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Research Article

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

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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 (R2) 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 (R2) 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.

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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: Wenser 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 λmax found to be 246 nm for Teneligliptin and 269 nm for Pioglitazone which match standard λmax 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 (R2=0.995) and Pioglitazone was y = -0.0047x-0.0087 (R2= 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 × (SD/Slope) and 10 × (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

Teneligliptin		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
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

Pioglitazone		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
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

Intraday precision of Teneligliptin		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
2	|-0.095|±0.0012	1.26
4	|-0.107|±0.0013	1.21
6	|-0.196|±0.0015	0.76
Interday precision forTeneligliptin		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
2	|-0.093|±0.0014	1.5
4	|-0.105|±0.0015	1.42
6	|-0.195|±0.0027	1.38
Repeatability of Teneligliptin		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
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 Recove-ry ± 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^2)	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

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

Table 13: Recovery Study Data

Name of Drug	% Level of recovery	Amt Taken (µg/ml)	Amt Added (µg/ml)	Total Amt (µg/ml)	Amt Recovered (µg/ml)	% Mean Recove-ry ± S.D. (n=3)
Teneligliptin	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
Pioglitazone	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

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

Table 15: Analysis of synthetic mixture

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

Table 16: Summary of validation parameters

Sr. No.	Parameters	Teneligliptin	Pioglitazone
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 (µg/ml)	0.078358	0.19056
9	LOQ (µg/ml)	0.028962	0.06289
10	%Assay	99.70	100.11

