

## Research Article

Received on: 05-04-2017  
Accepted on: 11-04-2017  
Published on: 15-04-2017

### Corresponding Author:

\* Jinal Alkesh Gheewala,  
Department of Quality Assurance,  
L.J. Institute of Pharmacy,  
Sarkhej Highway Road,  
Ahmedabad, Gujarat,  
India- 382210.  
Contact No. : 8866212551



\*Email Id- gheewalajinal@gmail.com

## Development and Validation of UV Spectrophotometric Method and RP - HPLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone In Synthetic Mixture

Jinal Alkesh Gheewala\*, Dilip Girish Maheshwari

### ABSTRACT

The present Article portrays simple, sensitive, accurate, precise and cost effective First order derivative Spectrophotometric method and RP-HPLC method for the simultaneous estimation of Teneligliptin and Pioglitazone in Synthetic Mixture. In The first order derivative method absorption at 228.5 nm (zero crossing point for Pioglitazone) was used for Teneligliptin and 269.2 nm (zero crossing point for Teneligliptin) was used for Pioglitazone. The linearity was taken in the concentration range of 2-10 µg/ml for Teneligliptin and 3-15 µg/ml for Pioglitazone with correlation coefficient (R<sup>2</sup>) 0.995 and 0.997, respectively. For The RP-HPLC method linearity was taken in the concentration range of 1- 5 µg/ml for Teneligliptin and 1.5-7.5 µg/ml for Pioglitazone with correlation coefficient (R<sup>2</sup>) 0.998 and 0.996, respectively. Proposed technique has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Teneligliptin and Pioglitazone in their Synthetic Mixture. The results of analysis have been validated statistically and by recovery studies.

**Key-words:** Teneligliptin, Pioglitazone, First order derivative, RP-HPLC, Synthetic Mixture, Validation method.

### Cite this article as:

Jinal Alkesh Gheewala\*, Dr. Dilip Girish Maheshwari, Development and Validation of UV Spectrophotometric Method and RP - HPLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone In Synthetic Mixture, Asian Journal of Pharmaceutical Technology & Innovation, 05 (23); 66-78, 2017. [www.asianpharmtech.com](http://www.asianpharmtech.com)

Dr. Dilip Girish Maheshwari,  
H.O.D Quality Assurance Department, L.J. institute of Pharmacy, Sarkhej Highway Road,  
Ahmedabad, Gujarat, India- 382210. Email Id: [dgmaheshwari@gmail.com](mailto:dgmaheshwari@gmail.com)

## INTRODUCTION

Teneligliptin and Pioglitazone is an Antidiabetic drug. Teneligliptin is a pharmaceutical drug for the treatment of type 2 Diabetes mellitus. It belongs to the class of anti-diabetic drug known as dipeptidyl peptidase -4 inhibitors or gliptin. It is used to control blood sugar level in patient affected by type 2 diabetes. It work by inhibiting the activity of certain enzyme known as DDP-4. The mechanism of DPP-4 inhibitors is to increase incretin levels (GLP-1 and GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, & Decrease the blood sugar level.

Poglitazone is an oral drug that reduces the amount of glucose (sugar) in the blood. It is in a class of anti-diabetic drugs called thiazolidinedione that are used in the treatment of type 2 diabetes. Pioglitazone is referred to as an "Insulin sensitizer" because it attaches to the insulin receptors on cells throughout the body and causes the cell to become more sensitive to insulin. 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.

According to clinical trial monotherapy of Pioglitazone leads to increase in body weight and edema incidences in the patient and in combination with Teneligliptin leads to lowering of incidence of edema and body weight in the patient.

## MATERIAL AND METHODS

### Method A

#### Instruments

UV Visible Spectrophotometer: A Shimadzu UV-visible double beam spectrophotometer model 1800 (Japan) with spectral width 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell.

Spectra were automatically obtained by UV probe system software (UV probe version 2.31)

Digital analytical weighing balance: Wensler DAB-220

Sonicator: Equitron

### Method B : RP-HPLC method

#### Chromatographic condition

- **Column:** Peerless C-18 (250×4.6 mm, 5 μm)
- **Mobile phase:** Methanol :Phosphate buffer: ACN (pH 3.3 adjusts with 10% ortho phosphoric acid) (50:25:25%v/v)
- **Flow rate:** 1ml/min
- **Detection Wavelength:** 225nm
- **Run time:** 10min
- **Detector:** UV detector
- **Injection volume :** 20μl

#### Chemicals and Materials:

- Teneligliptin (Purechem Pvt, Ahmedabad)
- Pioglitazone (Cadila, Ahmedabad)
- Methanol (Aventor Performance Material, India)

Synthetic mixture of Teneligliptin and Pioglitazone were prepared in the fixed dose of 20 mg Teneligliptin and 30 mg Pioglitazone respectively in laboratory scale as pilot batch.

#### Selection of a Solvent:

Both The Drugs were soluble in Methanol. So, Methanol was selected as a solvent for estimation of both the Drugs.

#### Preparation of standard stock solution

##### Preparation of standard stock solution of Teneligliptin (1000μg/ml):

Weighed accurately 100 mg of Teneligliptin and was transferred into 100 ml volumetric flask, diluted to half and sonicated and made up to the mark with Methanol. (1000 μg/ml)

**Preparation of working standard stock solution of Teneligliptin (100µg/ml):**

Pipetted out 10 ml from the stock solution and transferred into 100 ml volumetric flask and diluted with Methanol to obtain 100µg/ml.

**Preparation of standard stock solution of Pioglitazone (1000µg/ml):**

Weighed accurately 100 mg of Pioglitazone and was transferred into 100 ml volumetric flask, diluted to half and sonicated and made up to the mark with Methanol. (1000 µg/ml)

**Preparation of working standard stock solution of Pioglitazone (100µg/ml):**

Pipetted out 10 ml from the stock solution and transferred into 100 ml volumetric flask and diluted with Methanol to obtain 100µg/ml.

