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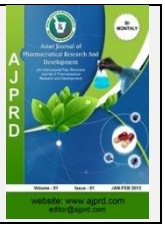

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Research Article

Development and Validation of RP-High Performance Liquid Chromatographic Method for Simultaneous Estimation of Teneligliptin and Pioglitazone In Tablet Formulation

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A B S T R A C T

A reliable, economical, sensitive and reproducible RP-HPLC was developed and validated for the simultaneous estimation of Teneligliptin (TENE) and Pioglitazone (PIO) in combined dosage form. In the RP-HPLC method the mobile phase used was Acetonitrile: 5mM potassium dihydrogen orthophosphate (KH2PO4) buffer (60:40) at PH 3.6 and flow rate was 1.0 ml per min. The method was scanned at isosbestic point 238 nm for both the drugs. The linearity range for TENE and PIO was found to be 0.1-500 µg/ml and 0.500 µg/ml with regression correlation coefficient of (R2) 1.000 and 0.999 respectively. The LOD and LOQ for TENE was found to be 0.002645 and 0.0801 and for PIO was found to be 0.017727 and 0.05371 respectively. The retention time for Teneligliptin andPioglitazone was found to be 2.678 min and 5.010 min respectively. The percentage Assay for TENE and PIO was found to be 97.90-100.69%w/w and 98.53-99.58% w/w respectively. The developed method RP-HPLC was validated as per ICH guidelines.

Key Words: RP-HPLC, Teneligliptin (TENE), Pioglitazone (PIO).

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INTRODUCTION:

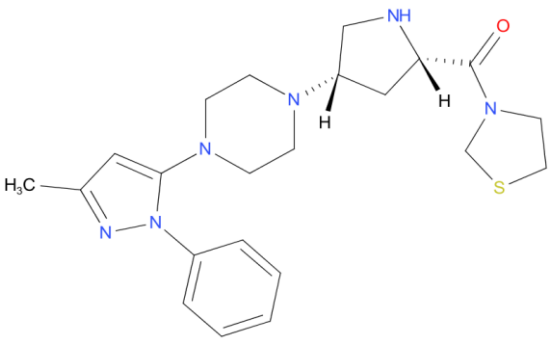
Teneligliptin, (TENE) an anti-diabetic drug known as dipeptidyl peptidase-4 inhibitors or gliptinregulates blood sugar levels in individuals with type 2 diabetes by impeding the activity of the enzyme DPP-4, it enhances the levels of incretin hormones (GLP-1 and GIP), resulting in the inhibition of glucagon release, heightened insulin secretion, slowing gastric emptying, and ultimately reduces blood sugar levels[1,2].

Pioglitazone, (PIO) an anti-diabetic drug called thiazolidinedione class referred to as an “Insulin sensitizer” because it attaches to the insulin receptors on cells throughout the bodyand causes the cell to become more sensitive to insulin[3]. As a result, more glucose is removed from the blood, and the level of glucose in the blood falls. Pioglitazone also lowers the level of glucose in the blood by reducing the production and secretion of glucose into the blood by the liver[4].

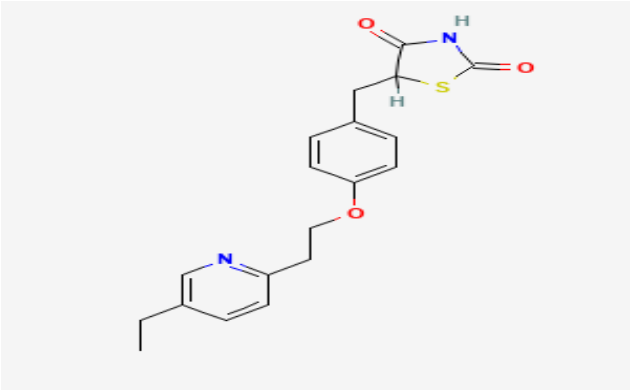
Literature survey cites many methods for estimation of Teneligliptin and Pioglitazoneindividually andin combination with other drugs.

Only one UV Spectrophotometric method has been reported in literature for estimation of Teneligliptin and Pioglitazone in combination, so there was need to develop and validate a new analytical RP-HPLC method for this combination product.

Hence in this project work, an attempt was made to develop and validate a New RP-HPLC methodfor simultaneous estimation of Teneligliptin and Pioglitazone in tablet formulation using different validation parameters as per ICH guidelines Q2(R1)[5,6].



