# Development of Stability Indicating and Robust RP-HPLC Method for Determination of Teneligliptin

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***Original Research Article***

## ABSTRACT

A simple and rapid reverse-phase HPLC method was developed for determination of Teneligliptin (TGP) in the presence of its degradation products generated from forced decomposition studies. The HPLC separation was achieved on a C18 ACE column (150x 4.6 mm i.d.; 5 µm) using mobile phase as a mixture of Phosphate buffer pH-7.2 using ortho-phosphoric acid: methanol (30:70v/v). The UV detection was carried out at 245nm at ambient temperature and the flow rate of 1.0 mL/min. The calibration curve was found to be linear in the concentration range of 10-50 µg/mL(r=0.9993). Force degradation study was performed under various conditions like acidic, alkaline, oxidative, photolytic and mass balance calculations were carried out from the degradation results. The developed method was validated as per ICH guidelines with respect to linearity, accuracy, precision, limit of detection and quantification. The robustness of the proposed method was evaluated by the Plackett Burman design. The purity of the degraded sample was checked by peak purity analysis. The peaks of degradation products did not interfere with that of pure Teneligliptin.

*Keywords: Teneligliptin; force degradation; mass balance; Plackett burman; robustness.*

## 1. INTRODUCTION

Teneligliptin Hydrobromide Hydrate used in the treatment of type-II diabetes mellitus. It is a highly potent, competitive and long-lasting DPP-4 inhibitor that improves postprandial hyperglycemia and dyslipidemia [1]. Chemically is [(2S, 4S)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]-2-pyrrolidinyl] (1,3-thiazolidin-3-yl)methanone Hydrate pentahydrobromide] (Fig. 1). Sound shelf life of the formulation can be proposed scientifically by carrying out force degradation studies (stress stability). International Conference on Harmonization (ICH) laid down the acceptance criteria that would meet specifications throughout lifetime of the pharmaceutical product the only way to demonstrate is Stability testing.

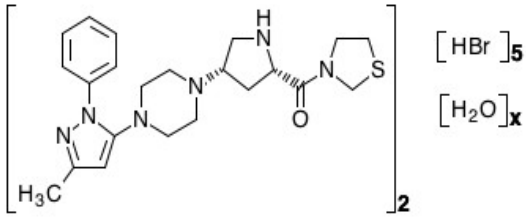


**Fig. 1. Structure of teneligliptin hydrobromide hydrate**

The information on intrinsic stability behavior of new drug substance and the stability assay method to protect these elements from exploitation are usually keep secret by the inventors. Analytical methods should be

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validated so as to demonstrate that impurities unique to the new drug substance do not interfere with or are separated from specified drug substance.

Similarly Mass balance studies in forced degradation of related compound method to prove specificity and capability to quantify degradation impurities; if known impurities are present. Mass balance helps to establish competence of a stability indicating method though it may not be possible in all circumstances. Lack of mass balance calculation leads a doubt on capability of method to accurately quantify all degradation products generated. It is challenging to evaluate Mass balance accurately always. The mass imbalance can be due variety of reasons from varying responses of drug peak and degradation product peaks, may also happen due to potential loss of volatile degradation products, formation of non-chromophoric compounds, formation of early eluents, and retention of compounds in the column [2]. The availability of known impurity standards helps accurately to calculate a mass balance during the quantitative determination through corrected response factors. Literature survey reveals HPLC [3-5] and spectrophotometric method [6,7] are reported for estimation of Teneligliptin but none of the reported methods have utilised the mass balance approach neither robustness was evaluated using experimental design (Plackett Burman Method). Considering the importance of Force degradation studies on drug substance and drug product as well as mass balance calculation; we have developed a method for determination of Teneligliptin in formulation.

## 2. EXPERIMENTAL

### 2.1 Materials

Pharmaceutical grade Teneligliptin Hydro-bromide Hydrate was gifted by Micro Labs Ltd. Bangalore. Methanol (HPLC grade) was purchased from Merck Chemical Company (India).Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, o-phosphoric acid; hydrochloric acid, sodium hydroxide, Sodium Chloride and Hydrogen peroxide used were of GR grade.

### 2.2 HPLC Instrumentation and Chromato-graphic Conditions

The Shimadzu HPLC system comprising of SPD-20M was used for detection, a manual injector with 20 µL capacity per injection. Column used was ACE C18 (150×4.6×5µ). Chromatographic separation of TGP was achieved at ambient temperature using the mobile phase comprising of Methanol: Phosphate Buffer (70:30) at a flow rate of 1.0 mL/min. pH of mobile phase was adjusted with o-phosphoric acid to pH 7.2. Before use, the mobile phase was filtered through a 0.45µ membrane filter and sonicated for 15-20 min. Injection volume was 20 µL, and the optimum wavelength selected for quantification was 245nm. Prior to injection of drug solution, the column was equilibrated for 30-40 min with mobile phase.

### 2.3 Preparation of Phosphate Buffer Solution (pH 7.2)

Weighed and dissolved 2.38g of Disodium Hydrogen Phosphate, 0.19g of Potassium Dihydrogen Phosphate and 8.0g of sodium Chloride in 600 mL of double distilled water and sonicated for 15 min., the volume made up to 1000 mL and pH was adjusted with 1% ortho Phosphoric acid It was filtered through 0.45µ membrane filter.

### 2.4 Preparation of Mobile Phase

Methanol 70mL and 30mL of Phosphate buffer pH 7.2 were mixed and sonicated for 15min. to remove the air bubbles. Each mobile phase was sonicated and filtered through 0.45µm membrane filter. Mobile phase was used as diluent.