The solutions were scanned in the range 200-400 nm and  $\lambda_{max}$  found to be 246 nm for Teneligliptin and 269 nm for Pioglitazone which match standard  $\lambda_{max}$  Teneligliptin and Pioglitazone.

**Procedure of selection of wavelength:**

0.6 ml working standard stock solution of Teneligliptin (100 µg/ml) and 0.9 ml working standard stock solution of Pioglitazone (100 µg/ml) was transferred into different 10 ml volumetric flask and dilute up to mark with Methanol to get 6 µg/ml of Teneligliptin and 9 µg/ml of Pioglitazone. Each solution was scanned in the range of 200-400 nm. Zero Order spectra were converted into First Order spectra. Teneligliptin shows ZCP (Zero Crossing Point) at 269.2 nm and Pioglitazone show ZCP at 228.5 nm. Hence, these wavelengths 228.5 and 269.2 were selected as analytical wavelengths.

**Method Validation**

Method validation was performed following ICH guidelines. The proposed technique has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

**1) Linearity (Calibration curve)**

The linearity of Teneligliptin and Pioglitazone was found to be in the range of 2-10 µg/ml and 3-15µg/ml, respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

All D1 spectrums were recorded using above spectrophotometric condition. D1 absorbance at 228.5 nm and 269.2 nm were recorded for Teneligliptin and Pioglitazone, respectively (n=6). Calibration curve were obtained by plotting average absorbance versus concentrations for both the drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Teneligliptin was  $y = -0.0071x + 0.00052$  ( $R^2=0.995$ ) and Pioglitazone was  $y = -0.0047x - 0.0087$  ( $R^2= 0.997$ ).

**2) Accuracy**

Accuracy of the developed method was confirmed by doing recovery study by addition of standard drug to the pre-quantified sample preparation at three different concentration levels 50 %, 100 % and 150 %, taking in to consideration percentage purity of added drug sample. The amounts of Aripiprazole and Clozapine were estimated by applying obtained values to the respective regression line equations. Each concentration was analyzed 3 times and average recoveries were measured.

**3) Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility. The repeatability was evaluated by assaying 6 times of sample solution of 4µg/ml Aripiprazole and 10µg/ml Clozapine prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Teneligliptin and Pioglitazone was carried out by estimating different concentration of Aripiprazole (2, 4, 6µg/ml) and Clozapine (3, 6, 19µg/ml), 3 times on same day and on 3 different day (first, second and third).

**4) Limit of Detection (LOD) and Limit of Quantification (LOQ)**

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the  $3.3 \times (SD/Slope)$  and  $10 \times (SD/Slope)$  criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

**Chromatography:**

The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. The pKa value for Teneligliptin and Pioglitazone is 10.44 and 6.66 respectively. After number of trial experiments, it was established that the mobile phase Methanol: ACN: potassium dihydrogen Ortho phosphate buffer (pH 3.3 adjusts with Ortho phosphoric acid) (50:25:25) shows good peak shape and resolution.

**System suitability parameters**

The resolution, tailing factor and number of theoretical plates are shown in table. The values obtain confirmed the suitability of the system for the analysis of these drugs in combination.

**TABLE: 1 Linearity data of Teneligliptin at 228.5 nm**

<b>Teneligliptin</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Absorbance ± SD (n=6)</b>	<b>% RSD</b>
2	-0.096 ±0.0016	1.66
4	-0.107 ±0.0017	1.58
6	0.196 ±0.0023	1.17
8	-0.223 ±0.0024	1.07
10	-0.314 ±0.0028	0.89

**TABLE 2: Linearity data of Pioglitazone at 269.2 nm**

<b>Pioglitazone</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Absorbance ± SD (n=6)</b>	<b>% RSD</b>
3	-0.064 ±0.0010	1.56
6	-0.086 ±0.0013	1.51
9	-0.112 ±0.0016	1.42
12	-0.123 ±0.0017	1.38
15	-0.176 ±0.0021	1.19

**Table 3: Precision study of Teneligliptin at 228.5 nm**

<b>Intraday precision of Teneligliptin</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Absorbance ±SD (n=3)</b>	<b>% RSD</b>
2	-0.095 ±0.0012	1.26
4	-0.107 ±0.0013	1.21
6	-0.196 ±0.0015	0.76
<b>Interday precision for Teneligliptin</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Absorbance ±SD (n=3)</b>	<b>% RSD</b>
2	-0.093 ±0.0014	1.5
4	-0.105 ±0.0015	1.42
6	-0.195 ±0.0027	1.38
<b>Repeatability of Teneligliptin</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Absorbance ± SD (n=6)</b>	<b>% RSD</b>
4	-0.107 ±0.0014	1.30

**Table 4: Precision study of Pioglitazone at 269.2nm**

Intraday precision of Pioglitazone		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
3	-0.063 ±0.0008	1.23
6	-0.086 ±0.0010	1.16
9	-0.112 ±0.0011	0.98
Interday precision of Pioglitazone		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
3	-0.065 ±0.0009	1.38
6	-0.086 ±0.0011	1.27
9	-0.112 ±0.0012	1.07
Repeatability of Pioglitazone		
Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD
6	-0.086 ±0.0013	1.51

**Table 5: LOD and LOQ data for Teneligliptin and Pioglitazone of first order derivative method**

Parameter	Teneligliptin	Pioglitazone
LOD(µg/ml)	0.33	0.24
LOQ(µg/ml)	0.97	0.72

**Table 6: Recovery study**

Name of Drug	% Level of recovery	Amt Taken (µg/ml)	Amt Added (µg/ml)	Total Amt (µg/ml)	Amt Recovered (µg/ml)	% Mean Recovery ± S.D. (n=3)
Teneligliptin	50	4	2	6	5.9	98.37±0.0709
	100	4	4	8	7.89	98.68±0.0971
	150	4	6	10	9.83	98.366±0.2081
Pioglitazone	50	6	3	9	8.80	97.8±0.2645
	100	6	6	12	11.7	98.33±0.3785
	150	6	9	15	14.7	98.33±0.4015