TENELIGLIPTIN



PIOGLITAZONE

MATERIALS AND METHOD

High Performance Liquid Chromatograph 10 AT SHIMADZU- SPD20A Detector System, injector Rheodyne-7725i (Fixed capacity loop of 20 µl), syringe is Hamilton 50µl and LC-solutions software.

UV-Visible Spectrophotometer Shimadzu-1900i. Software Version Lab Solution UV-Vis was used for data processing.

CHEMICALS AND REAGENTS

TENE and PIO API were obtained from Microlabs Private Ltd. Bengaluru. Acetonitrile HPLC Grade (FINAR), Water (FINAR), Potassium dihydrogen orthophosphate (FINAR)was used.

Preparation of Phosphate buffer

5mM of Phosphate buffer was prepared by dissolving 340.2 mg of potassium dihydrogen orthophosphate in 500 mL of water and adjusting the pH to 3.6 with 1% OPA.

Preparation of Mobile Phase:

The working mobile phase was prepared in the ratio of 60:40 (Acetonitrile: Phosphate buffer) filtered, degassed and sonicated for 10 min.

Preparation of Standardsolution:

Accurately weighed 10mg of TENE standardand transferred into 10mL volumetric flask,3-5mL of Mobile phase was added and sonicated for 2 min to dissolve it completely,then the volume was made up to 10 ml with Mobile phase to get 1000µg/mL of standard TENE solutionand labelled as STD STOCK A.

Accurately weighed 10mg of PIO standard and transferred to 10mL volumetric flask,3-5mL of Mobile phase was added and sonicated for 2 min to dissolve it completely, then the volume was made up to 10 ml with mobile phase to get 1000µg/mL of standard PIO solution and labelled as STD STOCK B.

Preparation of SampleTeneligliptin and Pioglitazone solution.

A quantity of 10 tablets (**TENEPRIDE-P**) were weighed and their average weight was calculated. The tablets were finely triturated and accurately weighed a quantity of powder containing 20mg of TENE and 15mg of PIO and transferred to 100ml volumetric flask, solubilized in 25ml of mobile phase and sonicated for 10mins. After sonication the volume was made up to the mark with the mobile phase to obtain final concentration of200µg/mL and 150µg/mL of TENE and PIO respectively and was labelled as 'SMP STOCK'.

Mixture of Standardsolution (20µg/ml of TENE + 15µg/ml of PIO) inmobile phase were scanned in UV region of 200-400nm by using UV-Visible Spectrophotometer. The Spectra obtained is presented in **Fig 1.**

Then, the above std sol^{n}was filtered through 0.45µm nylon membrane filter and 20µL was injected into the HPLC system under standardized chromatographic conditions to get a stable baseline and to observe for peak of Teneligliptin and Pioglitazone. The chromatograms obtained is presented in **Fig 2**.

VALIDATION OF HPLC METHOD

Specificity

Solutions of standard and sample were prepared and 20µL was injected into HPLC. It was observed that other substances present in the formulation did not interfere with the peak of Teneligliptin and Pioglitazoneand thus the method was specific. The peak purity of Teneligliptin and Pioglitazonewas checked by comparing the spectra at different level viz. peak start, peak apex and peak end position of the spot.

Linearity

Series of standard solution of TENE and PIO were prepared in 10ml volumetric flask in mobile phase to obtain the concentration of 0.1-500µg/mL for TENE and 0.5-500µg/mL for PIO. Peak areas were calculated and the graph were plotted against concentration. The correlation coefficient (r^2) of least square linear regression of TENE and PIO were

calculated. The calibration graph for TENE and PIO is presented in **Fig 3**.

Limit of detection and Limit of quantification

Detection limit was determined based on the standard deviation of peak area and was calculated by formula

$$LOD = 3.3 \times \frac{\text{Standard Deviation of Y-intercept}}{\text{Average slope of six calibration curves}}$$

Quantification limit was determined based on the standard deviation of peak arca and was calculated by formula

$$LOQ = 10 \times \frac{\text{Standard Deviation of Y-intercept}}{\text{Average slope of six calibration curves}}$$

Robustness

The robustness was carried out by deliberately varying some method parameters by changing wavelength and change in flow rate by ±3% and mean, S.D and % RSD were calculated.

The data obtained for all validation parameters is tabulated in **Table-2.**

Precision

Precision was considered at three levels: repeatability, intermediate precision and reproducibility.The standard solutions of 20 and 15 µg/mL were selected for inter-day and intraday. The %RSD for Intraday and Inter- day studies for Teneligliptin and Pioglitazone was within the acceptance criteria of less than 2%. The data obtained is tabulated in **Table-1**.

