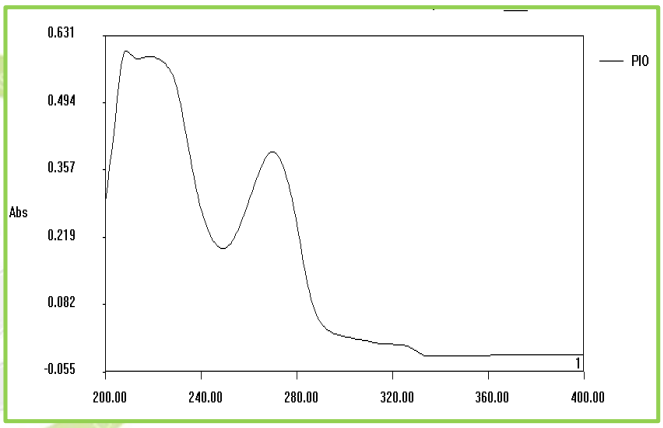

Figure 4: UV spectrum of PIO

RP-HPLC Method

Standard Stock Solution of STG

10mg of standard STG was weighed and transfered to a 10ml volumetric flask then dissolved in the water and the volume was made up to the mark with water to obtain conc. of 1000µg/ml of STG and labelled as 'Std Stock STG'.

Standard Stock Solution of PIO

10mg of standard PIO was weighed and transferred to a 10ml volumetric flask then dissolved in the methanol and the volume was made up to the mark with solvent to obtain conc. of 1000µg/ml of PIO and labelled as 'Std Stock PIO'.

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Combined Standard Stock Solution of STG and PIO

1ml of 'Std Stock STG' (1000µg/ml) and 0.3ml of 'Std Stock PIO' (300µg/ml) transferred to 10 ml volumetric flask and diluted to 10 ml with methanol to get 'Std Stock MIX AP' (100µg/ml STG and 30µg/ml PIO).

Selection of Analytical Wavelength

To investigate the appropriate wavelength for simultaneous determination of STG (10µg/ml) and PIO (10µg/ml) individual solutions in the mobile phase were scanned in the range of 200-400nm.

Selection of Mobile Phase and its Strength

The solutions of STG (10µg/ml) and PIO (10µg/ml) were prepared in water and methanol filtered through syringe filter, then injected into HPLC system. The chromatograms were analysed using different combination of Acetonitrile: Methanol: Water at a flow rate of 1ml/min for 10-30min at 270nm.

Mixed solution of STG (10µg/ml) and PIO (10µg/ml) was prepared in methanol and filtered through syringe filter, then injected into the HPLC system, after the column saturated with mobile phase and constant back pressure.

Selection of Column (Stationary Phase)

To get well resolved, symmetric peak with highest no. of theoretical plates the solution of the STG and PIO were analysed using C18 column as a stationary phase.

Chromatographic Conditions

- ✓ **Analytical Column:** Phenomenex C18 column (150 mm × 4.6 mm, 5 µm)
- ✓ **Mobile Phase:** Acetonitrile**:** Methanol: Water (30:30:40)
- ✓ **Flow Rate:** 1ml/min
- ✓ **Injection Volume:** 20 µl
- ✓ **Detection Wavelength:** 270nm

Identification of Separated Peak of the Drugs

For identification of peak of the drugs; the standard solutions of STG (10µg/ml) and PIO (10µg/ml) were injected separately into HPLC system and retention time were matched with retention time of mixture.

Method Validation

Method A

The UV spectrophotometric method was validated as per ICH guidelines Q2 R1 for method validation. The parameters evaluated were linearity, precision, accuracy, LOD and LOQ.

Linearity

This was studied by diluting standard stock solution (1000µg/ml) of STG to 50-250µg/ml and PIO (100µg/ml) to 5-25 µg/ml concentrations. Concentration curves of concentration against absorbance were plotted at their respective wavelengths for both the drugs and the obtained data was subjected to regression analysis. The standard curves for STG and PIO are shown in figure 5 and 6.

