# DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF METFORMIN AND TENELIGLIPTIN IN SOLID DOSAGE FORM

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| ARTICLE INFO | ABSTRACT |
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**Key Words**

RP-HPLC,
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A simple, rapid, accurate, precise and economical reverse phase High performance liquid chromatographic method was developed for simultaneous quantification of two anti-diabetic drugs, viz., Metformin and Teneligliptin. The separation of both the drugs was achieved on an INERTSIL ODS column C8 (4.6mm x 250mm, 5μm particle size) using a mobile phase of phosphate buffer (at pH 3): Acetonitrile (50:50 v/v). The flow rate was 1.0 ml/min and detection was done at 240 nm. The retention time of Metformin and for Teneligliptin was 3.608 mins and 5.148 mins respectively. The proposed method was validated as per ICH guidelines. The linearity of the method was evaluated at a range of 250 to 1250μg/ml and 10 to 50μg/ml for Metformin and Teneligliptin respectively. The Correlation Coefficient of Metformin and Teneligliptin were 0.999 each. Precision studies were carried out and % RSD of peak areas of Metformin and Teneligliptin was about 0.4and 0.8 respectively. The percentage recoveries of both the drugs Metformin and Teneligliptin from the tablet formulation were 99.86% and 99.96% respectively. Results obtained for LOQ, LOD and Robustness were well within the acceptance criteria. Validation results indicated that the method is linear, accurate, precise, and robust. The simple mobile phase composition makes this method cost effective, rapid, and non-tedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

## INTRODUCTION

Metformin and Teneligliptin fixed-dose combination is an anti-hyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). Metformin is biguanide derivative it improves insulin sensitivity and decreases insulin resistance by inhibiting complex of the mitochondrial respiratory chain and inducing AMP activated protein kinase dependent signaling. Teneligliptin is peptide mimetic known as dipeptidyl peptidase-4 inhibitors or "gliptins". 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, and decreases blood glucose levels. **Metformin** is biguanide derivative with the chemical name (1- carbamimidamido - N, N-dimethyl methanimidamide). It is a White Crystalline powder, soluble in Water and Ethanol. **Teneligliptin** is pyrrolidine-based inhibitor of dipeptidyl peptidase 4 (DPP-4), The chemical name is 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4- [(3S,5S )- 5- (1, 3- thiazolidine-3-

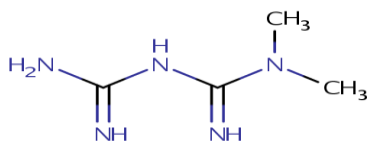carbonyl)pyrrolidin-3-yl]piperazine.soluble in Dimethylsulfoxide.



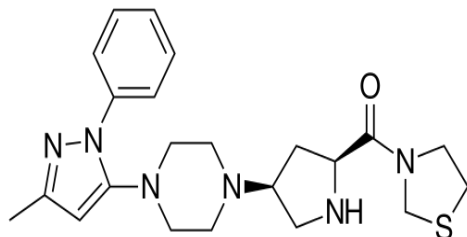**Figure 1: Structure of Metformin**



**Figure 2: Structure of Teneligliptin**

Some spectroscopic and chromatographic methods have been reported for the estimation of these drugs in individual dosage forms. Similarly a few more methods are reported for their estimation in combinations with other molecules .Literature also reveals reports on bio-analysis of these drugs from various biological matrices . But a very few RP-HPLC methods are known till date for the quantification of Metformin and Teneligliptin combination. Based on the above observations and the significance of this drug combination in the management of diabetes it was assumed worthy to develop a simple, reliable, economical and validated RP-HPLC method that can be conveniently adopted for the routine analysis [ 6, 7, 8,9,10].

## MATERIALS AND METHODS

**Instrumentation:** The present work utilized WATERS HPLC system with Empower software. A reverse phase column (make: INERTSIL ODS column C8 (250mm x 4.6mm , 5.0 μm particle size) was used. UV-spectra were obtained from LABINDIA 3000+ UV/VIS Spectrophotometer.