**Table 7: Analysis of synthetic mixture**

Name of Drug	Amount taken (µg/ml)	Mean Amount found (µg/ml)	% Assay ± S.D.
Teneligliptin	2	1.97	98.5±0.152
Pioglitazone	3	2.95	98.33±0.378

**Table 8: Summary of validation parameters**

Sr. No.	Parameters	Teneligliptin	Pioglitazone
1	Wavelength (nm)	228.5 nm	269.2 nm
2	Beer's Law Limit (µg/ml)	2-10	3 - 15
3	Regression equation (y = mx +c)	y =-0.0071x+0.00052	y =- 0.0047x-0.0087
4	Correlation Coefficient (r <sup>2</sup> )	0.9957	0.9971
5	Intraday Precision (%RSD, n=3)	0.76-1.26	0.98-1.23

6	Interday Precision (% RSD, n=3)	1.38-1.49	1.02-1.36
7	Repeatability (% RSD, n=6)	1.30	1.51
8	Accuracy (% Recovery, n=3)	098.3-98.66	97.8-98.3
9	LOD (µg/ml)	0.24	0.32
10	LOQ (µg/ml)	0.97	0.72
11	%Assay	98.5	98.33

**Table 9: System suitability parameter**

Sr. No.	Parameters	Teneligliptin	Pioglitazone
1	Retention Time	2.58	6.13
2	Theoretical Plates	2716	4435
3	Tailing Factor	1.346	1.354
4	Area (µV.s)	1716	2415
5	Resolution	12522	

**Table 10: Calibration Data for Teneligliptin (1-5 µg/ml) and Pioglitazone (1.5-7.5 µg/ml)**

Teneligliptin			Pioglitazone		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD	Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD
1	118351±325.639	0.27515	1.5	177918.8±856.4007	0.481343
2	183768±322.304	0.17539	3	293617.2±1149.586	0.391526
3	254581±328.551	0.12906	4.5	442323.8±1437.062	0.324889
4	334531±363.226	0.10858	6	557873.8±1547.955	0.277474
5	413946±356.097	0.08603	7.5	720947.5±1764.789	0.244787

**Table 11: Precision study of Teneligliptin**

TENELIGLIPTIN		
INTRADAY PRECISION		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=3)	% RSD
1	118597±319.85	0.26969
2	183768±321.308	0.17484
3	254581±328.551	0.12906
INTERDAY PRECISION		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=3)	% RSD
1	118385±320.87	0.27104
2	184055±322.304	0.17511
3	254781±327.557	0.12856
REPEATABILITY		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=3)	% RSD
3	254581±328.551	0.12906

**Table 12: Precision study of Pioglitazone**

<b>Pioglitazone</b>		
<b>INTRADAY PRECISION</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Peak Area (µV.*s) ± S.D. (n=3)</b>	<b>% RSD</b>
1.5	177786±853.25	0.479931
3	293645±1147.585	0.390807
4.5	441128±1444.069	0.327358
<b>INTERDAY PRECISION</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Peak Area (µV.s) ± S.D. (n=3)</b>	<b>% RSD</b>
1.5	178586±852.21	0.477199
3	293657±1149.586	0.391472
4.5	441421±1437.062	0.325554
<b>REPEATABILITY</b>		
<b>Conc. (µg/ml)</b>	<b>Mean Peak Area (µV.s) ± S.D. (n=3)</b>	<b>% RSD</b>
3	442323.8±1437.062	0.324889

**Table 13: Recovery Study Data**

<b>Name of Drug</b>	<b>% Level of recovery</b>	<b>Amt Taken (µg/ml)</b>	<b>Amt Added (µg/ml)</b>	<b>Total Amt (µg/ml)</b>	<b>Amt Recovered (µg/ml)</b>	<b>% Mean Recovery ± S.D. (n=3)</b>
<b>Teneligliptin</b>	50	2	1	3	2.98	99.66±0.2150
	100	2	2	4	3.97	99.70±0.2335
	150	2	3	5	4.99	100.15±0.2783
<b>Pioglitazone</b>	50	3	1.5	4.5	4.45	99±0.2081
	100	3	3	6	5.97	99.5±0.2309
	150	3	4.5	7.5	7.48	99.8±0.3511

**Table 14: LOD and LOQ Data**

<b>Parameter</b>	<b>Teneligliptin</b>	<b>Pioglitazone</b>
LOD(µg/ml)	0.028962	0.062885
LOQ(µg/ml)	0.078358	0.190561

**Table 15: Analysis of synthetic mixture**

<b>Name of Drug</b>	<b>Amount taken (µg/ml)</b>	<b>Mean Amount found (µg/ml)</b>	<b>% Assay ± S.D.</b>	<b>% RSD</b>
<b>Teneligliptin</b>	<b>2</b>	1.99	99.83±0.23065	1.16
<b>Pioglitazone</b>	<b>3</b>	2.98	99.5±0.27301	0.92

**Table 16: Summary of validation parameters**

<b>Sr. No.</b>	<b>Parameters</b>	<b>Teneligliptin</b>	<b>Pioglitazone</b>
1	Beer's Law Limit (µg/ml)	1-5	1.5-7.5
2	Regression equation (y = mx +c)	y=3710x +38351	y=44941x +33546

3	Correlation Coefficient ( $r^2$ )	0.998	0.9968
4	Intraday Precision (%RSD, n=3)	0.129-0.26	0.32-0.47
5	Interday Precision (% RSD, n=3)	0.128-0.27	0.32-0.48
6	Repeatability (% RSD, n=6)	0.129056	0.324889
7	Accuracy (% Recovery, n=3)	99.16-100.15	99-99.8
8	LOD ( $\mu\text{g/ml}$ )	0.078358	0.19056
9	LOQ ( $\mu\text{g/ml}$ )	0.028962	0.06289
10	%Assay	99.70	100.11

