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DEVELOPMENT AND VALIDATION OF RATIO SPECTRA DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE AND PIOGLITAZONE HYDROCHLORIDE

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ABSTRACT

By ratio first-order derivative spectroscopy, a method for simultaneous estimation of Teneligliptin hydrobromide hydrate (TNG) and Pioglitazone hydrochloride (PIO) in bulk medication and their combined dosage form has been devised. This approach is based on dividing the spectra of a mixture by the standard spectra of the analytes, and the spectra were recorded with = 16 nm and a scaling factor of 10. By estimating the first derivative signal at 238.60 nm and 262.85 nm, respectively, the amount of TNG and PIO in the binary combines was determined. In the concentration ranges of 2-12 g/mL for TNG and 1.5-9 g/mL for PIO, the calibration curve was linear. All validation parameters were carried out in accordance with ICH recommendations. The technology was successfully employed for simultaneous drug estimation.

Keywords: Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride, Ratio derivative method, Simultaneous estimation

INTRODUCTION

Type 2 diabetes, also known as non-insulin-dependent diabetes. The second-line therapies for type 2 diabetes are teneligliptin hydrobromide hydrate (TNG) and pioglitazone hydrochloride (PIO). Teneligliptin (2S,4S)-4-[4] pyrazol-5-yl (3-

methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] (1,3-thiazolidin-3-yl) pyrrolidin-2-yl (1,3-thiazolidin-3-yl) Methanogen hemi Penta has a peculiar peptidomimetic structure consisting of five consecutive rings. The critical contact between the pyrazole's phenyl ring and DPP-4's S2 extended subsite, which increases the drug's potency and selectivity, as seen in an X-ray co-crystal structure of teneligliptin with DPP-4 **[1-3]**.

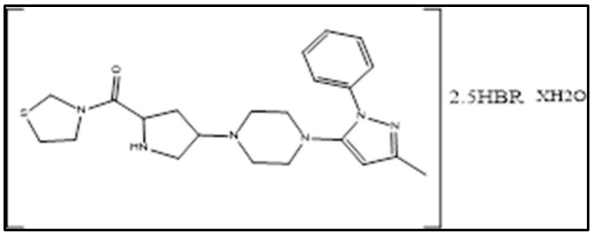

Figure 1: Teneligliptin hydrobromide hydrate structure

Teneligliptin lowers blood glucose levels through increasing incretin levels (GLP-1 and GIP), which simultaneously increases insulin secretion, slows stomach emptying, and lowers glucagon release **[4-6]**. Pioglitazone, also known as (RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2,4-dione), belongs to the thalidonedione class of oral hypoglycaemic medicines.

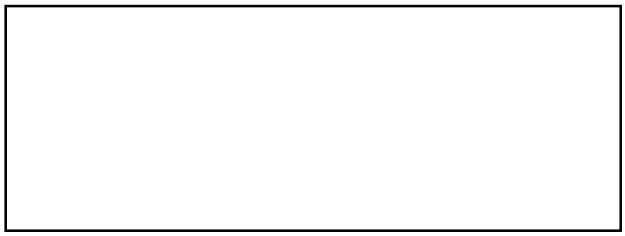

Figure 2: Pioglitazone hydrochloride structure

Thiazolidinediones have a high affinity for PPAR$_\gamma$, a nuclear receptor superfamily of ligand activated nuclear receptors. When a ligand activates PPAR$_\gamma$, it forms a heterodimer with the retinoid-X receptor, another nuclear receptor **[7, 8]**. This heterodimer initially binds to certain DNA regions and regulates transcriptional activities in order to control the transcriptional activity of target genes involved in glucose and lipid metabolism **[9, 10]**. diverse PPAR$_\gamma$ agonists, such as rosiglitazone, pioglitazone, and troglitazone, affect the regulation of over 100 PPAR$_\gamma$-responsive molecules in diverse ways. This could be due to the fact that various ligands have varied coactivator protein interactions and receptor

conformations. Pioglitazone's activation of PPAR results in increased peripheral, hepatic, and adipocyte insulin sensitivity, its active metabolites, the hydroxy derivatives MII and MIV, and the keto derivative MIII, improve disordered glucose homeostasis by reducing insulin resistance **[11]**.