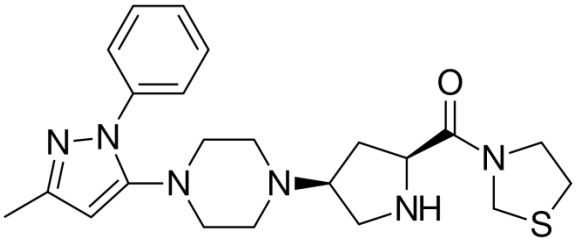

FIGURE 1: Structure of Teneligliptin

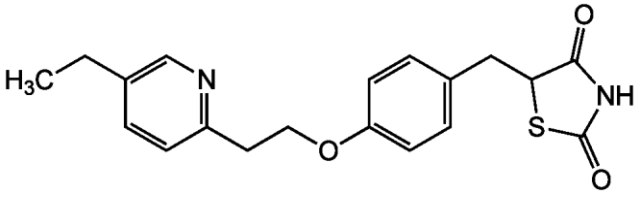

FIGURE 2: Structure of Pioglitazone

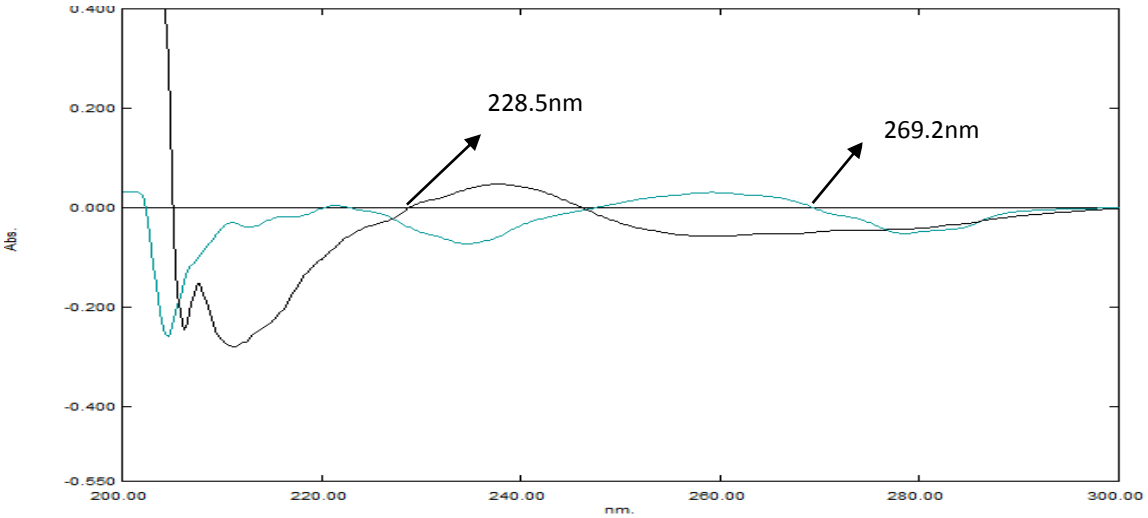

FIGURE 3: Overlain spectra of Teneligliptin (10µg/ml) and Pioglitazone (15 µg/ml) in methanol (First order)