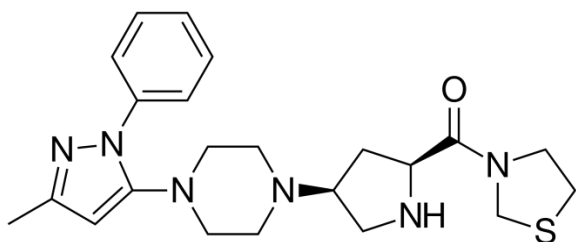


FIGURE 1: Structure of Telenigiptin

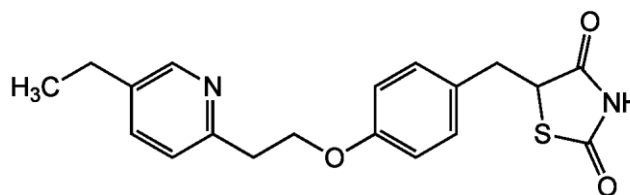


FIGURE 2: Structure of Pioglitazone

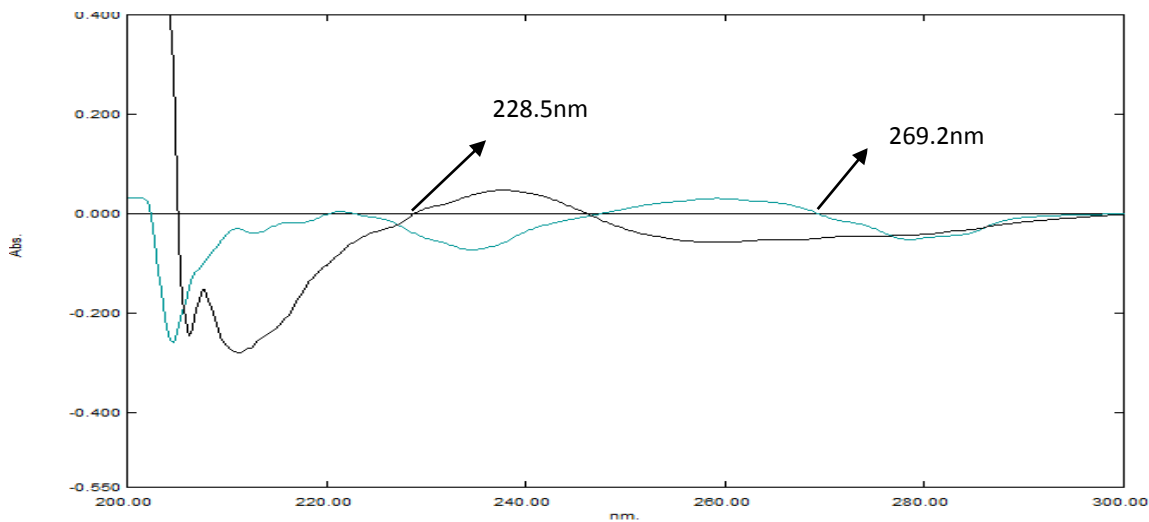
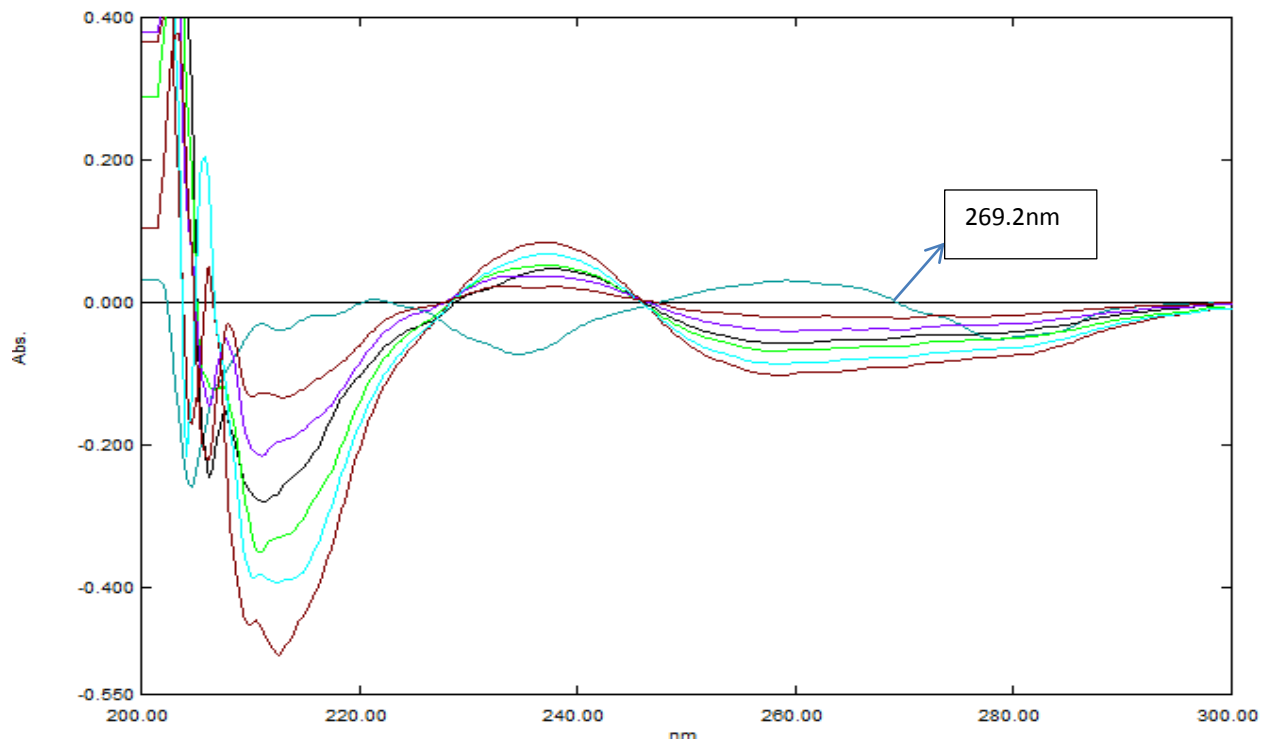
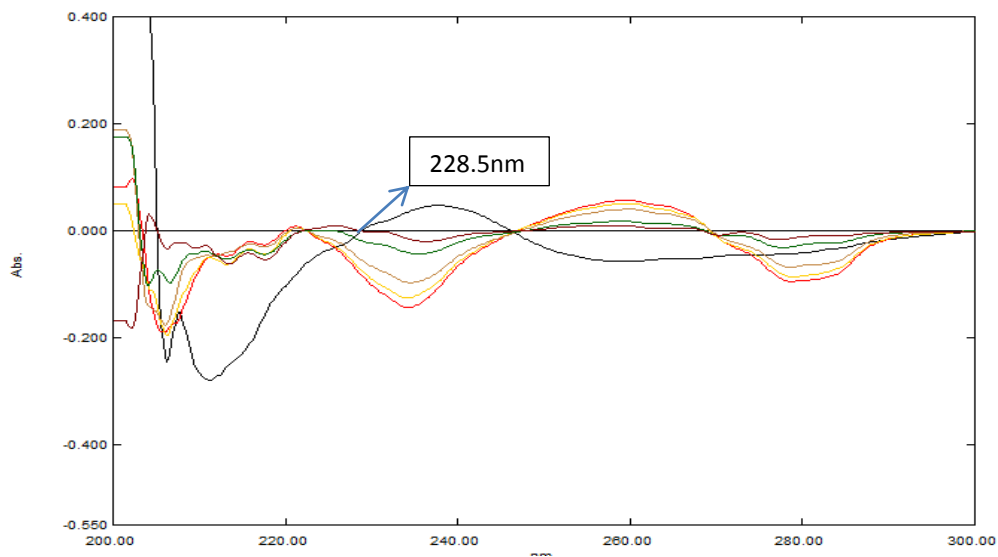