There have been several ways reported for calculating TNG and PIO in a combined dose form. Because of its sensitive results and low-cost analytical equipment, UV-visible spectrometry is an appropriate strategy for pharmaceutical dose control of pharmaceutical composition. As a result, an effort was made to develop and validate a precise, accurate, sensitive, and easy UV-spectrophotometric method for quantifying TNG and PIO in combination dose form. The described method is suitable for testing currently available combination dose forms.

MATERIALS AND METHODS

Apparatus

A UV-Vis spectrophotometer (Shimadzu UV- 1800) two matched quartz cells ,2 nm spectral width and 0.5 nm wavelength precision was used to determine the absorbance of all the solutions. UV probe software (version 2.42) and a digital electronic balance (Shimadzu) were used to capture spectra automatically.

Chemicals and reagents

Presice Chemipharma Pvt. Ltd. provided TNG bulk powder as a gift sample, and Abhilasha Pharma Pvt. Ltd. provided PIO. A commercial fixed-dose combination was purchased from the local market. High-purity methanol (S. D. Fine Chemicals Ltd., Mumbai, India) and distilled water were used.

Preparation of Standard Solution

TNG and PIO standard Stock solutions were prepared by transferring 10 mg of the respective drug to 10 ml separate volumetric flasks and dissolved in methanol to represent 1 mg/mL of each drug. Aliquot 1.0 ml from the above solution in separate 10 ml volumetric flasks and make up the volume with diluent

METHODOLOGY

Different solutions of TNG and PIO were used as a divisor for development of divisor concentration and selection of optimized wavelength. An accurate choice of both standard divisors and working wavelengths is fundamental for various reasons. Stored spectra of standard TNG solutions (2-12 µg/mL) were divided by a standard spectrum of PIO. PIO solutions (1.5-9 µg/mL) of different concentrations underwent same procedure and TNG were used as the divisor. Thus, a concentration of 10 µg/mL of TNG and 9 µg/ml of PIO as divisor were selected. The absorbance values at 238.60 nm and 262.85 nm were used for the determination of TNG and PIO in prepared mixtures respectively. **Figure 3** shows the first derivative ratio spectra of various concentration of TNG whereas 09 µg/mL of

PIO used as a divisor and the first derivative ratio spectra of various concentration of PIO is depicted in **Figure 4** when 10 µg/mL of TNG used as the divisor.

