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Method Development and Validation for The Simultaneous Estimation of Teneligliptin and Pioglitazone Using Mixed Hydrotropic Solubilizing Agents Using UV-VIS Spectroscopy

Parvin Shaikh[1], Neelam Singla[2]

[1]*Research Scholar, Department of pharmaceutical sciences, Suresh Gyan Vihar University, India*
[2]*Department of Pharmaceutics, Professor, Suresh Gyan Vihar University Jaipur, India*
Email: parishaikh15@gmail.com

Background: In this work, a UV-Vis spectroscopic approach for the simultaneous estimation of pioglitazone (PGL) and teneligliptin (TNG) utilizing a combination of hydrotropic solubilizing agents is developed and validated. Both medications' solubility was markedly improved; the greatest gains were shown when 2M sodium citrate and 2M ammonium acetate were combined, yielding an 18-fold increase for TNG and a 15-fold increase for PGL. Results: For both substances, the technique showed excellent linearity within the Beer's law limits (10-50 µg/ml), with correlation coefficients (r^2) of 0.999. Recovery experiments confirmed the accuracy of the approach with mean recoveries near 100%. Low percentage relative standard deviations for the validation parameters indicated strong reproducibility and precision. Conclusions: Results that were in line with label claims were obtained from the successful application of the approach to the analysis of marketed tablet formulations. This work develops a dependable and reasonably priced method for the simultaneous analysis of TNG and PGL that may be used for regular quality control in pharmaceutical environments.

Keywords: Teneligliptin, Pioglitazone, UV-Vis Spectroscopy, Solubility Enhancement, Hydrotropic Agents, Method Validation, Pharmaceutical Analysis, Quality Control.

1. Introduction

Since pioglitazone (PGL) and teneligliptin (TNG) target distinct routes to regulate blood glucose levels, their simultaneous estimation is crucial in the treatment of type 2 diabetes mellitus. The dipeptidyl peptidase-4 (DPP-4) inhibitor TNG raises incretin hormone levels, which results in a decrease in glucagon release and an increase in insulin secretion (Kaur & Jindal, 2016). On the other hand, PGL, a thiazolidinedione, decreases insulin resistance by increasing insulin sensitivity in muscle and adipose tissues. For quality assurance and

therapeutic monitoring, these medications must be identified analytically in pharmaceutical formulations and biological matrices. High-performance liquid chromatography (HPLC) and other conventional techniques are frequently used, however they frequently call for costly equipment and solvents. Thus, it would be ideal to create a more economical and effective technique that makes use of UV-Vis spectroscopy. The solubility of weakly soluble pharmaceuticals in aqueous solutions can be improved by mixed hydrotropic solubilizing agents, which makes UV-Vis spectroscopy a feasible choice for their simultaneous assessment. The solubility of TNG and PGL can be increased by hydrotropic agents as sodium benzoate and urea, allowing for precise spectral analysis [1–3].The purpose of this work is to create and verify a UV-Vis spectroscopic technique that uses a combination of hydrotropic solubilizing agents to estimate TNG and PGL simultaneously. To ensure the method's dependability for routine analysis, its validation characteristics will comprise specificity, linearity, accuracy, precision, and robustness.

2. Material and Methods

Solubility

TNG and PGL solubility was measured at 25±1°C. In a separate 10 ml volumetric flask with a different solvent, precisely weighed 10 mg of TNG and PGL were added, and the flask was then put on a mechanical shaker for eight hours. Both solutions were filtered via Whatman filter paper No. 41 after eight hours. After being appropriately diluted, the filtrates were examined visually[4].

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

To create standard stock solutions, 10 mg of each drug were separately dissolved in 8 mL of a mixed hydrotropic solution that contained 2M Ammonium Acetate: 2M Sod. Citrate (1:1). The flask was then sonicated for approximately 10 minutes to solubilize the drug, and the volume was increased to 10 ml with the mixed hydrotropic agent to obtain a concentration of 1000 µg/ml (Stock-A) for both drugs [5].

Preparation of Sub Stock Solution (Stock-B)

Using a pipette, 1 ml aliquots were taken out of standard stock solution A of TNG and PGL, placed into a separate 10 ml volumetric flask, and diluted with 10 ml of water to achieve a concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

Using a pipette, separate aliquots of 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml were taken out of the standard stock solution (Stock-B) and placed in a 10-ml volumetric flask. The volume was then increased to 10 ml using RO water. The results for TNG were 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml, respectively. A 10 ml volumetric flask was filled with 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml of the sub stock solution (Stock-B), and the volume was increased to 10 ml using RO water. Accordingly, the PGL solutions were 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml.