FIGURE 3: Overlain spectra of Telenigiptin (10 $\mu\text{g/ml}$ ) and Pioglitazone (15  $\mu\text{g/ml}$ ) in methanol (First order)





**FIGURE 4. Linearity of 1<sup>st</sup> Derivative Spectra of Telenigiptin**



**FIGURE 5 Linearity of 1<sup>st</sup> Derivative Spectra of Pioglitazone**

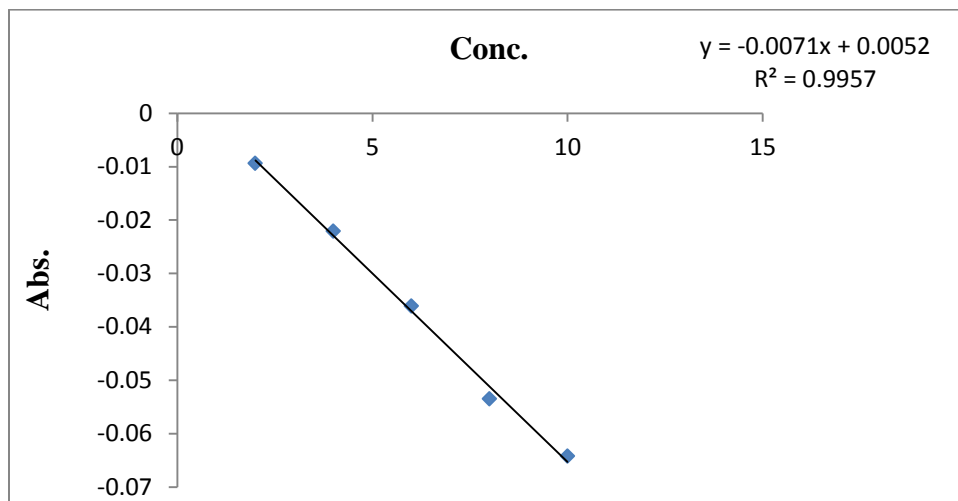


FIGURE 6 Calibration curve of Telenigiptin

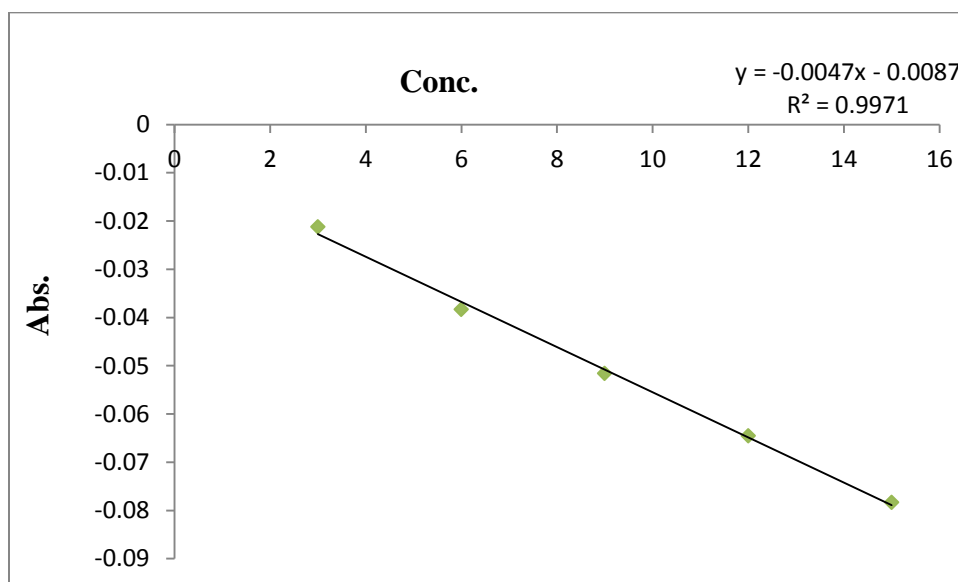


FIGURE 7 Calibration curve of Pioglitazone

RP-HPLC

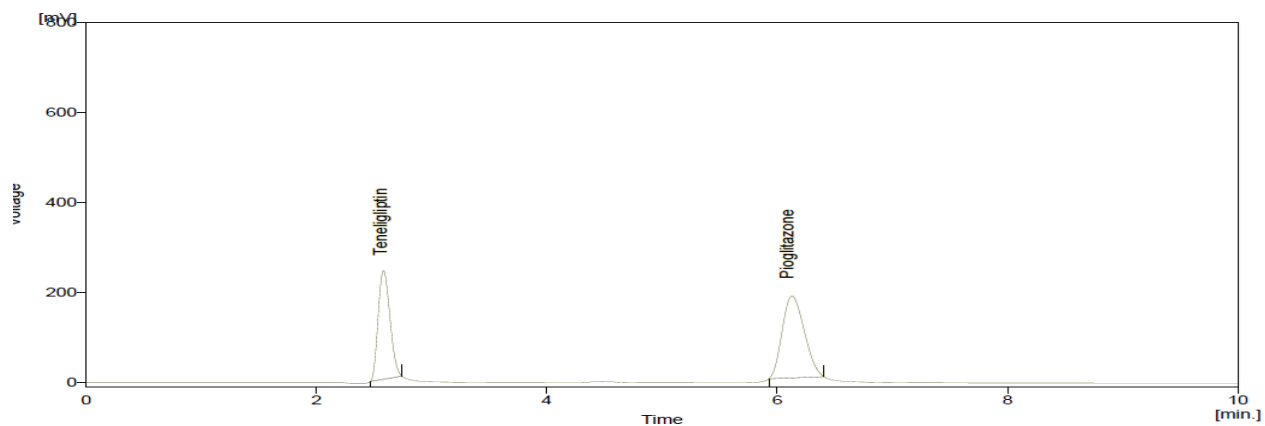