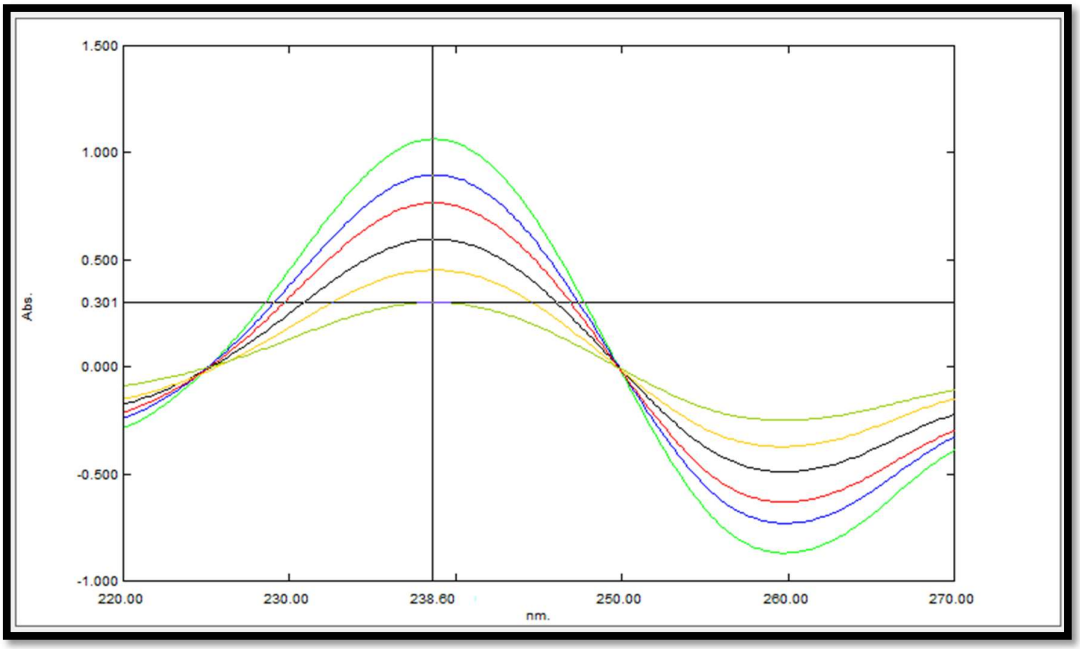

Figure 3: First Derivative ratio spectra of (2,4,6,8,10,12 µg/mL) of a solution of TNG when 09 µg/ml solution of PIO used as the divisor. (Δλ = 16 nm and scaling factor = 10)

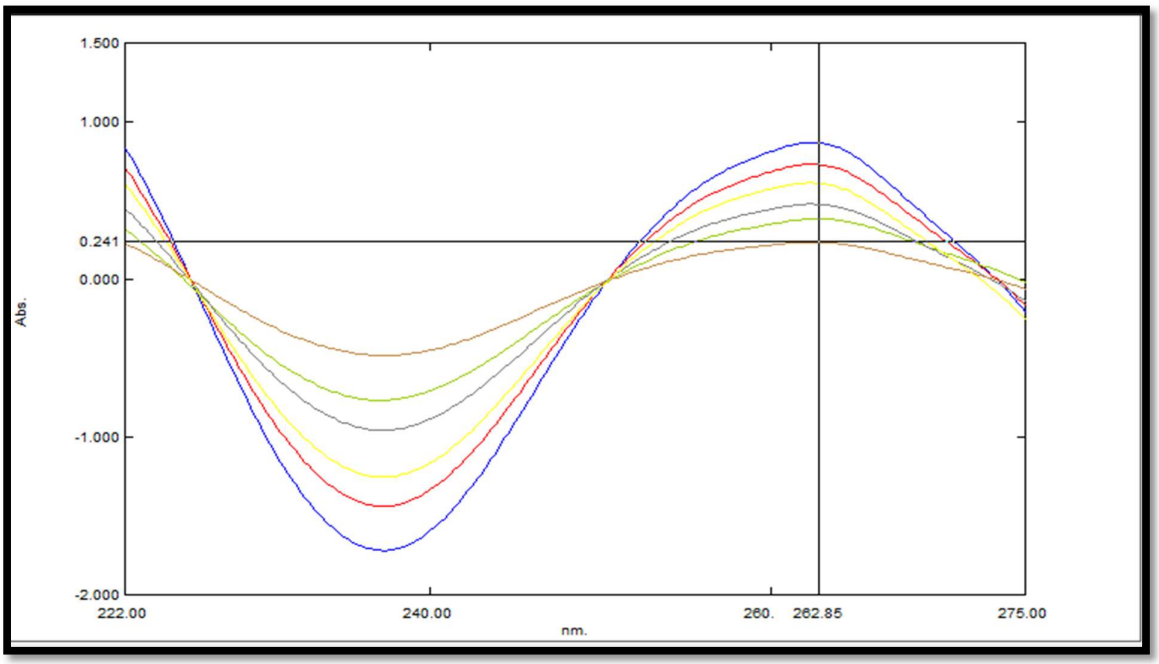

Figure 4: First Derivative ratio spectra of (1.5,3,4.5,6,7.5,9.0 µg/mL) of a solution of PIO when 10 µg/ml solution of TNG used as the divisor. (Δλ = 16 nm and scaling factor = 10)

Optimization of Derivative Intervals and Scaling Factor

Different scaling factor was used 10 was found optimal with respect to both slit width and wavelength interval Δλ was tested at different values (2, 4, 8, 16 nm)

Assay of Marketed Formulation

Ten tablets were weight calculate the average net content of blend. Precisely weigh powder and transfer a quantity of tablet equivalent to about 20 mg of TNG and 15 mg of PIO into 100 mL volumetric flask followed by the addition of 100 mL methanol and kept for 20 minutes in sonication. The resultant solution was filtered through Whatman filter paper No. 41. Pipette out a suitable amount from the above solution to obtain 4, 6, 8 µg/mL of TNG **(Figure 5)** and 3, 4.5, 6 µg/mL of PIO **(Figure 6)**. For the quantitative determination of TNG and PIO, the absorbance (n = 3) was taken at selected wavelengths The concentration of drugs in the tablet sample was determined by the respective regression line equation of both methods.