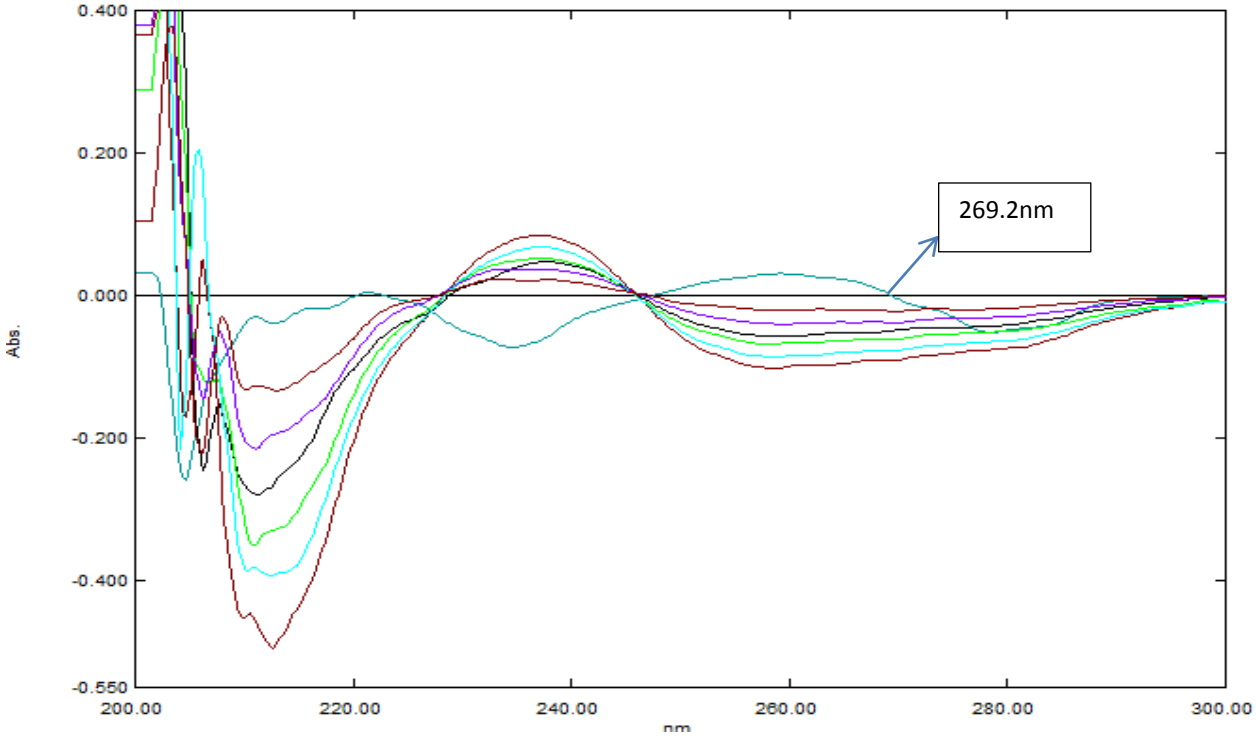

FIGURE 4. Linearity of 1st Derivative Spectra of Teneligliptin

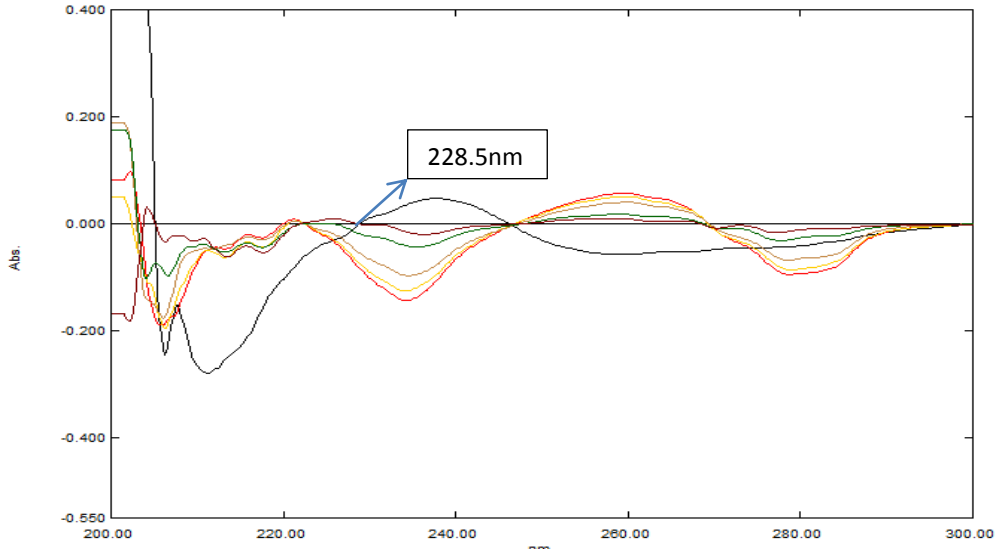

FIGURE 5 Linearity of 1st Derivative Spectra of Pioglitazone

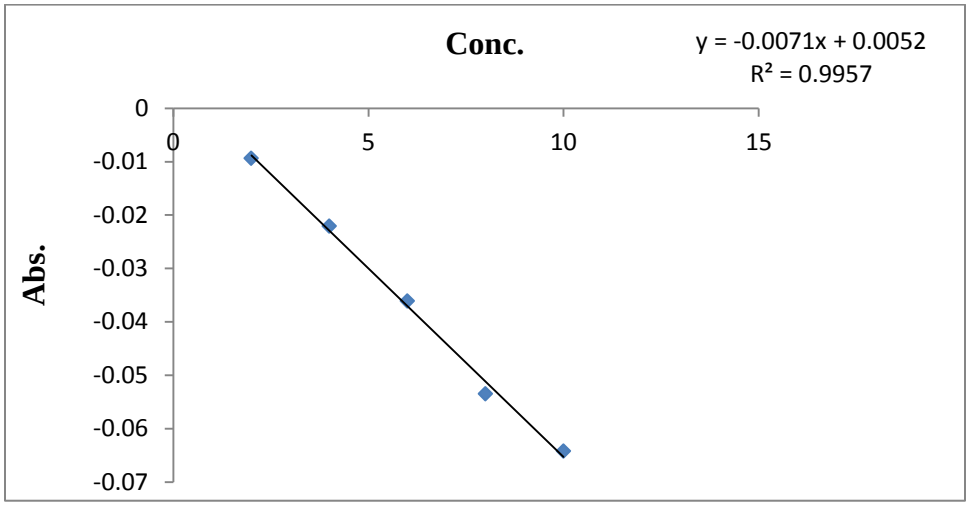

FIGURE 6 Calibration curve of Teneligliptin