Accuracy

Recovery studies were carried out by standard addition method by adding known amount of SIT and PIO (working standard) to pre-analysed sample at three different concentration levels that is 80%, 100% and 120% of assay concentration and percent recoveries were calculated.

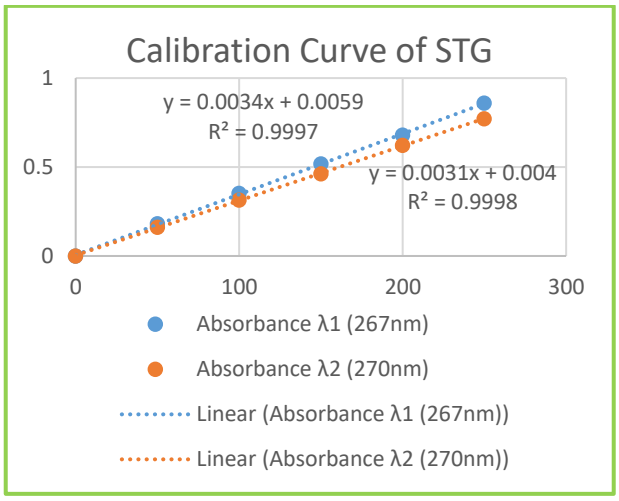

Figure 5: Calibration curve of STG of UV-Spectrophotometric method at λ_1 (267nm) and λ_2 (270nm)

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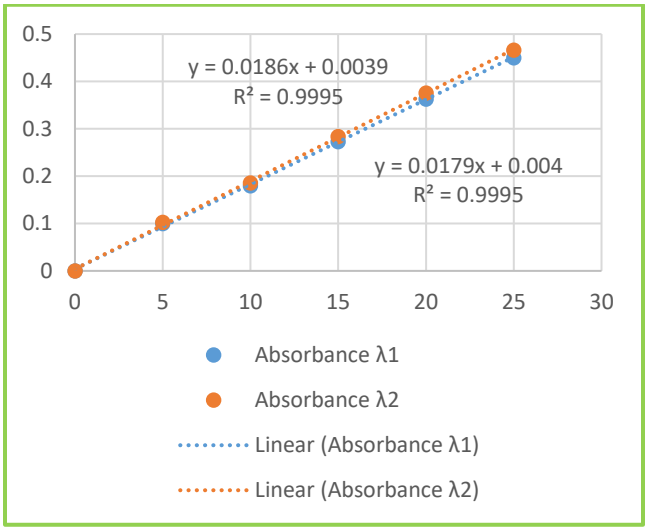

Figure 6: Calibration curve of PIO of UV-Spectrophotometric method at λ_1 (267nm) and λ_2 (270nm)

Table 1: Standard absorptivity for STG

Concentration ((µg/ml)	Absorbance		Specific Absorptivity Values	
	λ_1 (267 nm)	λ_2 (270 nm)	λ_1 (267 nm)	λ_2 (270 nm)
50	0.181	0.162	36.2	32.4
100	0.352	0.314	35.2	31.4
150	0.517	0.462	34.47	30.8
200	0.697	0.622	34.85	31.1
250	0.860	0.771	34.4	30.84
Mean			**35.024**	**31.31**

Table 2: Standard absorptivity for PIO

Concentration ((µg/ml)	Absorbance		Specific Absorptivity Values	
	λ_1 (267 nm)	λ_2 (270 nm)	λ_1 (267 nm)	λ_2 (270 nm)
5	0.100	0.103	200	206
10	0.180	0.186	180	186
15	0.273	0.284	182	189.33
20	0.363	0.376	181.5	188
25	0.450	0.466	180	186.4
Mean			**184.7**	**191.15**

Precision

Precision was ascertained by determination of six replicates of same concentrations of sample and standard for method precision and system precision. Both intraday and inter-day precisions were carried out.