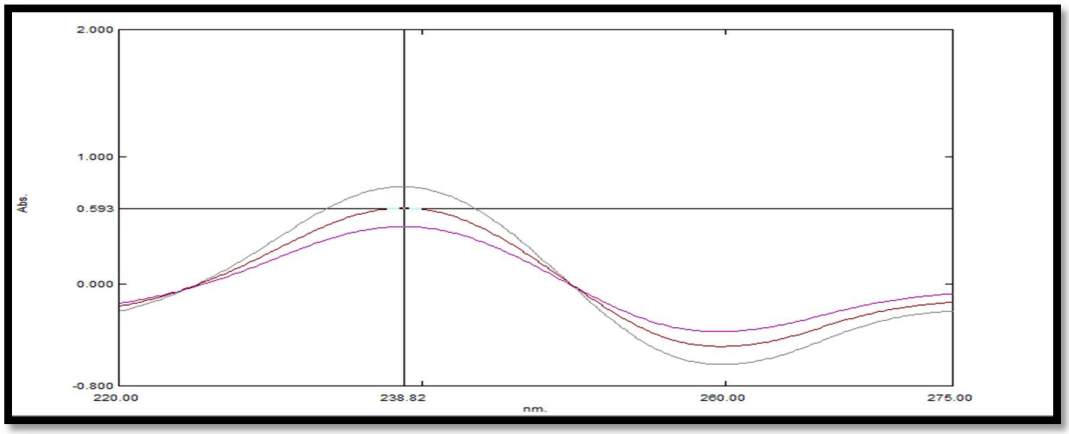

Figure 5: Spectra of TNG of Marketed formulation

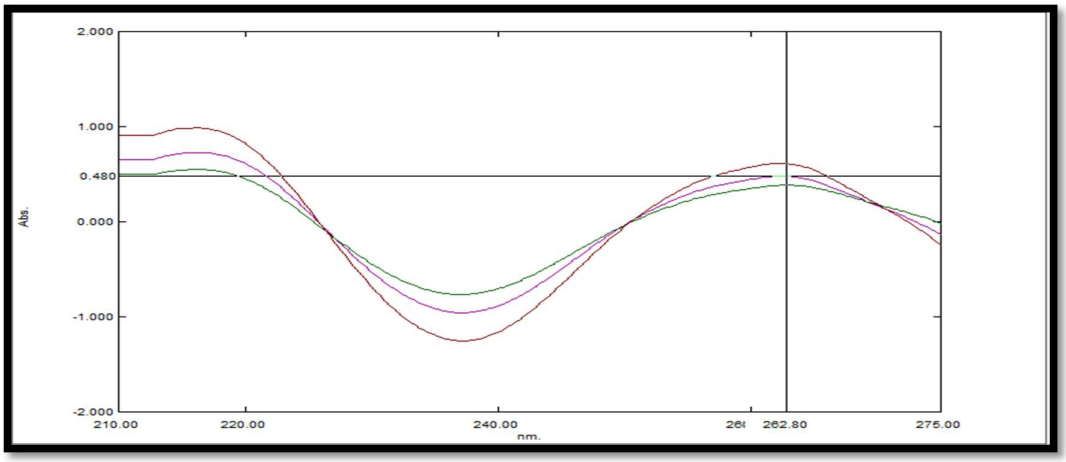

Figure 6: Spectra of PIO of Marketed formulation

Table 1: Data of marketed formulation analysis

Drug	Label claim	Conc. (µg/ml)	% Amount found			Mean % Amount found	SD	%RSD
			1	2	3			
TNG	20	4	98.02	99.69	99.02	98.91	0.84	0.851
		6	99.93	98.81	100.15	99.63	0.71	0.719
		8	98.54	99.04	100.05	99.21	0.77	0.772
PIO	15	3	98.26	99.11	100.80	99.39	1.29	1.298
		4.5	99.30	100.71	99.59	99.87	0.74	0.746
		6	100.67	99.40	100.80	100.32	0.79	0.797

Validation of the Proposed Method

Validation was done according to ICH Q2 (R1)

Linearity

The linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analysing 6 independent level of calibration curve in the range of 2-12 µg/ml for TNG and 1.5-9 µg/ml for PIO. The calibration curve of amplitude vs. concentration was plotted and correlation coefficient was determined and regression line equation obtained **(Figure 7 and 8)**.