Accuracy

Recovery studies were determined by adding known amounts of Std TENE and PIO to pre-analysed samples at three different concentration levels i.e., 80 %, 100 %, 120% of assay concentration. The percentage recovery, standard deviation and % RSD was calculated. The chromatograms obtained is presented in **Fig 4.**

RESULTS AND DISCUSSION

The standard solutions of Teneligliptin (20µg/mL) and Pioglitazone(15µg/mL) in mobile phase Acetonitrile: Phosphate Buffer (5mM) pH 3.6 (60:40v/v) were scanned in the UV region of 200 to 400 nm using Shimadzu 1900i UV-Visible Spectrophotometer and the spectra obtained is presented in **Fig 1.**

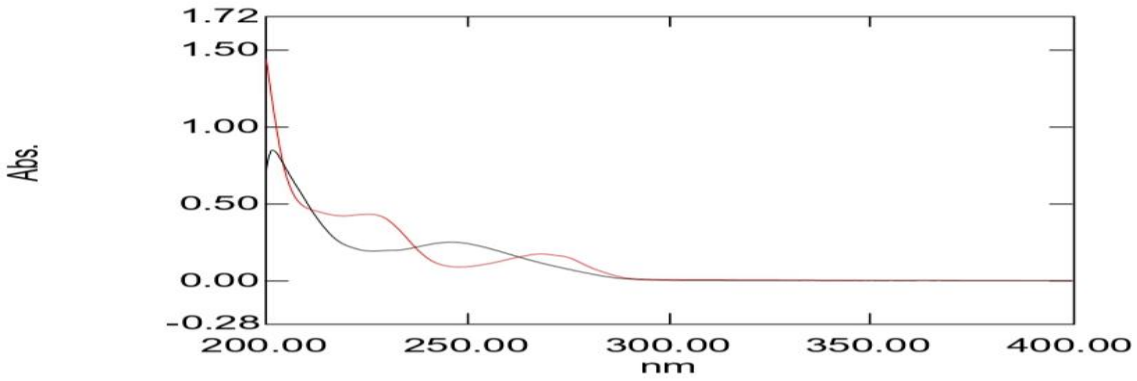


Figure 1: UV Spectra of TENE (20µg/mL) and PIO (15µg/mL).

From the UV Spectra, the isosbestic point was found to be 238nm.

Mobile Phase consists of Acetonitrile: Phosphate Buffer (5mM) pH 3.6 (60:40v/v) at a flow rate of 1mL/min, at 238 nm shows good resolution of TENE and PIOpeak, hence it was standardized and selected for the project work.

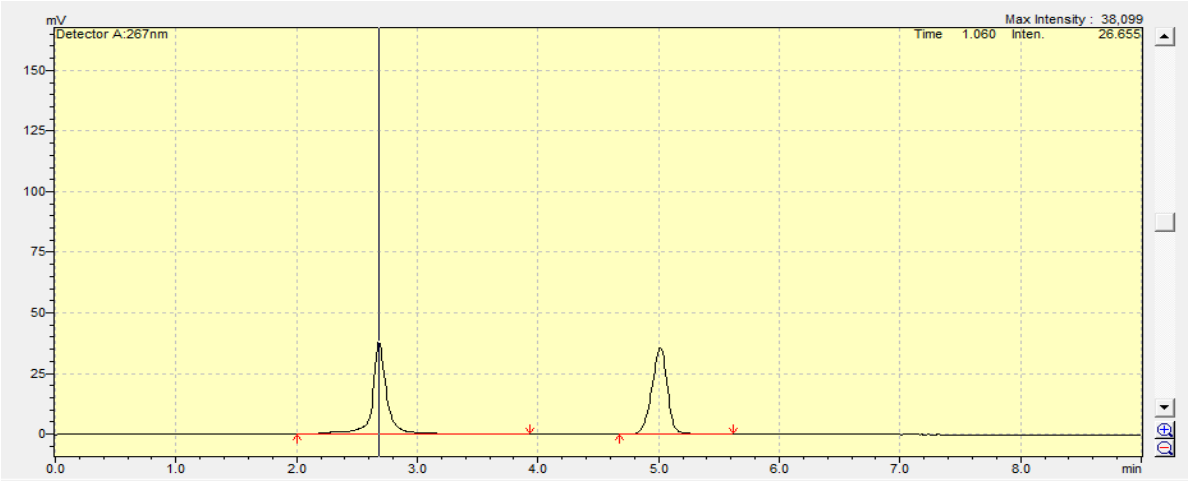


Figure 2: Chromatogram for TENE (20µg/mL) and PIO (15µg/mL) in MP containing Acetonitrile: Phosphate Buffer (5mM) pH 3.6 (60:40v/v).