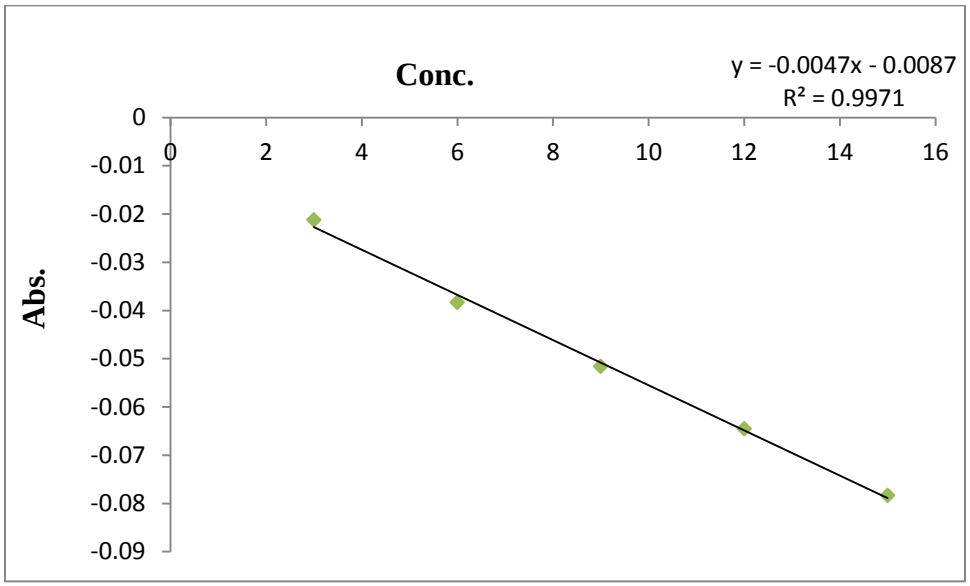

FIGURE 7 Calibration curve of Pioglitazone

RP-HPLC

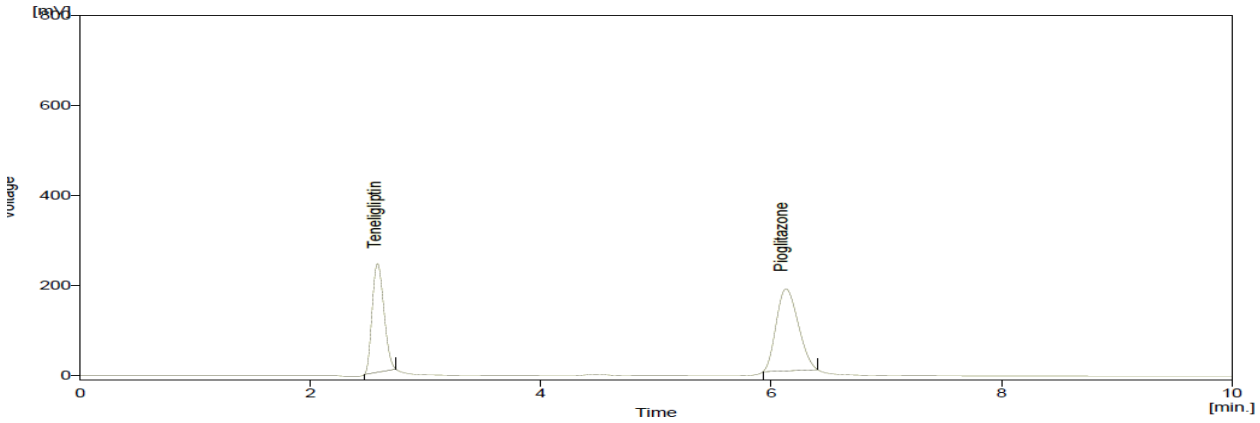

Figure 8: Chromatogram of Teneligliptin(3µg/ml) and Pioglitazone (3 µg/ml) in Methanol: ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.3) (50:25: 25)