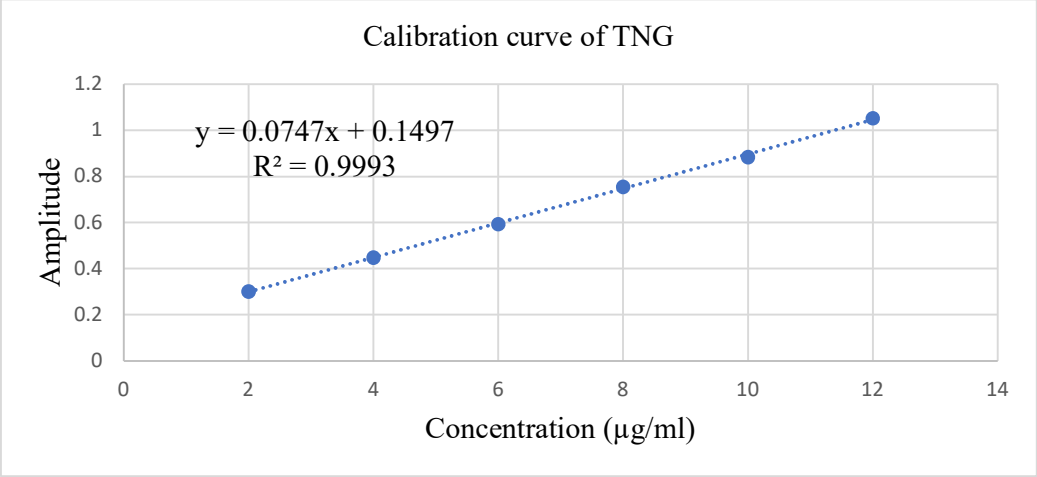

Figure 7: Linearity curve of TNG

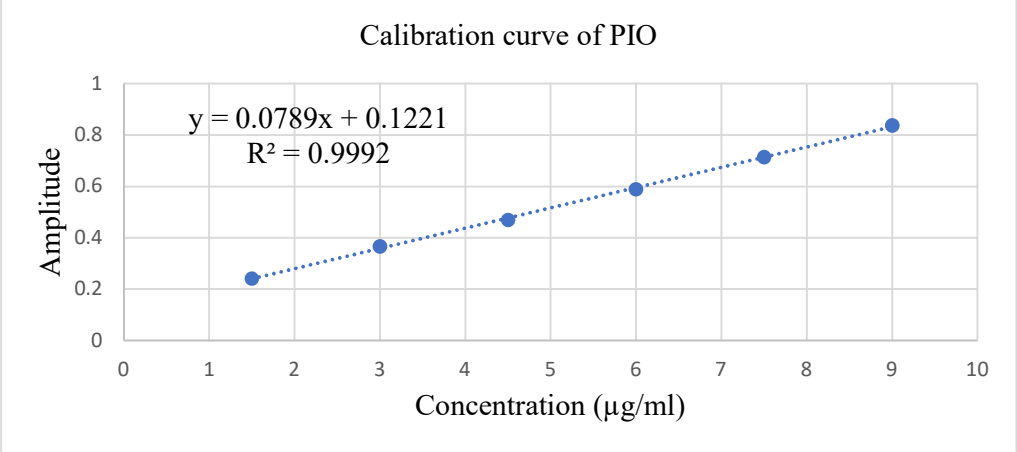

Figure 8: Linearity curve of PIO

Table 2: Linearity data for TNG

Concentration (µg/ml)	Amplitude					Average amplitude	S. D.	% RSD
	1	2	3	4	5			
2	0.299	0.305	0.301	0.300	0.302	0.301	0.002	0.76
4	0.446	0.452	0.447	0.450	0.453	0.449	0.003	0.67
6	0.596	0.592	0.598	0.594	0.589	0.593	0.003	0.58
8	0.750	0.753	0.759	0.754	0.762	0.755	0.004	0.63
10	0.885	0.880	0.886	0.884	0.892	0.885	0.004	0.48
12	1.056	1.057	1.052	1.054	1.054	1.053	0.002	0.27