Selection of wavelength for linearity

Separate solutions of 10 µg/ml TNG and 10 µg/ml PGL were made. The spectrum mode was used to scan both solutions between 200 and 400 nm. TNG and PGL showed their highest absorbances at 256.0 nm and 230.0 nm, respectively. At their respective maxima, TNG and PGL demonstrated linearity in the concentration range of 10–50 µg/ml. An absorbance versus concentration calibration curve was plotted [6].

Study of overlay spectra

The overlain spectra of the working standard solution and the standard stock solution, which were generated with concentrations of 10µg/ml of TNG and 10µg/ml of PGL, were recorded after scanning the spectrum mode over the 200–400 nm range against RO Water as a blank. PGL displayed an absorbance peak at 230.0 nm, while TNG displayed one at 250.0 nm. Isoabsorptive spots at 242.0 nm were also seen in the overlain spectra. Both drugs can be evaluated concurrently using the simultaneous equation technique because their absorbance maxima differ and do not interfere with one another. The basis of the simultaneous equation approach is the absorption of medications X and Y at the other's wavelength maximum. Two wavelengths, 250.0 nm and 230.0 nm, which correspond to the λ_{max} of TNG and PGL, respectively, were chosen for the approach. The absorbances were measured at the chosen wavelengths, and the mean of five independent measurements was used to calculate the absorptivities ($A^{1\%,\,1\,cm}$) for both medicines at both wavelengths. The following equations were used to determine the sample's concentrations [7, 8].

$$\mathrm{CTNG} = \frac{\mathrm{A1ay2} - \mathrm{A2ay2}}{\mathrm{ax1ay2} - \mathrm{ax2ay1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathrm{Eq.\,(1)}$$

$$\mathrm{CPGZ} = \frac{\mathrm{A1ax2} - \mathrm{A2ax2}}{\mathrm{ax1ay2} - \mathrm{ax2ay1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathrm{Eq.\,(2)}$$

where A_1 and A_2 represent the mixture's absorbances at 216.0 and 232.0 nm, respectively; ax_1 and ax_2 represent the TNG absorptivities at λ_1 (250.0 i.e. λ_{max} of TNG) and λ_2 (230.0 i.e. λ_{max} of PGL), respectively; and ay_1 and ay_2 represent the PGL absorptivities at λ_1 and λ_2, respectively. TNG and PGL concentrations are denoted by C_{PGL} and C_{TNG}, respectively. The overlapping spectra of the two medications in a 20:15 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method as determined by this approach were found to be outside the range of 0.1-2.0, which is satisfied for both the TNG and PGL [9].

Validation of simultaneous equation method (ICH Q2 (R1), 2005)[10-13]

Linearity

The drugs' response ratios demonstrated the linearity of both medications. Divide the absorbance by the corresponding concentration to determine the drug's response ratio. The concentration and response ratio were then plotted on a graph.

Accuracy

Recovery experiments were used to evaluate the accuracy of the suggested approaches at three different levels: 80%, 100%, and 120%. To conduct the recovery tests, preanalyzed tablet

solutions were mixed with a known quantity of standard TNG and PGL solution. Proposed methodologies were then used to re-analyze the resulting solutions. The entire analysis process was performed in order to determine whether the additional drug sample was recovered. At three replicates of five concentration levels, this recovery analysis was conducted again.

Precision

Three levels of method precision were examined: reproducibility, intermediate precision (day-to-day and analyst-to-analyst), and repeatability. By analyzing the same drug concentration five times, repeatability was achieved. Day to Day was carried out by examining five distinct drug concentrations throughout three days of the week.

Analysis of tablet sample

Twenty commercially available TNG and PGL tablets were weighed and ground into a fine powder, and 20 mg of TNG was added to a 10-milliliter volumetric flask. This quantity of tablet powder contained 15 mg of PGL. The medication in the tablet powder was then solubilized by adding 8 ml of a 2M Ammonium Acetate: 2M Sod. Citrate (1:1) solution, and the flask was sonicated for roughly 10 minutes. The volume was then adjusted to the mark using hydrotropic solution. Using Whatman filter paper No. 41, after sonication filtering was carried out. To get the final concentrations of both medications within the working range, the filtrate was gathered and further diluted with water. The concentrations were determined using the simultaneous equation approach, and the absorbances of the final dilutions were measured at certain wavelengths. Five repetitions of the process were made [14-16].

3. Results

Solubility

In terms of solubility enhancement, linearity, recovery, precision, and repeatability, the development and validation of the UV-Vis spectroscopic method for the simultaneous measurement of Teneligliptin (TNG) and Pioglitazone (PGL) (figures 1 and 2) showed encouraging results. The efficiency of different mixed hydrotropic agents in improving TNG and PGL solubility is displayed in Table 1. The combination of 2M Ammonium Acetate and 2M Sodium Citrate (1:1) produced the greatest solubility boost, with TNG and PGL showing remarkable 18- and 15-fold increases, respectively. Such large increases in solubility suggest that these compounds help the pharmaceuticals dissolve more readily, which is essential for precise UV-Vis spectrophotometric measurement. The results are consistent with earlier research highlighting the function of hydrotropic agents in enhancing the solubility of poorly soluble substances (Dhaneshwar & Sutar, 2018).