Table 4: Repeatability study data for STG and Pio (n=6)

Sr No.	Concentration of STG (µg/ml)	Absorbance of STG	Concentration of PIO (µg/ml)	Absorbance of PIO
1	100	0.352	15	0.273
2		0.345		0.271
3		0.355		0.267
4		0.349		0.270
5		0.353		0.269
6		0.349		0.267
SD		0.0036		0.0023
RSD		1.03		0.852

LOD

Detection limit was determined based on the standard deviation of peak areas of same concentrations that is standard solutions of STG (150µg/ml) and PIO (15µg/ml) prepared six times and LOD calculated by

$$LOD = 3.3x (SD/S)$$

Where, SD= Standard Deviation; S= Slope of Curve

LOQ

Detection limit was determined based on the standard deviation of peak areas of same concentrations that is standard solutions of STG

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(150µg/ml) and PIO (15µg/ml) prepared six times and LOQ calculated by

LOQ= 10x (SD/S)

Where, SD= Standard Deviation; S= Slope of Curve

Table 3. Accuracy for UV- spectrophotometric Method

Sr. No.	Level of % Recovery	Amount of 'Sample Stock-A' (ml)	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Amount Recovered (µg/ml)		% Recovery	
			STG	PIO	STG	PIO	STG	PIO	STG	PIO
1	0	0.5	0	0	4.88	1.52	0	0	0	0
2	80	0.5	4	1.2	8.8	2.7	3.92	1.18	98	98.33
3	100	0.5	5	1.5	10	3.1	5.12	1.58	102.4	105.33
4	120	0.5	6	1.8	10.92	3.34	6.04	1.82	100.66	101.11

Method B:

Specificity

The chromatogram of standard solution of mixture of STG and PIO was compared with formulation to observe the interference of excipient.

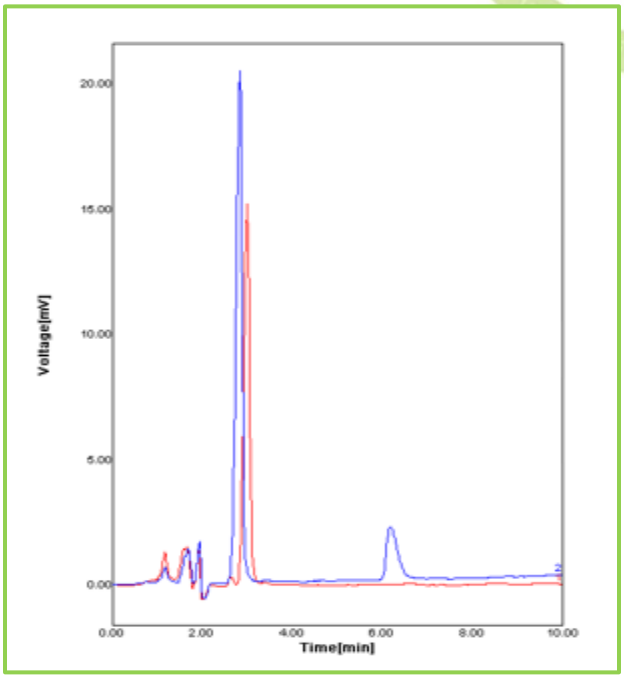

Figure 7. Overlain Chromatograms of sample and standard solution of drugs

Linearity

1, 2, 3, 4 and 5ml of 'Std Stock MIX AP' were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with methanol to obtain the conc. of 10, 20, 30, 40 and 50µg/ml of STG and 3, 6, 9, 12 and 15µg/ml of PIO. The solutions were filtered through 0.45µ syringe filter and 20µl injected into the HPLC system and their chromatogram were recorded for 10mins under the chromatographic conditions as described above after getting a stable baseline. Peak areas were recorded for all the peaks. Calibration curves of STG and PIO were constructed by plotting the peak area of STG *v/s* conc. of PIO and peak area of STG *v/s* conc. of PIO, respectively. The correlation coefficient (r^2) of least square linear regression for STG and PIO was calculated.