Table 3: Linearity data for PIO

Concentration (µg/ml)	Amplitude					Average amplitude	S. D.	% RSD
	1	2	3	4	5			
1.5	0.244	0.243	0.242	0.239	0.241	0.241	0.001	0.79
3	0.369	0.366	0.367	0.365	0.368	0.367	0.001	0.43
4.5	0.469	0.473	0.468	0.464	0.472	0.469	0.003	0.75
6	0.588	0.587	0.589	0.597	0.585	0.589	0.004	0.78
7.5	0.716	0.712	0.719	0.720	0.706	0.714	0.005	0.80
9	0.834	0.840	0.835	0.839	0.840	0.837	0.002	0.34

Limit of Detection and Limit of Quantification

The limit of detection (LoD) and the limit of quantification (LoQ) of the drug were calculated using equations given in ICH Q2 (R1) guidelines.

Table 4:Linear regression parameters for TNG and PIO by ratio first orderderivative UV-Spectrophotometric method

Regression equation	Y = 0.0747x + 0.1497	Y = 0.0789x + 0.01221
Regression coefficient	0.9993	0.9992
Standard deviation of slope	0.00032	0.00064
Standard deviation of intercept	0.00229	0.00297
Limit of detection (µg/ml)	0.101	0.124
Limit of quantification (µg/ml)	0.306	0.377

Method Precision (Repeatability)

The precision of method was checked by repeated (n = 6) scanning and measurement of the absorbance of binary mixture containing TNG (4 µg/ml) and PIO (3 µg/mL) without altering the parameter of the proposed method.

Table 5: Repeatability study of TNG and PIO

Conc (µg/ml)	Repeatability						Mean amplitude	SD	%RSD
	TNG								
4	0.449	0.446	0.448	0.454	0.450	0.447	0.449	0.002	0.629
	PIO								
3	0.368	0.365	0.364	0.371	0.365	0.371	0.367	0.002	0.751

Intermediate Precision (Reproducibility)

It was demonstrated by Interday and intraday variation study and performed by measuring the absorbance value at respective wavelengths for the respective method for three concentrations on three different days, thrice and three times on the same day, respectively. The analysis was carried out on a binary mixture containing three concentration range of TNG (4, 8, 12 µg/mL) and PIO (3, 6, 9 µg/mL).

Table 6: Intraday study of TNG and PIO

Conc (µg/ml)	Intraday precision Amplitude			Mean Amplitude	SD	%RSD
	1	2	3			
TNG						
4	0.443	0.446	0.451	0.446	0.004	0.904
6	0.594	0.590	0.598	0.594	0.004	0.673
8	0.759	0.754	0.762	0.758	0.004	0.532
PIO						
3	0.368	0.370	0.369	0.369	0.001	0.271
4.5	0.475	0.472	0.478	0.475	0.003	0.631
6	0.585	0.581	0.589	0.585	0.004	0.683

Table 7: Interday study of TNG and PIO

Conc (µg/ml)	Interday precision Amplitude			Mean Amplitude	SD	%RSD
	Day-1	Day-2	Day-3			
TNG						
4	0.449	0.450	0.460	0.452	0.006	1.42
6	0.598	0.605	0.608	0.603	0.005	0.85
8	0.762	0.768	0.779	0.779	0.008	1.12
PIO						
3	0.370	0.374	0.376	0.373	0.003	0.81
4.5	0.478	0.482	0.489	0.483	0.005	1.15
6	0.582	0.590	0.598	0.590	0.008	1.35

Accuracy (Recovery Study)

It is carried out to determine the reliability of the proposed method. The accuracy of method was determined by calculating the % recovery of TNG and PIO from the marketed tablet formulation by the standard addition method. In pre analysed sample of 4 µg/ml TNG and 3 µg/ml PIO, known amount of standard solution of TNG (3.2, 4, 4.8 µg/ml) and PIO (2.4, 3, 3.6 µg/ml) at 80%, 100%, 120% levels were added. The procedure was repeated 2 more times and the recovered amounts of TNG and PIO were calculated at each level and % recovery was reported.