Figure 8: Chromatogram of Telenigiptin(3µg/ml) and Pioglitazone (3 µg/ml) in Methanol: ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.3) (50:25: 25)

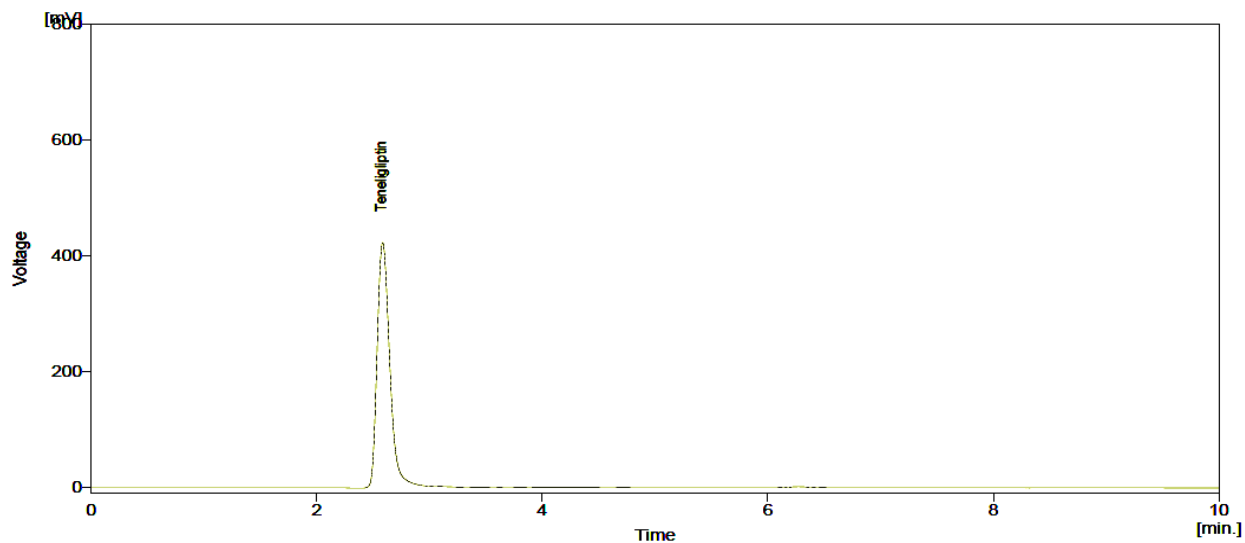


FIGURE 9 Chromatogram of Teneiglipitin (3 µg/ml) in Methanol: ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.3) (50:25:25)

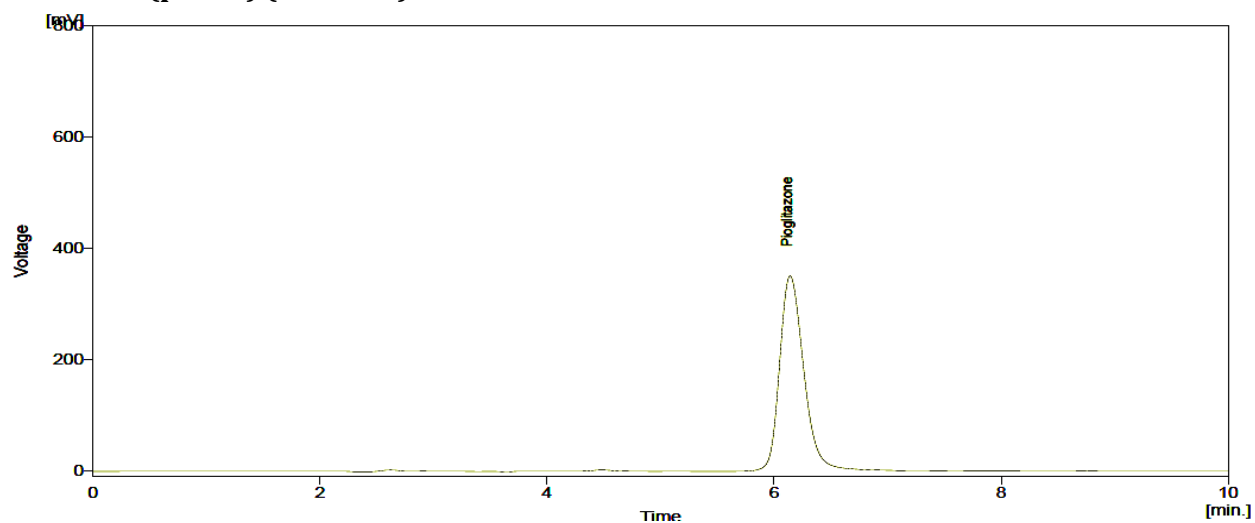


FIGURE 10: Chromatogram of Pioglitazone (3 µg/ml) in Methanol: ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.3) (50:25:25)