Table 5. Response of STG at various linearity levels

Conc. of STG (µg/ml)	Peak Area (mV)
0	0
10	48
20	92

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30	138
40	185
50	232

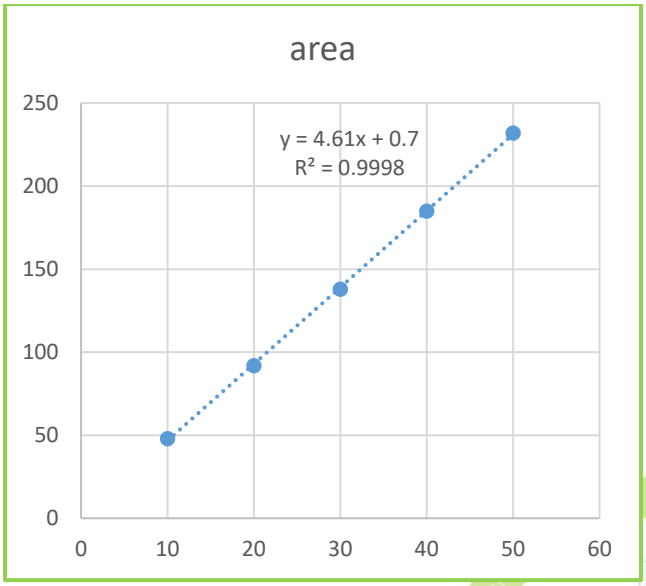

Figure 8: Calibration curve of STG of RP-HPLC method

Table 6: Response of PIO at various linearity levels

Sr. No.	Conc. of PIO (µg/ml)	Peak Area (mV)
1.	3	54
2.	6	109
3.	9	160
4.	12	217
5.	15	266

Accuracy

20 Tablets (PIOZ 30) were weighed and finely powdered, an accurately weighed tablet powder (183.1mg) equivalent to 15 mg of PIO was dissolved and diluted to 100ml with methanol. 0.5 ml of above solution was transferred in four different 10ml volumetric flask labeled as 0%, 80%, 100% and 120%. Then 0, 0.8, 1, 1.2ml of 'Std Stock MIX AP' (100µg/ml STG and 30µg/ml PIO) were added and volume was made up to the mark with mobile phase. All the solutions were filtered through syringe filter and injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions after getting a stable baseline. Peak areas were recorded and percent recoveries were calculated.