Linearity

The linearity of an analytical procedure specifies the results which are directly proportional to the concentration of analyte in the sample. The linearity and range were determined from coefficient of correlation (R^2) obtained by plotting AUC vs. CONCENTRATION at 238 nm. The linearity was observed in concentration range of 0.1-30 µg/ml and Calibration graph is presented in **Fig 3.**

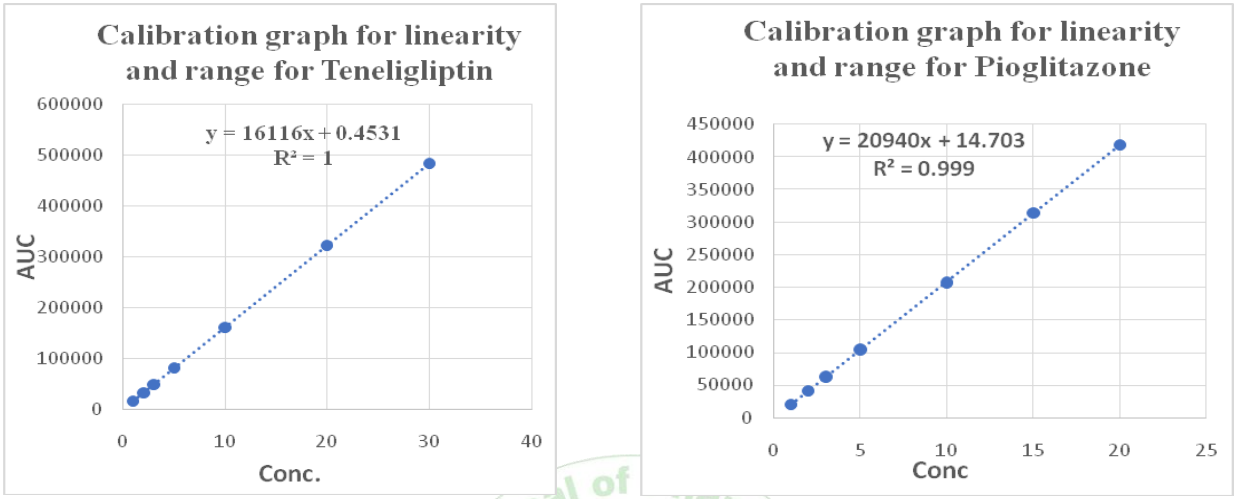


Figure 3: Calibration graph for linearity and range for Teneligliptin and Pioglitazone

Precision

Table-1: Intraday and Inter-day data for TENE and PIO.

Intraday			**Inter-day**		
TIME (Hrs)	**MEAN AUC (n=3)**		**TIME (Days)**	**MEAN AUC (n=3)**	
	TENE (20µg/mL)	**PIO (15µg/mL)**		**TENE (20µg/mL)**	**PIO (15µg/mL)**
0	**272768**	**285683**	***1***	**293010**	295688
2	271578	286073	***2***	297906	308344
4	286420	297138	***3***	301849	303180
MEAN	**776926**	**6254995**	MEAN	**813781**	**6256086**
%RSD	**0.883**	**0.785**	***%RSD***	**0.664**	**0.384**

Limits of detection and limit of quantification

The **LOD** and **LOQ** for TENE and PIOwere found to be **0.002645 and 0.0801 µg/ml**and **0.017727 and 0.053719 µg/ml**respectively.

System Suitability

System Suitability was performed to confirm that the system was appropriate for the analysis to be performed.

Accuracy

The percentage recovery of TENE and PIO at three different levels (80%,100%,120%) was found to be from 101.23%w/w-100.83%w/w for Teneligliptin and Pioglitazone which is well within the acceptance criteria limits (95-105%w/w) and the overlain chromatogram is presented in **Fig 4.**

Table 2: Validation parameters for TENE and PIO by HPLC method.