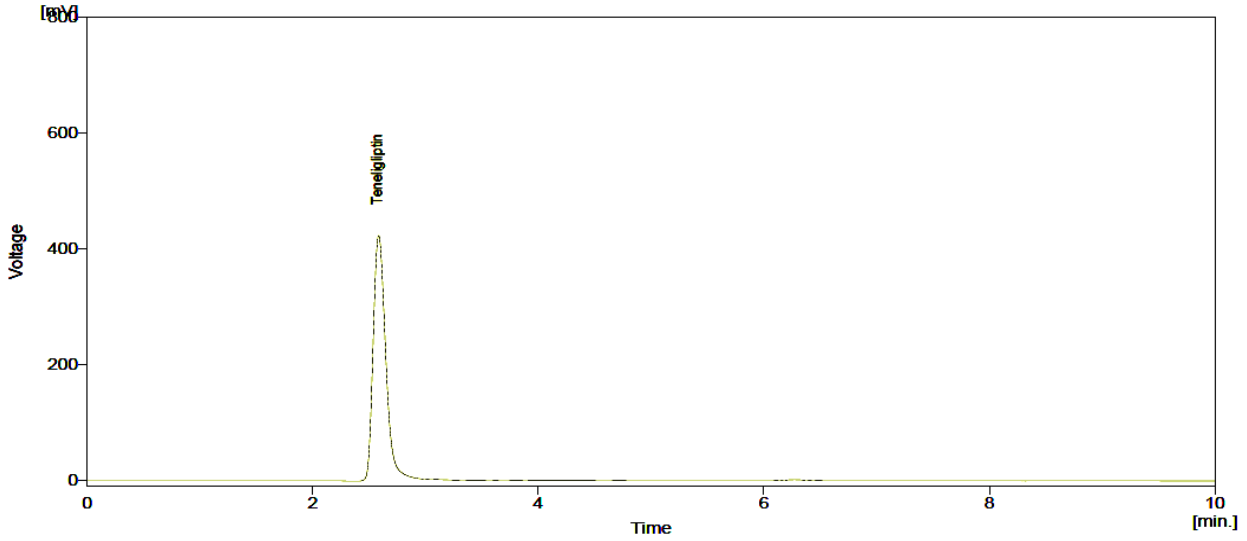

FIGURE 9 Chromatogram of Teneligliptin (3 µg/ml) in Methanol: ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.3) (50:25:25)

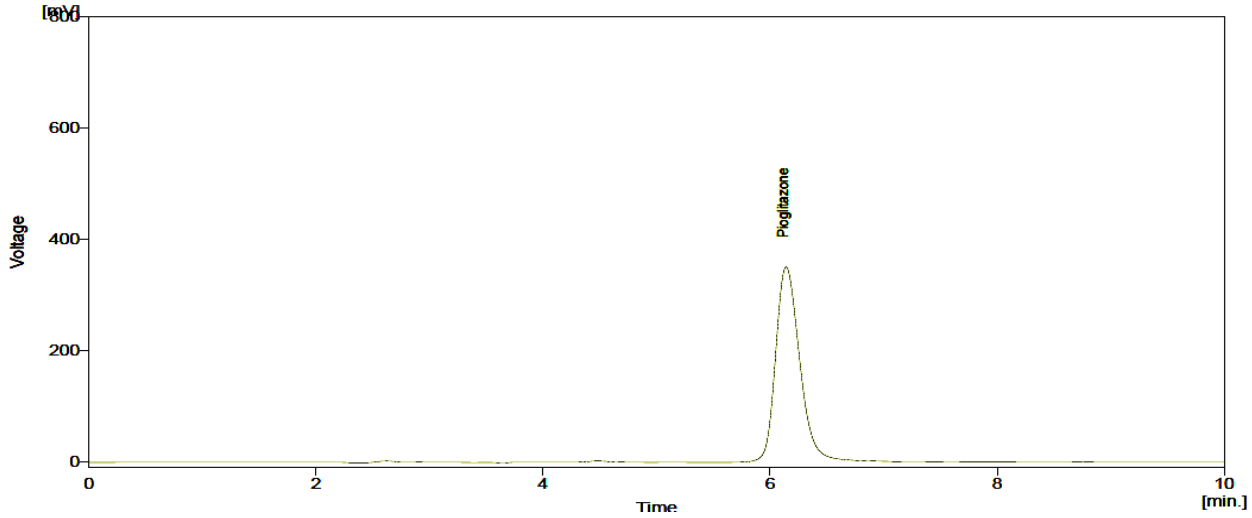

FIGURE 10: Chromatogram of Pioglitazone (3 µg/ml) in Methanol: ACN: potassium dihydrogen Ortho phosphate Buffer (pH 3.3) (50:25:25)