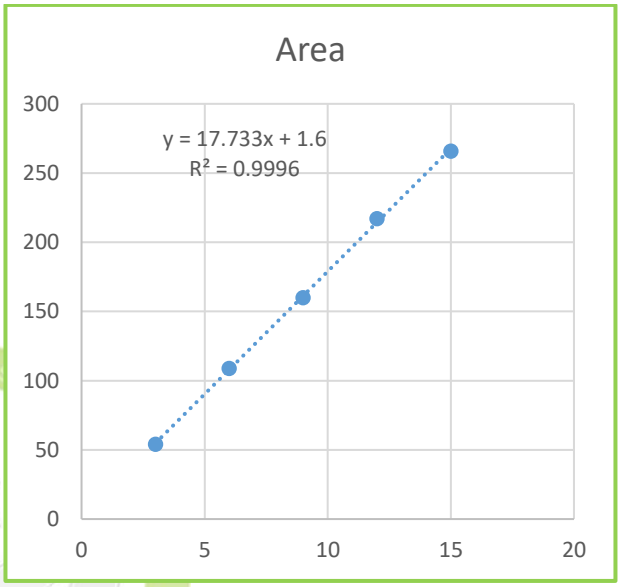

Figure 9: Calibration curve of PIO of RP-HPLC method

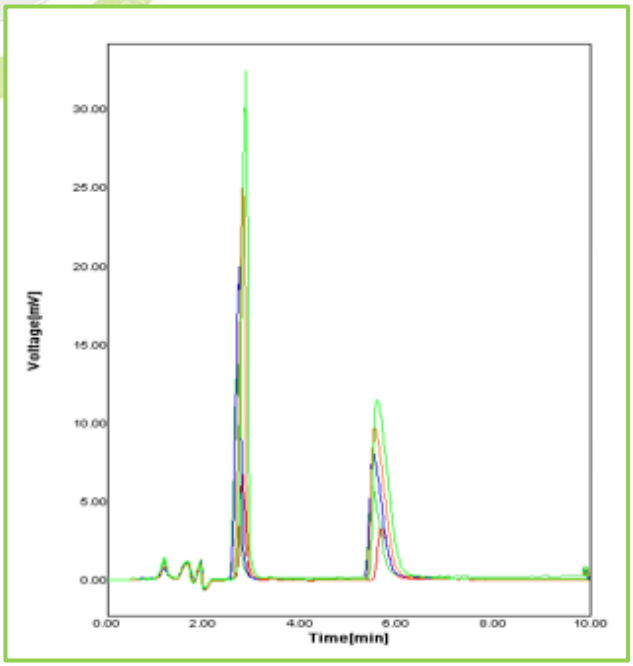

Figure 10: Overlain Chromatograms of serial dilutions of STG and PIO in optimized chromatographic conditions

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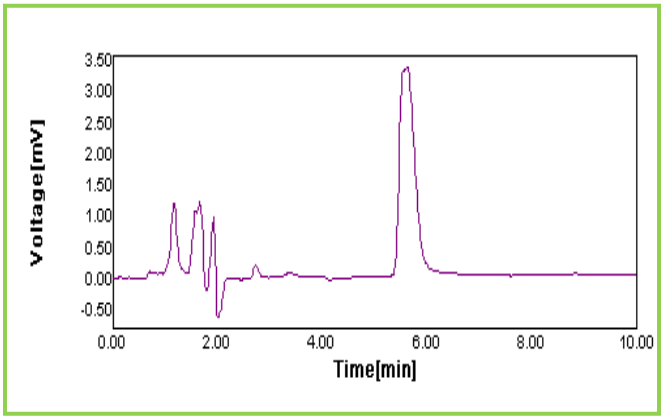

Figure 11: Chromatogram of STG (10 µg/ml) in optimized chromatographic conditions

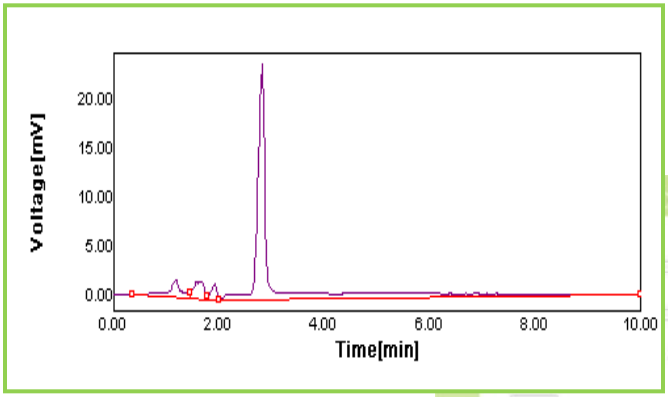

Figure 12: Chromatogram of PIO (10 µg/ml) in optimized chromatographic conditions

Precision

The precision of an analytical method was studied by performing Repeatability and intermediate precision.