Parameters	**Data for Teneligliptin**	**Data for Pioglitazone**
Regression Equation	16115x +59.519	20940x +14.703
Regression coefficient(R^2)	1	0.999
Limit of detection (µg/ml)	0.002645	0.017727
Limit of Quantification (µg/ml)	0.0801	0.053719
Precision (% RSD) Inter-day Intra-day	 0.664 0.883	 0.384 0.785
Assay(%w/w)	97.90-100.69%w/w	98.53-99.58%w/w

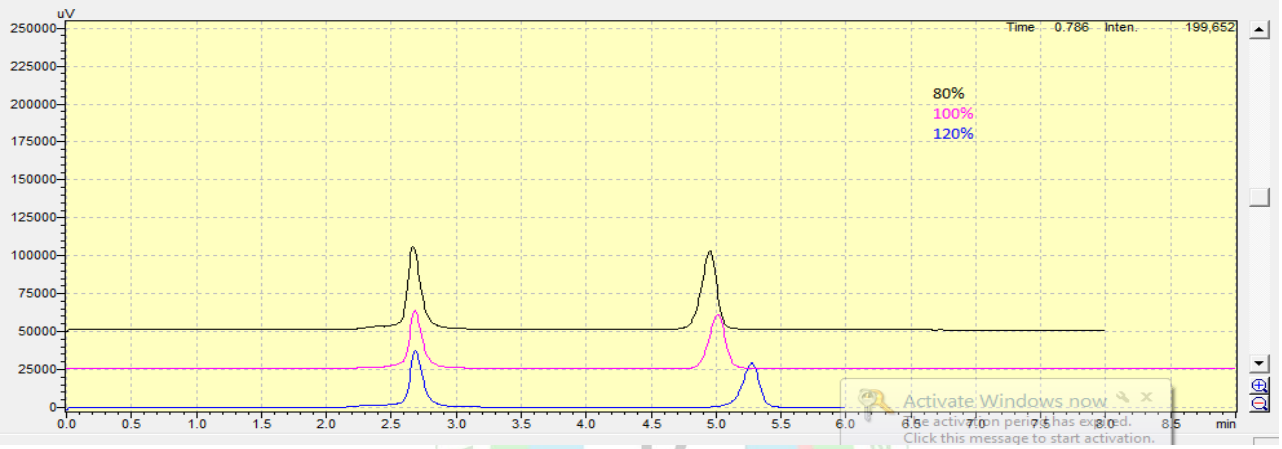


Figure 4: Overlain Chromatogram for Accuracy studies of TENE and PIO at three different levels.

Table 3: Percentage Recovery data for accuracy studies at three different levels.

DRUGS	**Conc.of STD (µg/ml) A**	**Conc.of Sample (µg/ml) B**	**Total Conc (A+B) (µg/ml)**	**Peak Area*for Mixture STD+ Sample**	**Total amount (A+B) from graph (µg/ml)**	**Recovery of Std (µg/ml)**	**%Recovery of Std (%w/w)**
TENE	16	20	36	584003	36.23	16.23	101.43
	20	20	40	652793	40.50	20.50	101.25
	24	20	44	716038	44.42	24.42	100.95
PIO	12	15	27	567768	27.04	12.04	100.33
	15	15	30	632361	30.12	15.12	100.8
	18	15	33	706503	33.65	18.65	103.6

***Average of three readings**

CONCLUSION

The retention time for Teneligliptin (20µg/mL) and Pioglitazone (15µg/mL) by RP-HPLC method was found to be 2.678min and 5.010min. The developed method was validated as per ICH Q2 (R1) guidelines. The linearity and range, was performed on series of concentration and was found to be linear in the concentration ranges of 0.1-500µg/mL for TENE and 0.5-500µg/mL for PIO with correlation coefficients of 1.000 and 0.999 (Fig 2). The **LOD** and **LOQ** for TENEwas found to be **0.002645 and 0.0801** and for PIO was found to be **0.017727 and 0.05371** respectively. The %RSD for precision of proposed method was found to be less than 2% indicating that the method was stable during Inter and Intraday studies. Accuracy was determined by standard addition method at three different levels. (80%, 100% and 120%) and the mean percentage recovery at three different levels was found to be **100.95-101.43** %w/w for TENE and

100.33-103.6%w/w for PIO which is well within the acceptance criteria limits (95-105%), hence the method was found to be accurate. The percentage Assay for TENE and PIO was found to be **97.90-100.69**%w/w and **98.53-99.58**% w/w respectively and are well within acceptance criteria of (95-105%). Hence the developed and validated method was found to be specific, accurate, precise, linear and robust and thus can be routinely applied for determination of Teneligliptin and Pioglitazone in bulk and pharmaceutical dosage form.

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