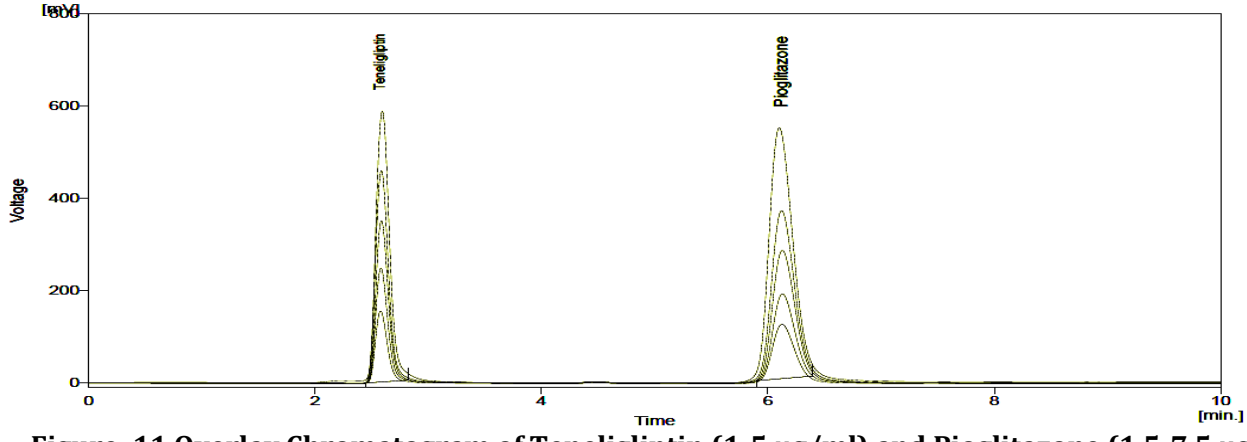

Figure: 11 Overlay Chromatogram of Teneligliptin (1-5 µg/ml) and Pioglitazone (1.5-7.5 µg/ml)

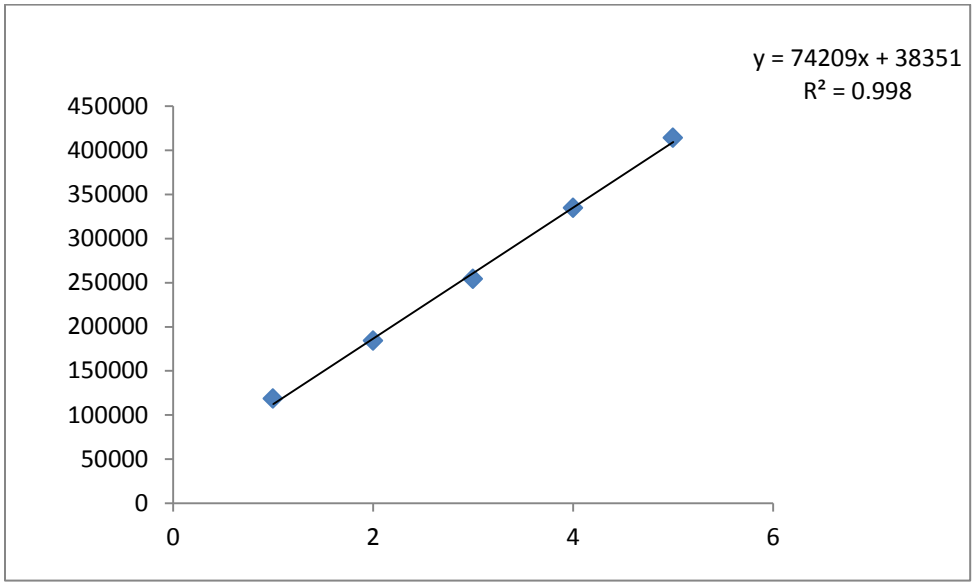

Fig 12: Calibration Curve of Teneligliptin (1-5µg/ml)