Table 1: Results of solubility enhancement by UV Vis. Spectroscopy

Sr. No.	Solvents	Solubility Enhancement (folds)	
		TNG	PGL
1	2M Sodium acetate	3	2
2	8M Urea	4	4
3	2M Sodium Citrate	7	4

4	2M Sodium Benzoate	4	3
5	2M Ammonium Acetate	3	6
6	2M Sod. Citrate	5	5
7	2M Sodium acetate: 2M Sodium Benzoate (1:1)	5	3
8	2M Urea:2M Sodium acetate (1:1)	3	5
9	2M Sodium citrate:8M Urea (1:1)	4	4
10	2M Sodium citrate:8M Urea (1:1)	6	5
11	2M Ammonium Acetate: 2M Sod. Citrate (1:1)	18	15

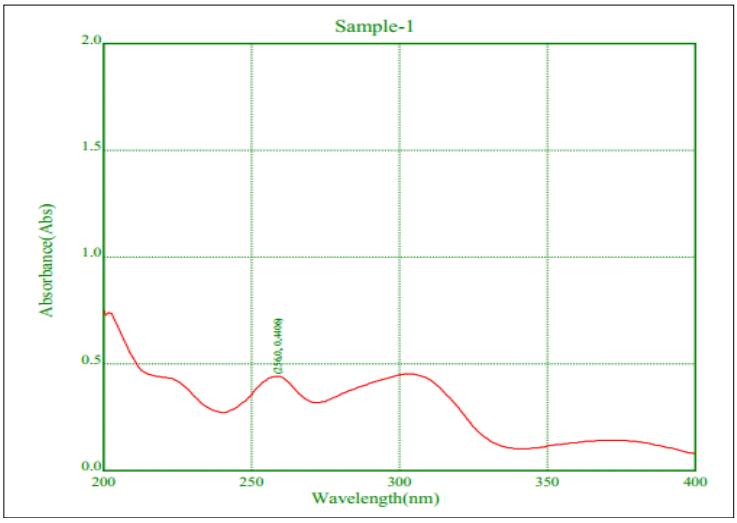

Figure 1: Determination of λ_{max} of TNG

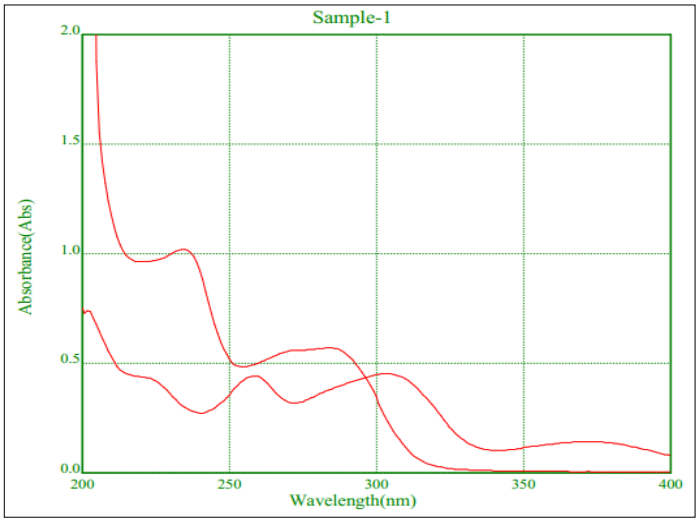

Figure 2: Overlay Spectra of TNG and PGL

Linearity range and calibration graph

The linearity results are shown in Table 2, where correlation coefficients (r^2) of 0.999 show significant correlations for both TNG and PGL. This excellent level of linearity within the designated Beer's law ranges (10–50 µg/ml) attests to the method's dependability for quantitative analysis. Figure 3 further confirms the accuracy and precision of the approach with consistent slope and intercept values.