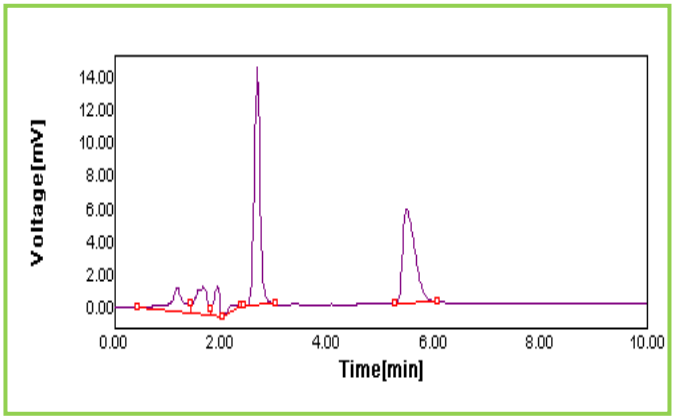

Figure 13: Chromatogram of combination of STG (10µg/ml) & PIO (10µg/ml) in optimized chromatographic conditions

Repeatability

20µg/ml of STG and 6µg/ml of PIO solution was filtered through 0.45µ syringe filter and 20µl injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated for six times.

Robustness

Combined standard solution of STG (20µg/ml), PIO (6µg/ml) was prepared and analysed at different flow rates (0.9, 1.0, 1.1 ml/min) and different wavelengths (269, 270, 271nm) separately.

Table 7. Recovery Studies

Sr. No.	Level of % Recovery	Amount of 'Sample Stock-A' (ml)	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Amount Recovered (µg/ml)		% Recovery	
			STG	PIO	STG	PIO	STG	PIO	STG	PIO
1	0	0.5	0	0	0	7.63	0	0	0	0
2	80	0.5	8	2.4	8.1	9.93	8.1	2.3	101.25	95.83
3	100	0.5	10	3	9.56	10.75	9.56	3.12	95.6	104
4	120	0.5	12	3.6	11.94	11.05	11.94	3.42	99.5	95

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Table 8: Results of Repeatability Study for STG and PIO

Inj.	Peak Area(mV) of STG	Peak Area(mV) of PIO
1	95	110
2	93	109
3	93	106
4	96	108
5	95	109
6	92	109
SD	1.54	1.37
RSD	1.5	1.2

System Suitability

Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

RESULTS AND DISCUSSION

The method has been employed successfully for quantitative determination of STG and PIO by UV spectrophotometric method and Reverse Phase High Performance Liquid Chromatographic method for the simultaneous estimation of STG and PIO and to validate the developed methods according to ICH Q2 (R1) guidelines.

Table 9: Summary of UV- Spectrophotometric Method of STG and PIO

Sr. No.	Parameters	STG	PIO
1.	Linearity Range (µg/ml)	50-250	5-25
2.	Regression Equation (y = mx+c)	y=0.0034x+0.0059	y=0.0179x+0.004
3.	Correlation Coefficient (r^2)	0.9997	0.9995
4.	LOD (µg/ml)	3.6	0.413
5.	LOQ (µg/ml)	10.90	1.26
6.	% Recovery	98-101	98-102
7.	Repeatability(%RSD)	1.03	0.852

Table 10: Summary of RP-HPLC Method of STG and PIO

Sr. No.	Parameters	STG	PIO
1.	Linearity Range (µg/ml)	10-50	3-15
2.	Regression Equation (y = mx+c)	y=4.61x+0.7	y=17.73x+1.6
3.	Correlation Coefficient (r^2)	0.9998	0.9996
4.	LOD (µg/ml)	1.1	0.25
5.	LOQ (µg/ml)	3.34	0.77
6.	% Recovery	95-104	95-102
7.	Repeatability(%RSD)	1.5	1.2

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CONCLUSION

The UV- spectrophotometric and HPLC methods are simple, accurate, precise for estimation of STG and PIO in bulk and pharmaceutical formulation. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method shows no interference by the excipients. The statistical parameters and recovery data reveals the good accuracy and precision. This method can be useful and suitable for the estimation of the STG & PIO in bulk and pharmaceutical formulations.

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