**Reagents and Chemicals:** HPLC grade solvents methanol, orthophosphoric acid and Acetonitrile were obtained from Merck Specialties Pvt Ltd, India. AR grade Potassium dihydrogen Orthophosphate and HPLC grade

water, alliance were obtained from Rankem Pharmaceuticals India Ltd. Metformin and Teneligliptin were obtained as gift samples from Saffola Life Sciences Pvt Ltd, Hyderabad, India and samples were obtained as tablets of Metformin (500mg) and Teneligliptin (20mg) from TENDIA M.

**Preparation of buffer (pH 3):** Accurately weighed 6.8gms of Potassium Di hydrogen Ortho Phosphate is transferred into 1000ml of water and Sonicated for 2 minutes and adjusted the PH for 3.0.

**Standard preparation:** Accurately weighed and transferred 5 mg of Teneligliptin and 125 mg of Metformin working standard into a 10ml clean dry volumetric flask. Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 0.6 ml of Teneligliptin & Metformin is pipetted from the above stock solution into a 10ml volumetric flask and diluted up to the mark with Diluent.

**Sample preparation:** Accurately weighed and transferred 5mg of Teneligliptin and 125 mg Metformin equivalent weight of the sample into a 10ml clean dry volumetric flask, about 7ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 0.6 ml of Teneligliptin and Metformin is pipetted from the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

**Selection of wavelength maxima:** Metformin and Teneligliptin showed isobestic point at wavelength 240 nm. Therefore 240nm was adopted as detection wavelength. The spectrum was shown in Fig**. 3.**

**Method Development:** Method development was initiated with a series of trials and reported. At each trial mixture of known components were injected and observed for resolution and tailing factor. Varied proportions of various solvents were tried as mobile phase and finally phosphate buffer: Acetonitrile at 50:50 gave improved peak symmetry and resolution. Finally, the chromatographic conditions were optimized at flow rate 1.0ml/min, injection volume of 20 μl, run time of 12 minutes, at ambient with Inertsil ODS, C8, (4.6mm x 250mm), 5μm column. The

retention time for Metformin and Tenegliptin was found to be 3.608 minutes and 5.148 minutes respectively. For both the drugs Metformin and Tenegliptin the tailing factor was found to be < 2. Further the method was validated as per ICH guidelines under the proposed chromatographic conditions. The results are given in **Table 1**. The chromatograms were represented as **Fig no: 4**.

**Method Validation:** Once chromatographic conditions were established, the method was validated in compliance with ICH guidelines. The following parameters like system suitability, specificity, linearity, precision, and accuracy, limits of detection and limit of quantification were performed.

**System Suitability:** The standard solution was prepared by using working standard as per the method. For six replicate injections system suitability parameters like number of theoretical plates, USP Tailing was found to be within specified limits. The results are given in **Table 2**.

**Specificity:** It is the ability of the method to measure the analyte of interest specifically in presence of matrix and other components. Samples of blank, standard and sample were injected as per the test procedure. The chromatograms were represented as **Fig no: 5& 6**

**Linearity:** Linearity of detector response was established by plotting graph between concentrations versus peak areas of the analytes. Data is shown in **Table 3** and represented graphically in **Fig 7 and Fig 8.**

**Accuracy:** Accuracy was determined by recovery studies at three different levels equivalent to 50%, 100%, 150%. Sample at each level is injected in triplicate. The concentration of the drug product in the solution was determined using assay method. The % RSD, mean recoveries was calculated, which shows that method is accurate. Data was shown in **Table 4.**

**System precision:** Six replicate injections of standard solution were injected into the HPLC system. The %RSD of peak areas for six replicate injections was found to be in the limits. Data is given in **Table 5.**

**Method precision:** The precision of test method was evaluated by analyzing assay for six individual samples prepared from same batch by the proposed method. The average % Assay and the relative standard deviation for the six sample preparation were found to be in the specified limits. Data was shown in **Table 6.**

**Limit of Detection (LOD) & Limit of Quantification (LOQ):** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOD & LOQ are calculated based on signal to noise ratio and the results are shown in **Table 7 & 8.**

**Robustness:** Robustness of the method was investigated by varying the instrumental conditions such as flow rate ($\pm 10\%$) & organic content in mobile phase ($\pm 2\%$). Standard solution was prepared and analyzed as per the test procedure and the system suitability parameters were monitored. The results are shown in **Table 9**

**Forced degradation studies**

**Preparation of stock:** 10 tablets were accurately weighed and crushed in mortor and pestle and transferred equivalent to 5 mg of Tenegliptin and 125 mg Metformin in sample into a 10ml clean dry volumetric flask add about 7 ml of Diluent and sonicate it up to 5 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.44 micron Injection filter. Further pipette 0.6ml of Tenegliptin & Metformin the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. The results are given in Table 10.

**Oxidation:** Pipette 0.6 ml above stock solution into a 10ml volumetric flask and 1ml of 12.5% v/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials. The chromatograms were represented as **Fig no: 11.**

**Acid Degradation Studies:** Pipette 0.6 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the

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volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials. The chromatograms were represented as **Fig no: 12.**

**Alkali Degradation Studies:** Pipette 0.6 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials. The chromatograms were represented as **Fig no: 13**.

**Dry Heat Degradation Studies:** Tenegliptin and Metformin sample was taken in petridish and kept in Hot air oven at $110^0$ C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed**.** The chromatograms were represented as **Fig no: 14**.

**Photo Stability studies:** The photochemical stability of the drug was also studied by exposing the 0.6ml stock solution in to 10ml volumetric flask and expose to UV Light by keeping the beaker in UV Chamber for 24 hours. For HPLC study, filter the solution with 0.45 microns syringe filters and place in vials were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatograms were represented as **Fig no: 15**

## RESULTS AND DISCUSSION

**Method Development:**

Metformin and Tenegliptin showed isobestic point at wavelength 240 nm.
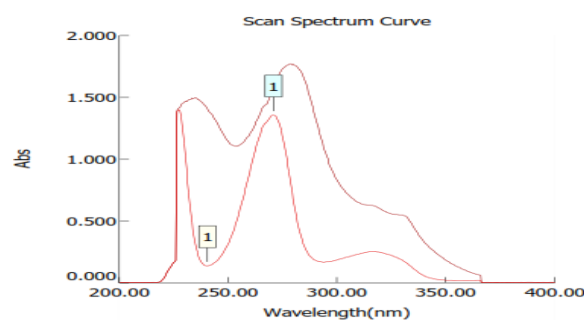


**Fig. 3: UV Spectrum of Metformin and Tenegliptin**

**Table 1:Optimised chromatographic conditions:**

| Parameters | Method |
|---|---|
| Stationary phase (column) | Inertsil -ODS $C_{18}$(250 x 4.6 mm, 5 μ) |
| Mobile Phase | Buffer: Acetonitrile at 50:50 |
| Flow rate (ml/min) | 1.0 ml/min |
| Run time (minutes) | 10 min |
| Column temperature (°C) | Ambient |
| Volume of injection loop (μl) | 20 |
| Detection wavelength (nm) | 240nm |

**1.System suitability*: S**ystem suitability studies were performed by replicate injections and the parameters like theoretical plates, tailing was recorded. The theoretical plates are more than 2000 and the tailing factor is less than 2 in each injection for both the analytes. The observed values were in agreement with the acceptance criteria.

**2. Specificity: S**pecificity studies were carried out by injecting sample, standard, and blanksolutions into the HPLC system. No interference was observed in blank chromatogram. The sample and standard chromatograms identical with same retention times.

**3. Linearity.** A graph was plotted with peak area versus concentration and the correlation coefficient was calculated. The $r^2$ values of both the drugs were found to 0.999 which were within the limits. The $r^2$ values confirmed the method was linear and the results were shown in table 9 & 10 and figures 7&8

**4. Accuracy:** Three target concentrations 50%, 100%, 150% were prepared and injected into HPLC system in triplicates. At each spike level the mean recovery values were between 98 to 102 % which satisfies the acceptance criteria. The recovery values indicate the method is

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accurate. The results are observed in table 7 & 8

**5. Precision:** System precision and method precision were performed and the % RSD of the peak areas were calculated and reported. The % RSD for the peak areas of six standard injections for system precision were 0.784 and 0.752 and for method precision 0.784 and 0.752 for Lumefantrine & Artemether respectively which were within the limits. The results are given in table 4 &5.

Intermediate precision was also performed on two different days and the results were observed in table 6. The % RSD for the peak areas of six standard injections were found to 0.8421 and 0.3471 for Lumafantrine and Artemether respectively, which were in agreement with acceptance criteria. The results were tabulated and presented in table 6.

**7.Robustness:** A study was carried out with variation in flow rate to evaluate the robustness of the method. The standard solutions were injected in the selected robust conditions and the system suitability parameters like theoretical plates, tailing factor and resolution were observed. The results showed that the theoretical plate count was more than 2000, tailing factor was less than 2 and resolution was found more than 2. The results of the study indicated that the method was robust and the results were shown in table 11 &12

**Forced Degradation Studies:**

The Data for Forced degradation are tabulated in **Table 10**. There was no interference of any peak at the retention time of analyte peaks from blank and placebo, Peak purity of all the treated samples was well within the limits. From this it has been concluded that the proposed method is specific and stability indicating for the estimation of Tenegliptin and Metformin, in the tablet dosage form.
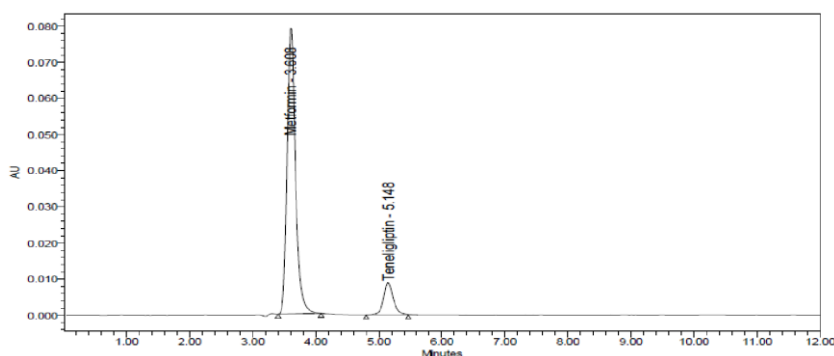


**Fig 4: Chromatogram for system suitability**

**Table 2: Peak characteristics of Metformin and Tenegliptin**

| S. No | Name | RT(min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|-------|------|---------|---------------|-------------|----------------|-------------|-----------------|
| 1 | *Metformin* | 3.608 | 854796 | 71179 | | 1.14 | 3281.91 |
| 2 | *Tenegliptin* | 5.148 | 114853 | 7911 | 5.75 | 1.08 | 5125.30 |

**Table 3: System suitability parameters of Metformin and Tenegliptin**

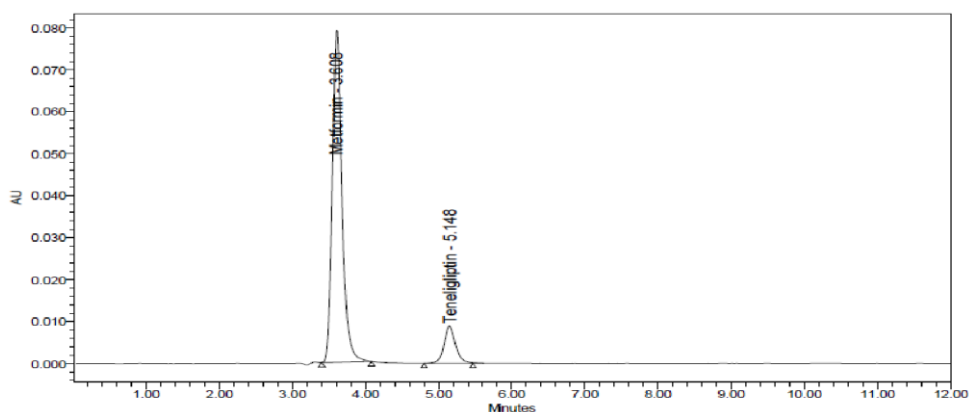| S. No | Name | RT(min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|-------|------|---------|---------------|-------------|----------------|-------------|-----------------|
| 1 | *Metformin* | 3.608 | 854796 | 71179 | | 1.14 | 3281.91 |
| 2 | *Tenegliptin* | 5.148 | 114853 | 7911 | 5.75 | 1.08 | 5125.30 |

**Fig-5 chromatogram of standard**
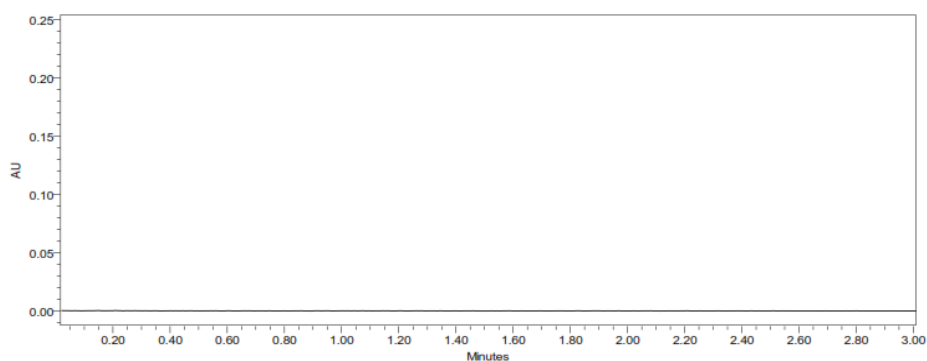


**Fig: 6: Chromatogram of blan**

**Table 4: Results of Linearity**

| Levels | Concentration (µg/ml) | | Peak areas | |
|---|---|---|---|---|
| | **Metformin** | **Teneligliptin** | **Metformin** | **Teneligliptin** |
| Level-1 | 250 | 10 | 244841 | 29672 |
| Level-2 | 500 | 20 | 525756 | 68336 |
| Level-3 | 750 | 30 | 856654 | 113345 |
| Level-4 | 1000 | 40 | 1150925 | 159680 |
| Level-5 | 1250 | 50 | 1435608 | 204473 |
| Average | | | 842756.8 | 115101.2 |
| Correlation Coefficient | - | - | 0.999 | 0.999 |

**Table 5: Results of System precision**

| System Precision | Metformin Areas | Teneligliptin Areas |
|---|---|---|
| 1 | 852828 | 111368 |
| 2 | 852337 | 112717 |
| 3 | 858355 | 112655 |
| 4 | 852839 | 113939 |
| 5 | 858513 | 112513 |
| 6 | 857582 | 112282 |
| AVG | 855409.0 | 112662.3 |
| SD | 12.524 | 845.7 |
| %RSD | 0.4 | 0.8 |

**Table 6: Results of Intermediate Precision**

| % Ass Metformin | % Ass Tenegliptin |
|---|---|
| 859453 | 112535 |
| 857162 | 111224 |
| 859458 | 112915 |
| 858377 | 113391 |
| 858482 | 113108 |
| 859771 | 112959 |
| 858783.8 | 112688.7 |
| 976.1 | 769.7 |
| 0.1 | 0.7 |
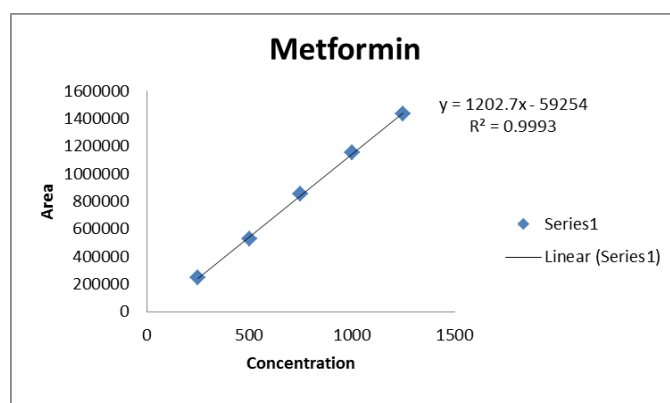


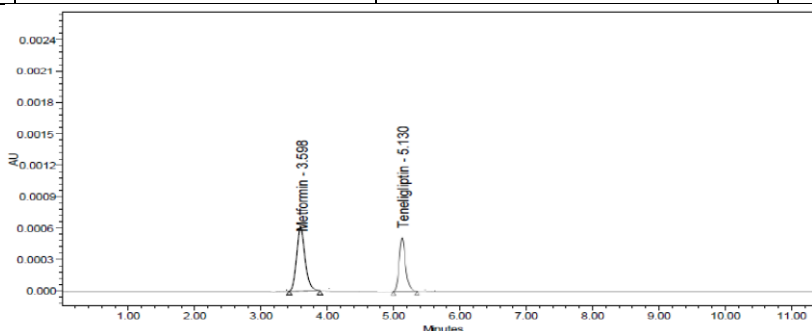**Fig. 7: Linearity Curve of Metformin**
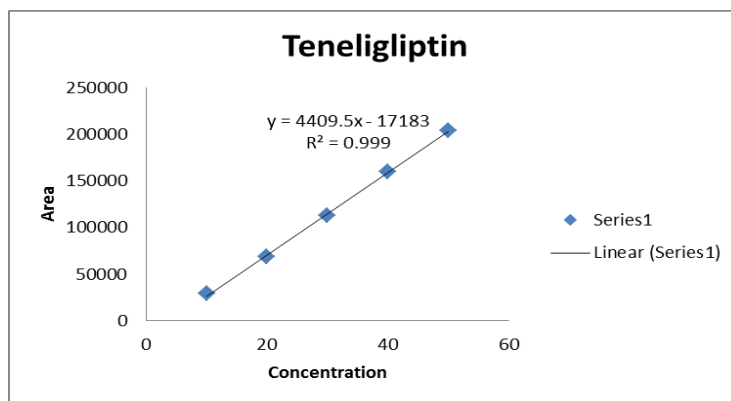


**Fig. 8: Linearity Curve of Tenelgliptin**
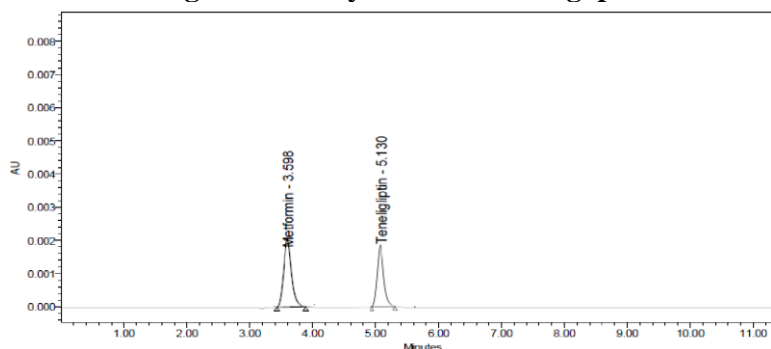


**Figure 9: Chromatogram of Metformin, Tenelgliptin showing LOD**

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**Table 7: Results of LOD**

| Drug name | Baseline noise(μV) | Signal obtained (μV) | S/N ratio |
|---|---|---|---|
| Metformin | 66 | 198 | 3.00 |
| Teneligliptin | 66 | 199 | 3.02 |



**Figure 10: Chromatogram of Metformin, Teneligliptin showing LOQ**

**Table 8: Results of LOQ**

| Drug name | Baseline noise(μV) | Signal obtained (μV) | S/N ratio |
|---|---|---|---|
| Metformin | 66 | 659 | 9.98 |
| Teneligliptin | 66 | 660 | 10.00 |

**Table 9: Results of Robustness**

| System suitability Parameters | | USP Tailing | | USP Plate count | |
|---|---|---|---|---|---|
| | | MET | TEN | MET | TEN |
| Flow Rate | 0.9 ml/min | 1.24 | 6.44 | 4361.01 | 5749.13 |
| | 1.0 ml/min | 1.14 | 5.66 | 3281.91 | 4959.43 |
| | 1.1ml/min | 1.18 | 6.11 | 4137.68 | 5286.06 |
| Change in organic composition in Mobile Phase | 10%less | 1.35 | 0.91 | 4962.22 | 6256.47 |
| | *Actual | 1.14 | 1.13 | 3281.91 | 4959.43 |
| | 10%more | 1.44 | 1.23 | 3940.49 | 52676.52 |
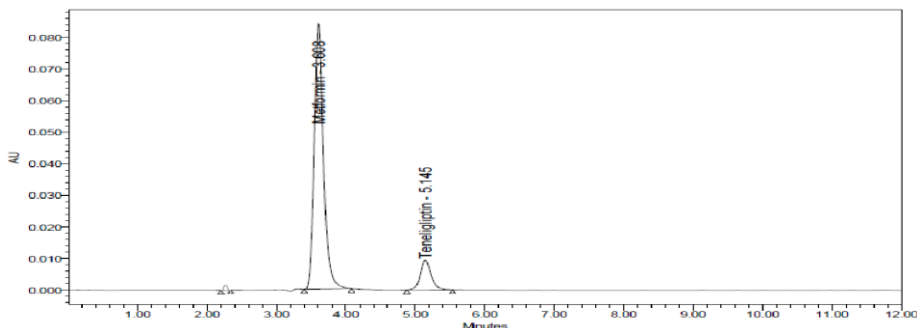


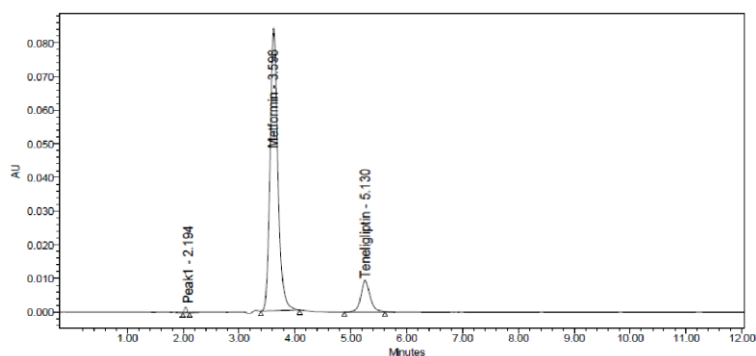**Figure 11: Chromatogram of Oxidative degradation**
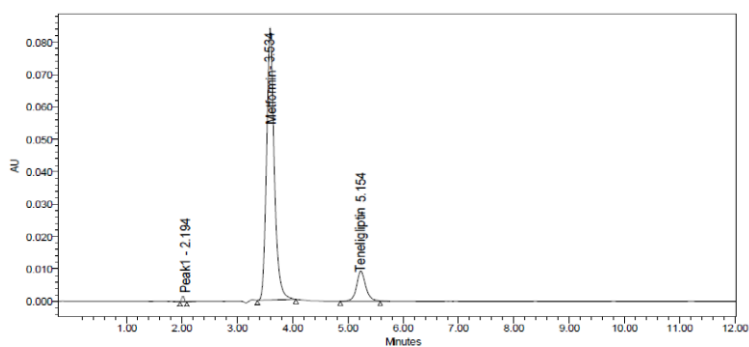
**Figure 14: Chromatogram of Thermal Degradation**



**Figure 15: Chromatogram showing Photo degradation**

**Table 10: Data of forced degradation studies**

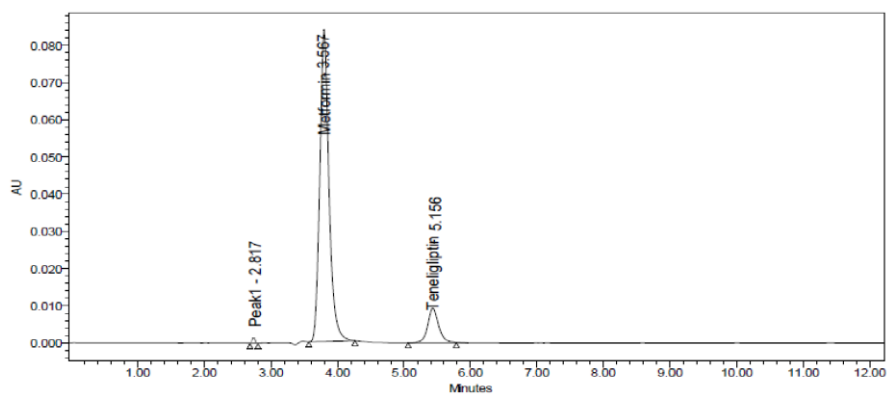| S.No | Sample | Components | % Degradation |
|------|--------|------------|---------------|
| 1 | Untreated sample | MET | - |
|   |  | TEN | - |
| 2 | Acid treated | MET | 5.6 |
|   |  | TEN | 7.91 |
| 3 | Alkali treated | MET | 5.2 |
|   |  | TEN | 6.5 |
| 4 | Peroxide treated | MET | 5.3 |
|   |  | TEN | 5.8 |
| 5 | Thermal /Dry heat exposed | MET | 5.3 |
|   |  | TEN | 4.2 |
| 6 | Photolytic degradation | MET | 5.5 |
|   |  | TEN | 7.0 |

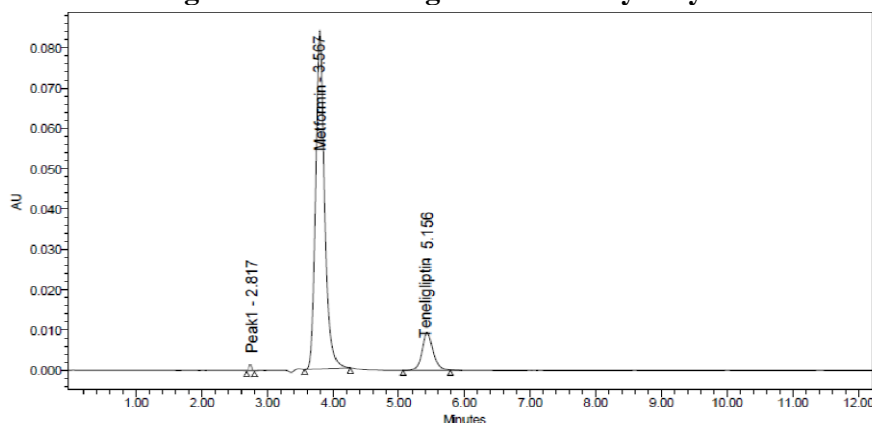**Figure 12: Chromatogram of Acid Hydrolysis**



**Figure 13: Chromatogram of Alkali Hydrolysis**

## CONCLUSIONS

An attempt was made to develop a stability indicating RP-HPLC method for the simultaneous estimation of Metformin and Teneligliptin. The method was optimized and the accountability of the newly developed method was established by validation as per ICH guidelines. Further the method was subjected to forced degradation studies and the percentage degradation at each degradation study was within the limits. The results of each validation parameter were in good agreement with acceptance criteria. Therefore the method has been proven to be linear, precise, accurate, specific, robust and stable. Hence we recommend that this method can be a good approach for the quantification of Metformin and Teneligliptin in combination dosage form and can be adopted for the routine quality control analysis of these drugs. **Acknowledgement:** The authors are thankful for the management, St.Pauls College of Pharmacy, Hyderabad for providing necessary facilities.

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