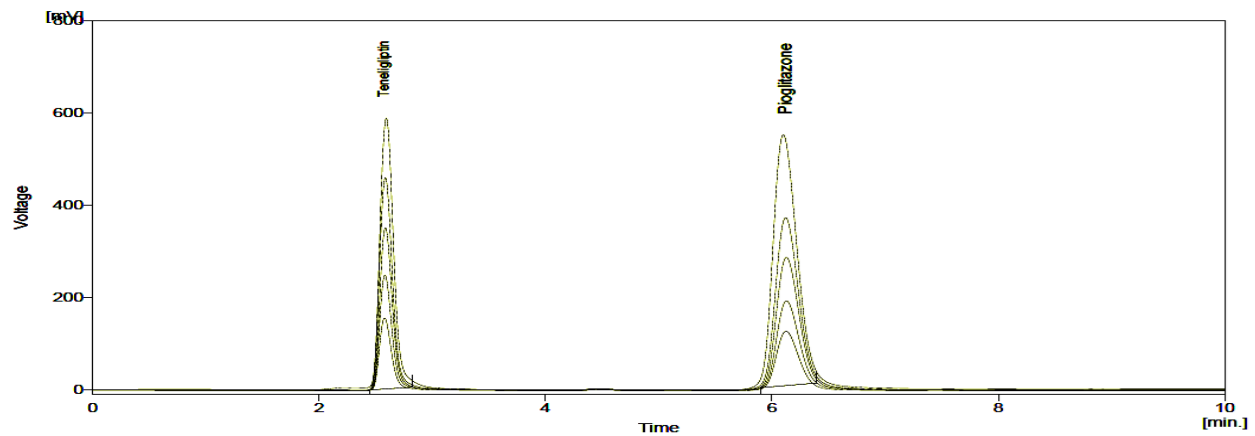
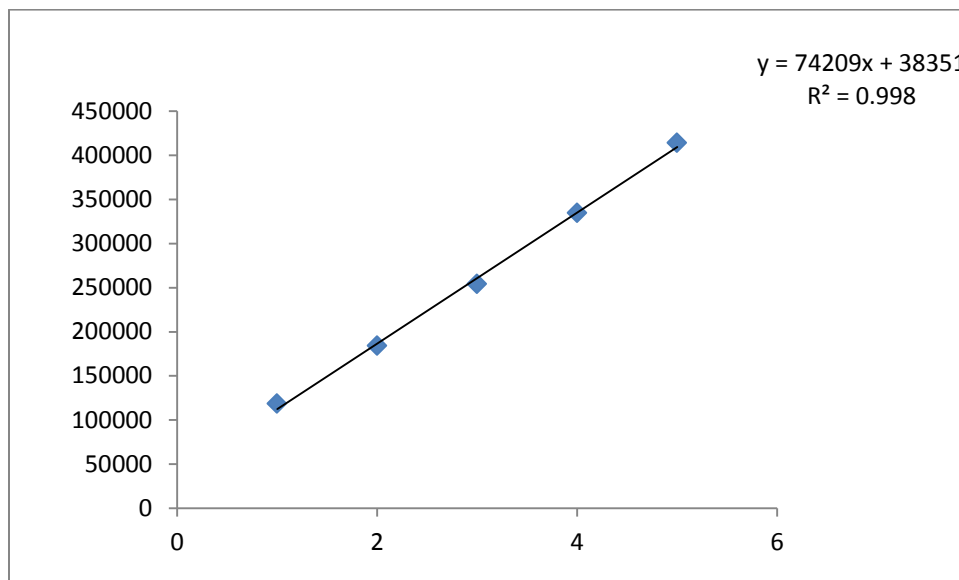
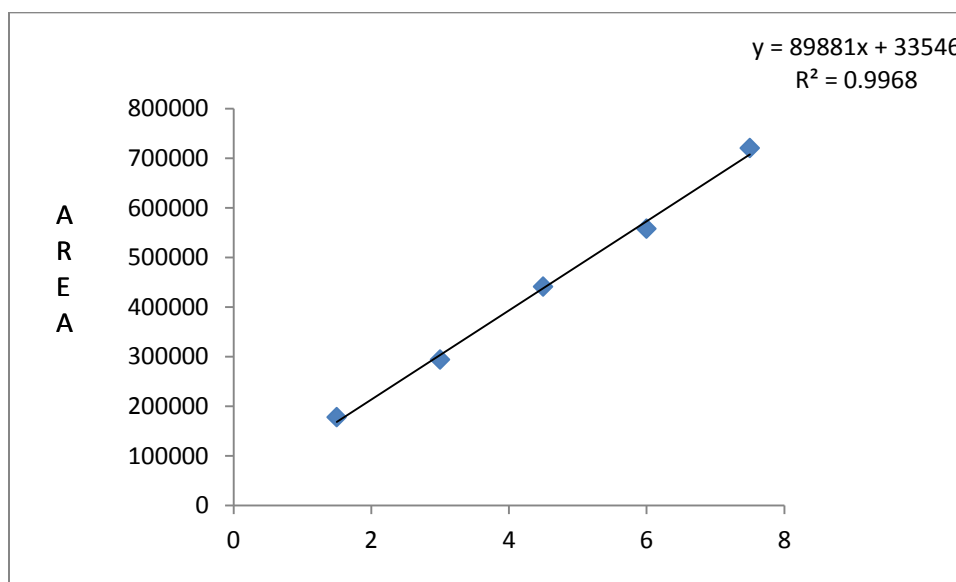


Figure: 11 Overlay Chromatogram of Teneiglipitin (1-5 µg/ml) and Pioglitazone (1.5-7.5 µg/ml)



**Fig 12: Calibration Curve of Telenigliptin (1-5µg/ml)**



**Fig 13: Calibration curve of Pioglitazone (1.5-7.5µg/ml)**

## RESULT AND DISCUSSION

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method have been developed for simultaneous estimation of Telenigliptin and Pioglitazone in Synthetic Mixture. Telenigliptin shows ZCP (Zero Crossing Point) at 269.2 nm and Pioglitazone show ZCP at 228.5 nm. At 228.5 (ZCP of Pioglitazone) Telenigliptin shows considerable absorbance while at 269.2 nm (ZCP of Telenigliptin) Pioglitazone shows considerable absorbance. Linearity Range of 2-10 µg/ml for Telenigliptin and 3-15 µg/ml for Pioglitazone with Correlation Coefficient of 0.995 and 0.997 for Telenigliptin and Clozapine respectively was obtained and the Precision data obtained with less than 2% RSD.