Table 8: Accuracy of TNG

% Spike	Conc. Of Sample (µg/ml)	Conc. Of Spiked (µg/ml)	Total Conc. Of drug taken(µg/ml)	Total Conc. Of drug found	% Recovery	Mean % Recovery	SD	%RSD
80%	4	3.2	7.2	7.09	98.52	98.95	0.46	0.472
	4	3.2	7.2	7.12	98.89			
	4	3.2	7.2	7.16	99.45			
100%	4	4	8	7.96	99.54	100.21	0.60	0.602
	4	4	8	8.05	100.71			
	4	4	8	8.03	100.38			
120%	4	4.8	8.8	8.68	98.71	99.57	0.83	0.841
	4	4.8	8.8	8.76	99.62			
	4	4.8	8.8	8.83	100.38			

Table 9: Accuracy of PIO

% Spike	Conc. Of Sample (µg/ml)	Conc. Of Spiked (µg/ml)	Total Conc. Of drug taken(µg/ml)	Total Conc. Of drug found	% Recovery	Mean % Recovery	SD	%RSD
80%	3	2.4	5.4	5.40	100.12	99.34	0.71	0.721
	3	2.4	5.4	5.33	98.71			
	3	2.4	5.4	5.35	99.18			
100%	3	3	6	5.90	98.35	99.40	0.92	0.926
	3	3	6	5.98	99.83			
	3	3	6	6.00	100.04			
120%	3	3.6	6.6	6.62	100.35	100.35	0.57	0.574
	3	3.6	6.6	6.66	100.93			
	3	3.6	6.6	6.58	99.78			

RESULT AND DISCUSSION

A simple, rapid, accurate, and precise first order ratio derivative approach was developed and validated in accordance with the ICH Q2 (R1) guideline for the simultaneous estimation of Teneligliptin hydrobromide hydrate (TNG) and Pioglitazone hydrochloride (PIO) in marketed formulation. By selecting the optimal concentration of divisor, zero order absorption spectra of mixture solution were obtained and translated into ratio spectra. Different concentrations of TNG (2,4,6,8,10, and 12 µg/ml) and PIO (1.5,3.4.5,6,7.5,9, µg/ml) were evaluated as a divisor, however the concentrations TNG 10 µg/ml and PIO 9 µg/ml provided the least noise in ratio spectra, the highest sensitivity, and the best linearity. Using Δλ 16 nm and scaling factor 10, the divisor concentrations for TNG (10 µg/ml) and PIO (9 µg/ml) produced good results for slope, intercept, and correlation coefficient. Furthermore, the ratio spectrums of both medications were transformed to first order derivatives in order to determine the best wavelength. The calibration range for TNG and PIO was determined by considering the practical range required by Beer-Lambert's law. In the provided concentration ranges of 2-12 g/ml for TNG at 238.60 nm and 1.5-9 µg/ml for PIO at 262.85 nm, the TNG and PIO showed

strong correlation coefficients R2 = 0.9993 and R2 = 0.9992, respectively. TNG and PIO had detection limits of 0.101 µg/ml and 0.124 µg/ml, respectively. TNG and PIO had quantification limits of 0.0.306 µg/ml and 0.377 µg/ml, respectively. TNG and PIO shows % recovery in the range of 98.95-100.21 and 99.34-100.35 respectively. Table 3 summarizes the suggested Ratio first order derivative UV-spectrophotometric method's analytical parameters. Based on the results, the proposed approach is effective for determining TNG and PIO in bulk or in commercial tablet formulations without interference from routinely used excipients and related substances.

CONCLUSION

Diabetes treated with a combination of teneligliptin hydrobromide hydrate and pioglitazone hydrochloride. An accurate, simple, quick, and precise approach for estimating both medicines in pharmaceutical formulations was developed and validated in accordance with ICH criteria. The method is capable of resolving spectral overlaps without prior separation, and both medications can be calculated without interfering with one another. The proposed method's results were found to be in close agreement with the label claim of the marketed product. The proposed method can be utilized to estimate both pharmaceuticals in bulk and pharmaceutical formulations at the same time.

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