Table 2: Results of Linearity of Teneligliptin and Pioglitazone

Parameter	Results of Linearity	
	TNG	PGL
Working λmax	256.0 nm	230.0 nm
Beer's law limit (µg/ml)	10-50	10-50
Correlation Coefficient (r2)*	0.999	0.999
Slope (m)*	0.022	0.020
Intercept (c)*	-0.000	-0.002

*Average of five determination

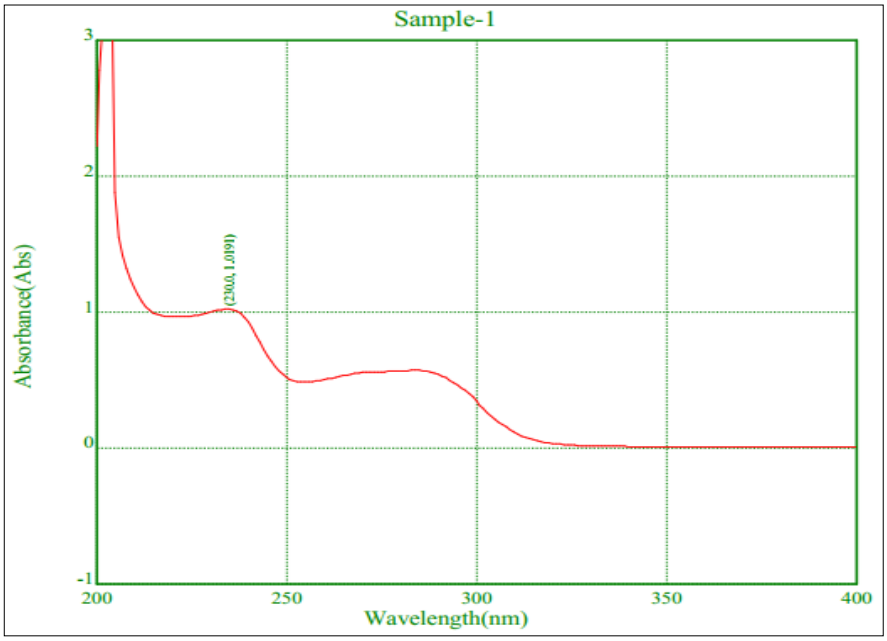

Figure 3: Linearity of PGL

Recovery studies

The mean percentage recoveries of both TNG and PGL were around 100% at all levels (80%, 100%, and 120%), with low standard deviations, according to recovery studies (Table 3). This proves the method's suitability for regular quality control in pharmaceutical analysis by showing that it can precisely recover the medicines from formulations.

Table 3: Results of recovery studies on marketed formulations

Recovery Level %	% Recovery (Mean±SD)*	
	TNG	PGL
80	97.824±1.453	98.862±0.352
100	98.267±0.729	98.463±0.524
120	98.900±0.577	99.181±0.594

* Value of 3 replicate and 5 concentrations

Validation results

The method's accuracy is demonstrated by the validation results (Table 4), which show low percentage relative standard deviations (%RSD) for repeatability, day-to-day, and analyst-to-analyst variability. The reliability of the established method is demonstrated by these findings, which are essential for guaranteeing consistent outcomes in a laboratory setting.

Table 4: Results of validation

Parameter (Mean±SD)*		TNG	PGL
Precision (%R.S.D.)*	Repeatability	97.535±0.979	98.834±0.108
	Day to Day	98.439±0.186	98.436±0.177
	Analyst to Analyst	98.891±0.083	98.711±0.105
	Reproducibility	98.844±0.128	98.791±0.100

*Average of five concentration

Analysis of tablet sample

The analysis results of TNG and PGL formulations that are marketed are shown in Table 5. TNG and PGL levels were 99.25% and 98.87%, respectively, which were in close agreement with the label claims. For routine analysis of these medications in tablet formulations, the method's dependability is further supported by the low percentage RSD values.

Table 5: Analysis of tablet formulation of TNG and PGL

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
TNG	20	19.85	99.25	0.125	0.136
PGL	15	14.83	98.87	0.223	0.228

4. Discussion

Accuracy, precision, and dependability are confirmed for the devised UV-Vis spectroscopic approach for the simultaneous determination of TNG and PGL employing mixed hydrotropic solubilizing agents. This method is appropriate for quality control in pharmaceutical applications because to the significant solubility increases that are obtained, as well as the high linearity and recovery. To increase its usefulness in clinical settings, more research might examine how this methodology might be applied to different drug combinations and

formulations [17,18].

5. Conclusion

Teneligliptin (TNG) and pioglitazone (PGL) in pure form and pharmaceutical formulations can be simultaneously estimated using the UV-Vis spectroscopy approach, which offers speed, convenience, accuracy, and precision. When compared to other documented methods, this approach is thought to be more cost-effective. It is a good choice for routine quality control of teneligliptin and pioglitazone in these pharmaceutical formulations because it is appropriate for tablet analysis [19, 20].

List of abbreviations

ICH	International Council for Harmonization
TNG	Teneligliptin
PGL	Pioglitazone
DMT2	Diabetes Mellitus Type 2
FDA	Food and Drug Administration
TEN	Teneligliptin
PIO	Pioglitazone
HPLC	High Performance Liquid Chromatography
HPTLC	High-Performance Thin-Layer Chromatography
RP-HPLC	Reverse Phase High Performance Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
RSD	Relative Standard Deviation
S.D	Standard Deviation
M.P	Mobile Phase

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