Accuracy was carried out by Recovery Studies and was obtained in the range of 98.3-98.66 for Telenigliptin and 97.8-98.3 for Pioglitazone. LOD and LOQ values were found to be 0.33 and 0.97 µg/ml respectively for Telenigliptin and for Pioglitazone value were found to be 0.24 and 0.72 µg/ml respectively.

A Simple, Precise and Accurate RP-HPLC Method have been developed for simultaneous estimation of Telenigliptin and Pioglitazone in Synthetic Mixture.

Linearity Range of 1-5 µg/ml for Teneligliptin and 1.5-7.5 µg/ml for Pioglitazone with Correlation Coefficient of 0.998 and 0.996 for Teneligliptin and Pioglitazone respectively was obtained and the Precision data obtained with less than 2% RSD.

Accuracy was carried out by Recovery Studies and was obtained in the range of 99.66-100.15 for Teneligliptin and 99-99.8 for Pioglitazone. LOD and LOQ values were found to be 0.029 and 0.078 µg/ml respectively for Teneligliptin and for Pioglitazone value were found to be 0.0628 and 0.1905 µg/ml respectively.

The proposed method was precise, accurate and reproducible with acceptable recovery, which can be applied for the analysis of Teneligliptin and Pioglitazone in synthetic mixture.

## CONCLUSION

The results of present study indicate that the proposed UV spectroscopic method is simple, precise and accurate. Statistical analysis proves that the method is repeatable and selective for the analysis of Teneligliptin and Pioglitazone in combination. It can therefore be concluded that the developed analytical method was precise & accurate and can be use for routine Analysis of both the drug in combination.

## ACKNOWLEDGEMENT

We are heartily thankful to Dr. K. Pundarikakshudu, Director of L.J Institute of Pharmacy, Ahmedabad for providing all the facilities and the valuable Guidance during the Research work.

## REFERENCES

1. Walker R., and Cate Whittlesea, In Clinical Pharmacy & Therapeutics; 4th Edn; Churchill Livingstone Elsevier; 2008, pp 523-533.
2. Mohan H., In Textbook of Pathophysiology; 6th Edn; Jaypee Brothers, Medical publishers Pvt, Ltd; pp 353-359.
3. Rang H. and Dale M., Rang & Dale's pharmacology; 7<sup>th</sup> Edn; Elsevier publication, 2012, pp517-521,528-533,827-856.
4. Tripathi KD., Essential of Medical pharmacology; 6<sup>th</sup> Edn; Jaypee Brother Medical publishers Ltd, 2008, pp254-257.
5. Wells Barbara., Dipiro.TJ.,Schwinghammer.LT.,and Dipiro.VC., Pharmacotherapy Handbook; 7<sup>th</sup> Edn;pp210-211.
6. Goyal RK., Mehta AA., Balaraman RD. and Gandhi's elements of pharmacology; 7<sup>th</sup> Edn; B.S.Shah Prakashan Pharamcology, 2007-208,pp322,330-333,265-266,269-272.
7. Drug Profile, "Description of Teneligliptin" [file.scirp.org/pdf/JDM\\_2016033116144017.pdf](http://file.scirp.org/pdf/JDM_2016033116144017.pdf) (Last Accessed : 15/8/2016)
8. Drug profile, "Description of Teneligliptin" [https://www.pharmacygeoff.md/Tenelia\\_Teneligliptin\\_Hydrobromide\\_Hydrate\\_20mg...](https://www.pharmacygeoff.md/Tenelia_Teneligliptin_Hydrobromide_Hydrate_20mg...) (Last Accessed : 15/8/2016)
9. Drug profile, "Contraindication of Teneligliptin" [www.tabletwise.com/medicine/teneligliptin/contraindications](http://www.tabletwise.com/medicine/teneligliptin/contraindications) (Last Accessed :16/8/2016)
10. Pubchem, "Physicochemical Properties of Teneligliptin" <https://pubchem.ncbi.nlm.nih.gov/compound/Teneligliptin> (Last Accessed:16/8/2016)
11. "Pioglitazone: Drug Profile" August 2016, <http://www.medicinenet.com/pioglitazone/index.htm> (Last Accessed: 13 Sep.2016)
12. "Pioglitazone:DrugProfile" August2016, <http://www.medicinent.com/script/main/hp.asp> (Last Accessed: 13,Sep.2016)
13. "Pioglitazone: Drug profile" August 2016, <http://en.wikipedia.org/wiki/pioglitazone> ( Last Accessed : 13,Sep.2016)
14. "Pioglitazone:Drugprofile" August2016, <http://www.nlm.nih.gov/medlineplus/druginfo/meds/a699016.html> (Last Accessed: 13, Sep.2016)
15. Snyder LR., Kirkland JJ. and Gajch JL. In Practical HPLC Method Development;2<sup>nd</sup> Edn; John wiley & Sons Inc. Publication, 1997, pp 21-56, 234-265.
16. Dong WM. Modern HPLC for practicing scientists; 7th Edn; Wiley interscience Publication, 2001, pp 4-11, 78-80, 84-86.
17. Hala A, Beniwal P., "Various detectors used in the high performance liquid chromatography with comparison of their sensitivity" December 2011, <http://www.pharmatutor.org/articles/details-on-various-detectors-used-in-high-performanceliquid-chromatography-in-compare-to-sensitivity>.
18. ICH, Q1 A (R2), Stability Testing of New Drugs and Product 2003, pp. 1-20  
ICH, Q2 (R1) (2005) Validation of Analytical Procedure: Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland