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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN HBR HYDRATE, METFORMIN HCL AND PIOGLITAZONE HCL IN TABLET DOSAGE FORM

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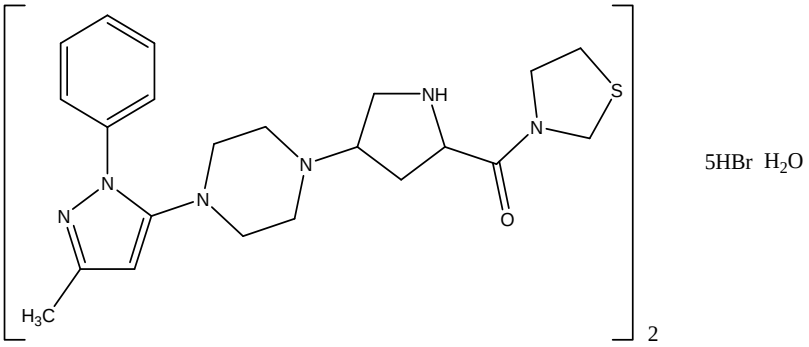
## ABSTRACT

A simple, specific, precise, accurate and robust stability indicating RP-HPLC method has been developed and subsequently validated for the separation and quantification of Teneligliptin Hydrobromide Hydrate, Metformin Hydrochloride and pioglitazone Hydrochloride in tablete dosage form. The quantification was carried out using Zodiac C18 (250 nm x 4.6 mm, 5µm) as stationery phase and mobile phase composition Acetonitrile: Methanol: Buffer pH 3 adjusted with 10% GAA in proportion of 25:25:50 v/v. The flow rate was adjusted to 1 ml/min and the iffluent was monitored at 225 nm using PDA detector. The retention time of Teneligliptin was 2.992 min, Metformin was 2.186 min and Pioglitazone was 11.483 min. The describe method was linear over a concentration range of 10-30 µg/ml for teneligliptin, 25-75 µg/ml for metformin and 7.5-22.5 µg/ml for pioglitazone. The percentage recovery of Tneligliptin was found to be 98.90% - 99.53%, Metformin was found to be 98.85% - 99.51% and Pioglitazone found to be 99.29% - 99.64%. Method was statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines. Forced degradation study was carried out under various stress conditions. The drug degraded in Acid, Base, Oxidative and Thermal conditions. All results was found satisfactory so, the stability indicating assay method can be applied to the tablet dosage form.

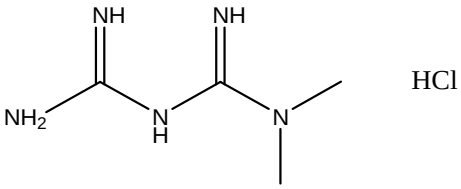
**KEYWORDS:** Teneligliptin Hydrobromide Hydrate, Metformin Hydrochloride, Pioglitazone Hydrochloride, RP-HPLC, Stability Indicating, Method Validation.

## Introduction[1-11]

Teneligliptin Hydrobromide hydrate [(2S,4S)-4-[4-(5-Methyl-2-phenylpyrazol-3yl) piperazin-1- yl] pyrrolidine-2-yl] -(1,3-thiazolidin-3- yl) methanone; hydrate; pentahydrobromide is a dipeptide peptidase-4 (DPP-4) inhibitor that belongs to the third generation, used in the management of type 2 diabetes. Teneligliptin works by increasing the insulin released by the pancreas and it helps in lowering the blood glucose level in the body Mainly use in type2 diabetes mellitus.

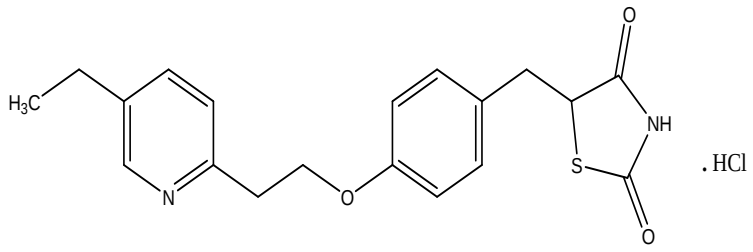



Metformin hydrochloride 3-(Diaminomethylidene)-1,1 dimethylguanidine; hydrochloride is a biguanide antihyperglycemic that is used in combination with diet and exercise to control blood sugar levels in type 2 diabetes. Metformin lowers blood glucose levels by reducing hepatic glucose production (also known as gluconeogenesis), decreasing intestinal glucose absorption, and increasing insulin sensitivity through increased peripheral glucose uptake and utilization.




Pioglitazone Hydrochloride 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione monohydrochloride helps to control your blood sugar levels by helping your body make better use of the insulin it produces. Pioglitazone is a selective agonist at peroxisome proliferator-activated receptor-gamma (PPARγ) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ increases the

transcription of insulin-responsive genes involved in the control of glucose and lipid production, transport, and utilization.




Literature review reveals that numbers of analytical methods are available for estimation of Teneligliptin hydrobromide hydrate, Metformin hydrochloride, Pioglitazone hydrochloride alone and with other combinations. However, there is no method reported for simultaneous estimation of Teneligliptin hydrobromide hydrate, Metformin hydrochloride, Pioglitazone hydrochloride in a tablet dosage form using RP-HPLC. Therefore, there is thought of interest to develop a chromatographic method for simultaneous estimation of Teneligliptin hydrobromide hydrate, Metformin hydrochloride, Pioglitazone hydrochloride in tablet dosage form. So, the aim of present work is to develop simple, accurate, precise, rapid, specific and sensitive RP-HPLC method for simultaneous estimation of Teneligliptin hydrobromide hydrate, Metformin hydrochloride, Pioglitazone hydrochloride in tablet dosage form.

## MATERIALS AND METHODS

Teneligliptin, Metformin HCl, and Pioglitazone HCl were procured from Steris Healthcare, Ahmedabad, and Gujarat, India. HPLC grade reagents Water, Methanol, Potassium dihydrogen phosphate, Acetonitrile of analytical grade were obtained from Merck specialists Pvt, Ltd Mumbai.

### Apparatus and chromatographic conditions

HPLC method development and validation was done on a HPLC instrument (YL 9100 HPLC System) UV Spectrophotometer (UV1800, Shimadzu, Japan), Stationary Phase used was Zodiac $C_{18}$ (250 mm X 4.6 mm, 5µm). When the individual solution, having concentration of 20 µg/ml of TEN and 15 µg/ml of PIO and 50 µg/ml of MET were scanned between 200-800 nm, it was observed that at 225 nm, adequate signal of all the components can be generated and hence it was selected as analytical wavelength for further method development. Mobile phase consisting of Acetonitrile: Methanol: Buffer pH 3 adjusted with 10% GAA (25:25:50) was used. The flow rate was 1.0 ml/min. The injection volume is 20 µL.

**Preparation of mobile phase**

**Preparation of 0.025 M $KH_2PO_4$ buffer**

0.025 M $KH_2PO_4$ buffer: Weighed about 1.70 gm of buffer and was dissolved in 500 ml of HPLC grade water.

**Composition:** Acetonitrile: Methanol: 0.025 M $KH_2PO_4$ buffer (25:25:50 v/v/v) Take 25 ml of Acetonitrile, 25ml of Methanol and 50ml of Potassium dihydrogenphosphate, add Glacial acetic acid up to pH measured at 3. Mix well and Sonicate in Sonicator for 15 minutes to degas it. Then Filter it with 0.45µ Membrane Filter Paper.

**Preparation of Standard stock solution**

**Teneligliptin Hydrobromide hydrate standard stock solution (200 µg.ml $^{-1}$)**

Accurately weighed 20 mg Teneligliptin Hydrobromide hydrate dissolved in 100 ml methyl alcohol (200 µg.ml $^{-1}$). Diluted 1 ml of this solution 10 ml with methyl alcohol, (20 µg.ml $^{-1}$)

**Pioglitazone Hydrochloride standard stock solution (30 µg.ml $^{-1}$)**

Accurately weighed 15 mg Pioglitazone Hydrochloride dissolved in 100 ml methyl alcohol (150 µg.ml $^{-1}$). Diluted 2 ml of this solution 10 ml diluent, (30 µg.ml $^{-1}$) Diluted 1 ml of this solution 10 ml with methyl alcohol, (15 µg.ml $^{-1}$).

**Metformin hydrochloride standard stock solution (500 µg.ml $^{-1}$)**

Accurately weighed 50 mg MET dissolved in 100 ml methyl alcohol (500 µg.ml $^{-1}$). Diluted 1 ml of this solution 50 ml with methyl alcohol, (50 µg.ml $^{-1}$).

**Prepration of sample solution**

Weighed accurately about 20 mg of TEN, 15 mg of PIO and 50 mg of MET and transfered into a 100 ml volumetric flask. Made up the volume of the flask to the mark with Methanol. (200 µg/ml TEN + 150 µg/ml PIO+ 500 µg/ml of MET). Withdraw 1 ml from Master Stock Solution and made up to 10 ml with methyl alcohol TEN+PIO+MET (20+15+50 µg.ml$^{-1}$)

**METHOD VALIDATION**

**Specificity**

Specificity of the method was adjudged by injecting the mobile phase in optimized chromatographic condition. Specificity of the method was adjudged by injecting the mobile phase in optimized chromatographic condition, it was observed that no interference observed from mobile phase.

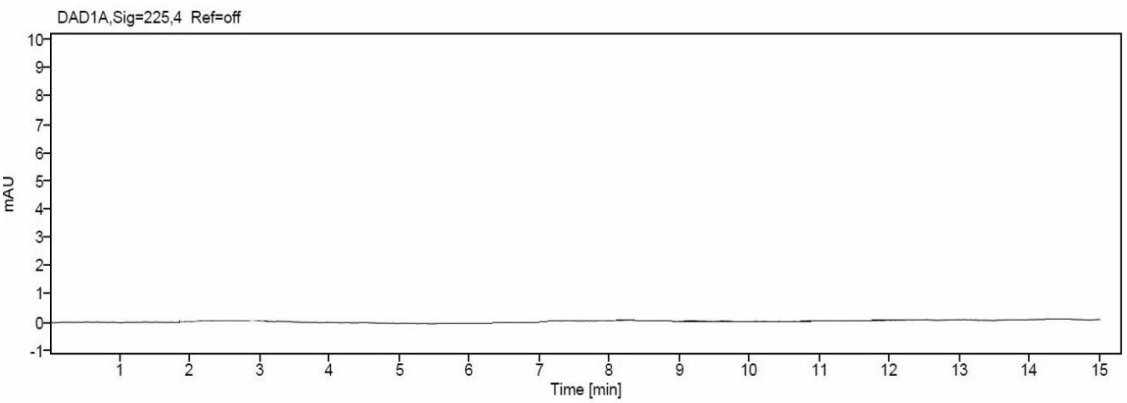



**Fig. 1: Chromatogram of Blank.**

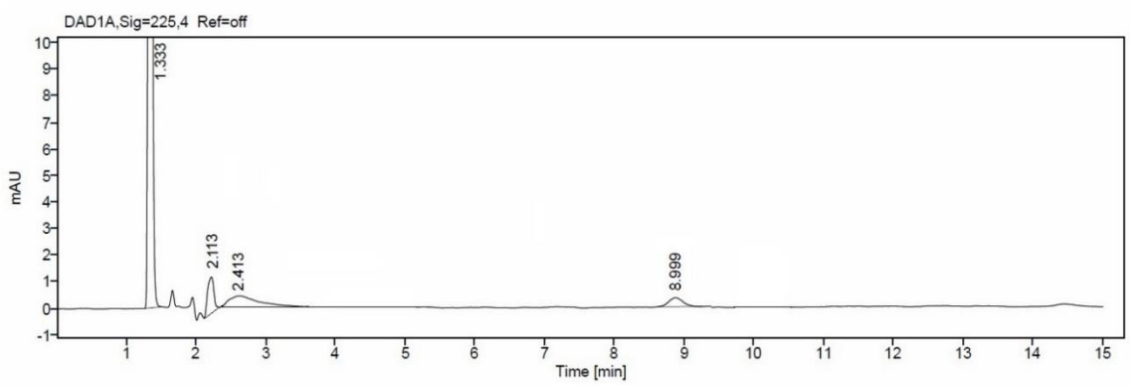



**Fig. 2: Chromatogram of TEN, MET and PIO Sample.**

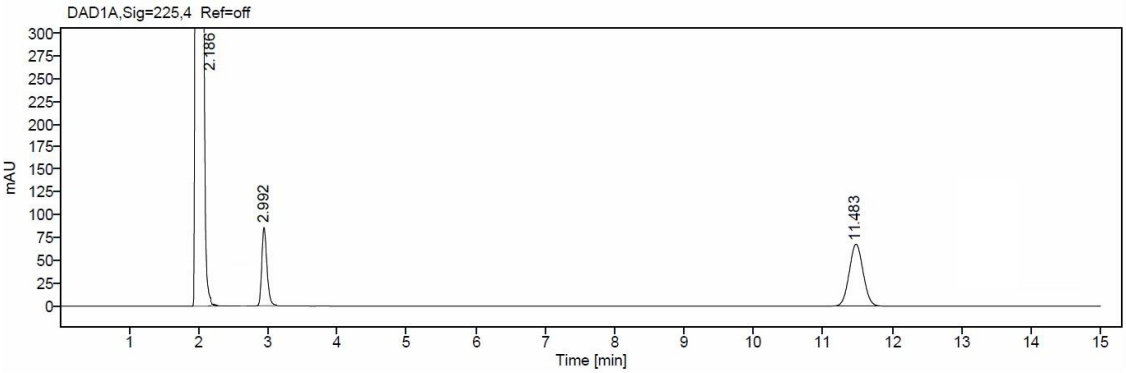



**Fig. 3: Chromatogram of TEN, MET and PIO Standard.**

### Linearity and Range

The linearity of Teneligliptin Hydrobromide Hydrate, Pioglitazone Hydrochloride and Metformin Hydrochloride assessed by analysis of combined standard solution in range of 10 – 30 µg/ml, 7.5 – 22.5 µg/ml and 25 – 75 µg/ml respectively. Correlation co-efficient for calibration curve of Teneligliptin Hydrobromide Hydrate, Pioglitazone Hydrochloride and Metformin Hydrochloride was found to be 0.9997, 0.9997and 0.9995 respectively.

**Table 1: Linearity Data of Teneligliptin.**

| Concentration (µg/ml) | Peak Area ± SD | %RSD |
|---|---|---|
| 10 | 11956.2 ± 164.57 | 1.38 |
| 15 | 18250.8 ± 209.64 | 1.15 |
| 20 | 24483.6 ± 263.53 | 1.08 |
| 25 | 30129.4 ± 255.58 | 0.85 |
| 30 | 36812.8 ± 232.63 | 0.63 |
| Linear regression Equation | | **y = 1223x – 110.77** |
| Regression coefficient ($r^2$) | | **$R^2$ = 0.9997** |

**Table 2: Linearity Data of Metformin.**

| Concentration (µg/ml) | Peak Area ± SD | %RSD |
|---|---|---|
| 25 | 90962.2 ± 1083.27 | 1.19 |
| 37.5 | 143880 ± 1403.20 | 0.98 |
| 50 | 194557.6 ± 1643.58 | 0.84 |
| 62.5 | 240661.8 ± 1594.97 | 0.66 |
| 75 | 291625 ± 1620.89 | 0.56 |
| Linear regression Equation | | **y = 3905.9x – 2466.3** |
| Regression coefficient ($r^2$) | | **$R^2$ = 0.9995** |

**Table 3: Linearity Data of Pioglitazone.**

| Concentration (µg/ml) | Peak Area ± SD | %RSD |
|---|---|---|
| 7.5 | 10189 ± 149.02 | 1.46 |
| 11.25 | 15128 ± 198.71 | 1.31 |
| 15 | 20305 ± 231.73 | 1.14 |
| 18.75 | 24962.2 ± 239.07 | 0.96 |
| 22.5 | 30573.6 ± 200.55 | 0.66 |
| Linear regression Equation | | **y = 1349 - 3.4143** |
| Regression coefficient ($r^2$) | | **$R^2$ = 0.9997** |

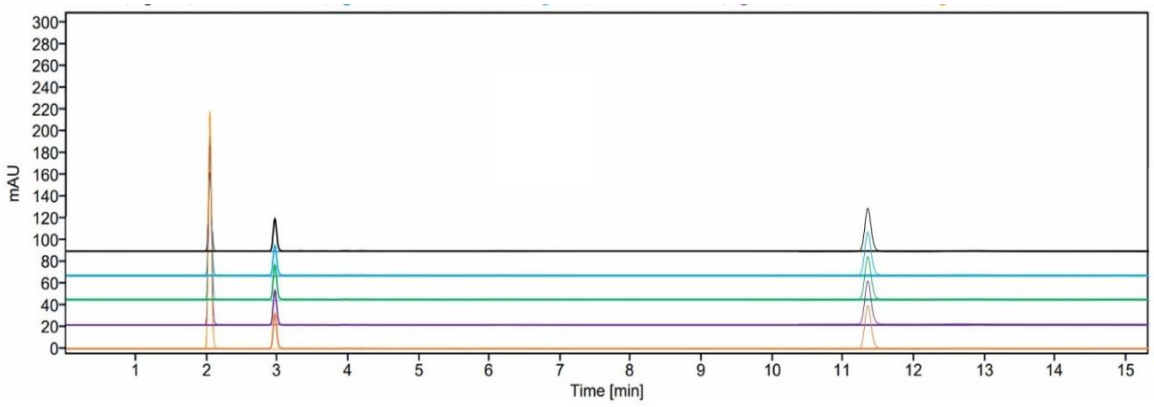



**Fig. 4: Overlain Chromatogram for linearity.**

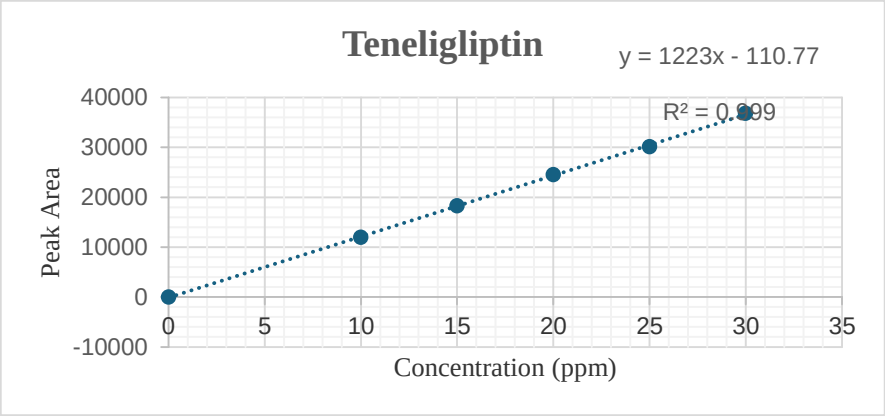



**Fig. 5: Calibration curve of TEN.**

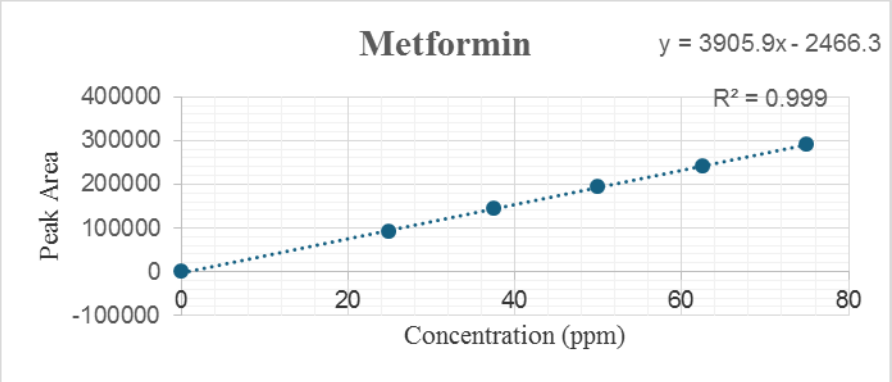



**Fig. 6: Calibration curve of MET.**

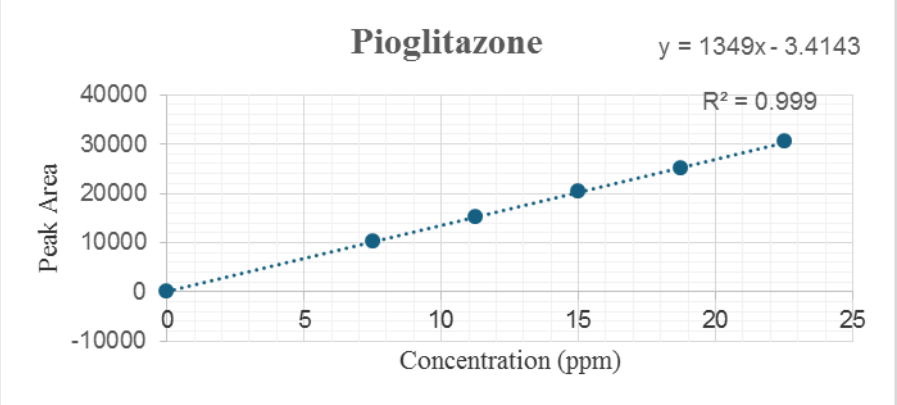



**Fig. 7: Calibration curve of PIO.**

**Accuracy**

Accuracy of the analytical method has been performed by spiking of sample with the standard. Spiking of the sample was performed at 50, 100 and 150 % of the target concentration. Each solution was chromatographed for 3 time and area obtained was subjected to statistical analysis to get idea about mean % recovery.

**Table 4: Accuracy data of TEN, MET, PIO.**

| Accuracy Data for TEN | | | | |
|---|---|---|---|---|
| **Level of Spiking** | **Amount of Drug Present (µg/ml)** | **Amount of drug added (µg/ml)** | **Amount of Drug recovered (µg/ml)** | **% Recovery** |
| **Unspiked** | 10 | - | - | - |
| **50 %** | 10 | 5 | 4.98 | 99.53 |
| **100 %** | 10 | 10 | 9.89 | 98.90 |
| **150 %** | 10 | 15 | 14.85 | 99.02 |
| **Accuracy Data for MET** | | | | |
| **Unspiked** | 25 | - | - | - |
| **50 %** | 25 | 12.5 | 12.36 | 98.85 |
| **100 %** | 25 | 25 | 24.86 | 99.43 |
| **150 %** | 25 | 37.5 | 37.32 | 99.51 |
| **Accuracy Data for PIO** | | | | |
| **Unspiked** | 7.5 | - | - | - |
| **50 %** | 7.5 | 3.75 | 3.72 | 99.29 |
| **100 %** | 7.5 | 7.5 | 7.46 | 99.51 |
| **150 %** | 7.5 | 11.25 | 11.21 | 99.64 |

**PRECISION**

**Repeatability**

Prepared standard working solution of mixtures having concentration of TEN (10 to 30 µg/ml), PIO (7.5 to 22.5 µg/ml) and MET (25 to 75 µg/ml) were injected at volume of 20 µL into column by employing optimized chromatographic conditions. Each standard mixture was injected 5 time and peak area was monitored. Each concentration was monitored for repeatability by RSD.

**Table 5: Repeatability data of TEN, MET & PIO.**

| **TEN** | | **MET** | | **PIO** | |
|---|---|---|---|---|---|
| **Conc.** | **20 µg/ml** | **Conc.** | **50 µg/ml** | **Conc.** | **15 µg/ml** |
| **Area 1** | 24544 | **Area 1** | 195632 | **Area 1** | 20536 |
| **Area 2** | 24753 | **Area 2** | 194231 | **Area 2** | 20454 |
| **Area 3** | 24312 | **Area 3** | 195863 | **Area 3** | 20163 |
| **Area 4** | 24122 | **Area 4** | 195224 | **Area 4** | 19973 |
| **Area 5** | 24687 | **Area 5** | 191838 | **Area 5** | 20399 |
| **Mean** | 24483.6 | **Mean** | 194557.6 | **Mean** | 20305 |
| **SD** | 263.53 | **SD** | 1643.58 | **SD** | 231.73 |
| **%RSD** | 1.08 | **%RSD** | 0.84 | **%RSD** | 1.14 |

**Intraday Precision**

Mixture that represents overall range (TEN+MET+PIO = 10+25+7.5, 20+50+15 and 30+75+22.5 µg/ml) were analyzed on same day at different time interval for intraday precision. (n = 3 determinations).

**Table 6: Intraday precision data for TEN, MET and PIO**

| TEN | | | MET | | | PIO | | |
|---|---|---|---|---|---|---|---|---|
| Conc. (µg/ ml) | Intraday Mean ± SD | %RSD | Conc. (µg/ ml) | Intraday Mean ± SD | %RSD | Conc. (µg/ ml) | Intraday Mean ± SD | %RSD |
| 10 | 11939 ± 150.06 | 1.26 | 25 | 91687.33 ± 1049.22 | 1.14 | 7.5 | 10139.33 ± 137.99 | 1.36 |
| 20 | 24511 ± 232.26 | 0.95 | 50 | 194015 ± 1738.66 | 0.90 | 15 | 20580 ± 263.77 | 1.28 |
| 30 | 36878.33 ± 306.35 | 0.83 | 75 | 290713.33 ± 2075.93 | 0.71 | 22.5 | 30886.33 ± 327.07 | 1.06 |

**Interday Precision**

Mixture that represents overall range (TEN+MET+PIO = 10+25+7.5, 20+50+15 and 30+75+22.5 µg/ml) were analyzed on different days for interday precision.

**Table 7: Interday precision data for TEN, MET and PIO.**

| TEN | | | MET | | | PIO | | |
|---|---|---|---|---|---|---|---|---|
| Conc. (µg/ ml) | Interday Mean ± SD | %RSD | Conc. (µg/ ml) | Interday Mean ± SD | %RSD | Conc. (µg/ ml) | Interday Mean ± SD | %RSD |
| 10 | 11941.33 ± 167.67 | 1.40 | 25 | 91641.67 ± 1419.62 | 1.55 | 7.5 | 10139 ± 145.08 | 1.43 |
| 20 | 24511.33 ± 281.43 | 1.15 | 50 | 193523 ± 2297.94 | 1.19 | 15 | 20568.33 ± 271.95 | 1.32 |
| 30 | 36584 ± 366 | 0.99 | 75 | 290311.67 ± 2462.12 | 0.85 | 22.5 | 30795.67 ± 339.69 | 1.10 |

**Intermediate Precision**

In the intermediate precision typical variation to be studied include different days, different conditions, different analyst and equipment as relevant.

**Table 8: Intermediate precision.**

| Sr.no | TEN | MET | PIO |
|---|---|---|---|
| 1 | 24356 | 20475 | 194745 |
| 2 | 24875 | 20675 | 193248 |
| 3 | 24255 | 20245 | 197451 |
| 4 | 24051 | 20347 | 198634 |
| 5 | 24899 | 20687 | 199745 |
| 6 | 24367 | 20844 | 198811 |
| Mean | 24467.17 | 20545.5 | 197105.67 |
| SD | 344.50 | 228.28 | 2560.61 |
| %RSD | 1.41 | 1.11 | 1.30 |

**Limit of Detection**

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 limit of detection (LOD) for both the drugs was determined based on the standard deviation of the response and the slope as per the equation designated by ICH guideline.

**Table 9: Result of LOD.**

| Sr. No. | Drug | LOD |
|---|---|---|
| 1 | TEN | 0.1776323 µg/ml |
| 2 | MET | 0.29745142 µg/ml |
| 3 | PIO | 0.084203 µg/ml |

**Limit of Quantification**

The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of Quantization (LOQ) for both the drugs was determined based on the standard deviation of the response and the slope as per the equationdesignated by ICH guideline.

**Table 10: Result of LOQ.**

| Sr. No. | Drug | LOD |
|---|---|---|
| 1 | TEN | 0.5382797 µg/ml |
| 2 | MET | 0.90136794 µg/ml |
| 3 | PIO | 0.255161 µg/ml |

**Robustness**

Determinations of TEN+MET+PIO = 20+50+15 µg/ml for each alteration was carried out and RSD was measured.

**Table 11: Robustness data for TEN, MET and PIO.**

| Parameter | Level of Change | Area (n = 3) | | |
|---|---|---|---|---|
| | | TEN | MET | PIO |
| Flow rate(± 0.1) | 0.98 ml/min | 24963 | 197247 | 20862 |
| | 1.0 ml/min | 24544 | 195632 | 20536 |
| | 1.02 ml/min | 24259 | 192534 | 20189 |
| | Mean ± SD | 24588.67 ± 354.12 | 195137.67 ± 2395.07 | 20529.00 ± 336.55 |
| | %RSD | 1.44 | 1.23 | 1.64 |
| Mobile PhaseComposition (± 2) | 27:23:50 | 24163 | 192376 | 20181 |
| | 25:25:50 | 24544 | 195632 | 20536 |
| | 25:27:48 | 24715 | 196242 | 20634 |
| | 23:25:52 | 24852 | 197246 | 20812 |

| | | | | |
|---|---|---|---|---|
| | Mean ± SD | 24568.50 ± 298.25 | 195374.00 ± 2106.53 | 20540.75 ± 265.65 |
| | %RSD | 1.21 | 1.08 | 1.29 |
| pH of mobilephase (±0.2) | 2.8 | 24897 | 196148 | 20758 |
| | 3 | 24544 | 195632 | 20536 |
| | 3.2 | 24189 | 191263 | 20093 |
| | Mean ± SD | 24543.33 ± 354.00 | 194347.67 ± 2683.83 | 20642.33 ± 338.57 |
| | %RSD | 1.44 | 1.38 | 1.65 |

**FORCE DEGRADATION STUDY**

| **Forced Degradation Condition** | **STEP–I** | **STEP–II** | **STEP–III** | **STEP–IV** | **STEP-V** |
|---|---|---|---|---|---|
| Acid Hydrolysis | Weigh accurately about 20 mg of TEN, 15 mg of PIO and 50 mg of MET and transfer into a 100 ml volumetric flask. Made up the volume of the flask to the mark with Methanol. (200 µg/ml TEN + 150 µg/ml PIO+ 500 µg/ml of MET). | Transfered 1 ml of above Solution into10ml Volumetric flask, add 2ml 2N HCl. | Solution was placed under reflux condition at 60°C for 24 hour. After that, cool the solution and neutralize with 2N NaOH. | Inject 20 µl the above solution under optimized chromatographic condition | %degradation was calculated by Comparison of obtained area of treated sample against standard solution (0-hour sample) |
| Base Hydrolysis | | Transfered 1 ml of above Solution into10ml Volumetric flask, add 2ml 2N NaOH. | Solution was placed under reflux condition at 60°C for 24 hour. After that, cool the solution and neutralize with 2N HCl. | | |
| Oxidative stress | | Transfered 1 ml of above Solution into10ml Volumetric flask, add 10% v/v $H_2O_2$ | Solution was placed at room temperature for 24 hours. | | |
| Thermal stability | Weighed accurately about 20 mg of TEN, 15 mg of PIO and 50 mg of MET | Transfered into petri dish and was kept in preheated oven at $105^0$C for 8 hours | Transfered into a 100 ml volumetric flask. Made up the volume of the flask to the mark with Methanol. (200 µg/ml TEN + 150 µg/ml PIO+ 500 µg/ml of MET). | Transfered 1 ml of above Solution into 10ml Volumetric flask to the mark with Methanol.<br><br>Inject 20 µl the above solution under optimized chromatographic condition | %degradation was calculated by Comparison of obtained area of treated sample against standard solution (0-hour sample) |

## CONCLUSION

RP-HPLC method was developed and validated as per International Conference on Harmonization (ICH) prescribed guidelines and Forced degradation study was carried out under various stress conditions. The drug degraded in Acid, Base, Oxidative and Thermal conditions. The developed method was validated and found to be simple, specific, precise, accurate and robust, as it separates components with good chromatographic criteria. All results were found satisfactory. So, the validated method can be applied for estimation of Teneligliptin, Metformin and Pioglitazone in their tablet dosage form.

## ACKNOWLEDGMENT

I pay my reverence to the Saraswati Institute of Pharmaceutical Sciences. I am undeniably proud to be associated with this college.

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