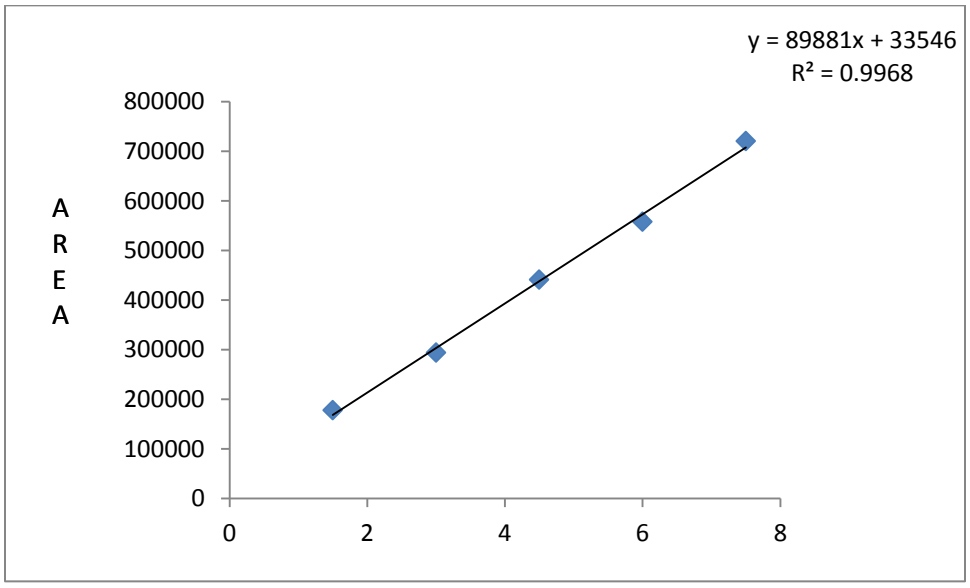

Fig 13: Calibration curve of Pioglitazone (1.5-7.5µg/ml)

RESULT AND DISCUSSION

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method have been developed for simultaneous estimation of Teneligliptin and Pioglitazone in Synthetic Mixture. Teneligliptin shows ZCP (Zero Crossing Point) at 269.2 nm and Pioglitazone show ZCP at 228.5 nm. At 228.5(ZCP of Pioglitazone) Teneligliptin shows considerable absorbance while at 269.2 nm (ZCP of Teneligliptin) Pioglitazone shows considerable absorbance. Linearity Range of 2-10 µg/ml for Teneligliptin and 3-15 µg/ml for Pioglitazone with Correlation Coefficient of 0.995 and 0.997 for Teneligliptin 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 Teneligliptin and 97.8-98.3 for Pioglitazone. LOD and LOQ values were found to be 0.33 and 0.97 µg/ml respectively for Teneligliptin 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 Teneligliptin 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 forTeneligliptin and for Pioglitazone value were found to be 0.0628 and 0.1905µg/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.

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REFERENCES

1. WalkerR., 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; 7th Edn; Elsevier publication, 2012, pp517-521,528-533,827-856.
4. Tripathi KD., Essential of Medical pharmacology; 6th Edn; Jaypee Brother Medical publishers Ltd, 2008, pp254-257.
5. WellsBarbara.,Dipiro.TJ.,Schwinghammer.LT.,and Dipiro.VC., Pharmacotherapy Handbook; 7th Edn;pp210-211.
6. Goyal RK., Mehta AA., Balaraman RD. and Gandhi's elements of pharmacology; 7th 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 (Last Accessed : 15/8/2016)
8. Drug profile, "Description of Teneligliptin" https://www.pharmacygeoff.md/Tenelia_Teneligliptin_Hydrobromide_Hydreate_20mg_... (Last Accessed : 15/8/2016)
9. Drug profile, "Contraindication of Teneligliptin"www.tabletwise.com/medicine/**teneligliptin/contraindications** (Last Accessed :16/8/2016)
10. Pubchem, "Physicochemical Properties of Teneligliptin" https:1//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 Giajch JL. In Practical HPLC Method Development;2nd 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