### 2.5 Preparation of Working Standard Stock Solution

Weighed and transferred accurately about 10mg of Teneligliptin (TGP) standard in a 50mL volumetric flask, 35mL of diluent was added, sonicated to dissolve and diluted up to mark with diluent. Aliquot portion of this solution was further diluted to 10mL with diluent (30µg/mL) (S1)

### 2.6 Preparation of Sample Solution

Twenty tablets were weighed and powdered, the average weight was calculated. An accurately weighed quantity equivalent to 10mg of Tenegliptin was transferred to 50mL of volumetric flask. To it 25mL diluent was added, sonicated for 30min. and volume made upto mark with diluent. Aliquot portion of above solution was further diluted to 10mL with diluent.

Solution S1 prepared above was scanned in the range of 200-400nm against solvent blank (methanol). The absorption maximum for Teneligliptin was found to be 245.4nm (**Fig. 2)** shows the absorption spectrum of Teneligliptin.

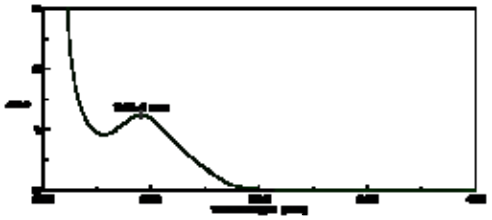

**Fig. 2. UV spectrum of Teneligliptin**

## 2.7 Initial Method Development

### 2.7.1 Choice of mobile phase

In order to choose the appropriate mobile phase, initial experimental runs were carried out as shown in **Table 1**. According to the observations obtained, mobile phase selected for further experimentation .was Methanol: Phosphate buffer (70:30) pH 7.2 which gave well defined symmetrical peak.

## 2.8 Application of the Proposed Method to Marketed Formulation

### 2.8.1 Preparation of sample

Twenty tablets were weighed and powdered. A quantity of tablet powder equivalent to about 10.0mg of TGP was weighed and transferred to 50mL of volumetric flask. A 1.5mL portion of above solution was further diluted to 10.0mL with diluent (30µg/mL). After equilibration of stationary phase, five sample solutions were injected separately and chromatogram were recorded **(Fig. 4).**

## 2.9 Method Validation

### 2.9.1 Study of system suitability parameter

The system suitability parameters monitored during the test includes the Area under Curve, Theoretical plates per column, Tailing factor and Resolution. After equilibration of the column with mobile phase, six replicate injections of 20µL solution were injected through the manual injection and the chromatogram were recorded**.**

**Table 1. Selection of moblie phase**

| Trials | Mobile phase | Retention time | Remarks |
|---|---|---|---|
| 1 | Methanol: Phosphate buffer without NaOH PH 6.8 (60:40) | 10.50 | Tailing observed |
| 2 | Methanol : phosphate buffer pH 7.2 (60:40) | 11.75 | Run time need to decreased |
| 3 | Methanol : phosphate buffer pH 7.2 (75:25) | 6.8 | Poor peak shape |
| 4 | Methanol : phosphate buffer pH 7.2 (70:30) | 5.7 | Sharp peak |

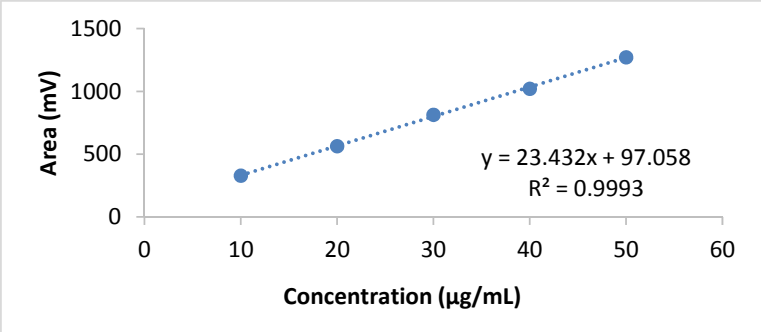


**Fig. 3. Calibration curve of Teneligliptin**

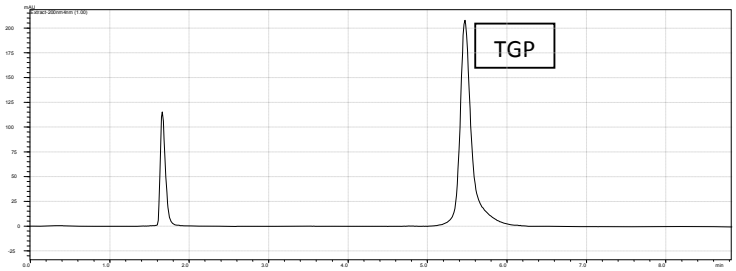


**Fig. 4. Chromatogram of formulation of TGP**

**2.9.2 Study of Linearity (Calibration curve)**

Aliquots of standard stock solution were diluted in range 0.5mL to 2.5mL in a series of 10mL volumetric flask with diluent (mobile phase) and volume was made up to mark with diluent to obtained concentration ranging from 10-50µg/mL of Teneligliptin **(Fig. 3).**

**2.9.3 Robustness testing (Placket Burman design) [8,9]**

The robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness testing was performed in order to evaluate the susceptibility of measurement due to deliberate variation. The study was accomplished through the Plackett-Burman design, which allows the execution of minimum no. of experiment for the study of selected factors.

The robustness study was performed with the help of Placket-Burman (PB) design because it examines the f selected factors in N ≥ f+1 experiments and it requires fewer runs (11 to 12 runs) as compared to other designs.

According to this design, total 12 runs were taken. For investigating the effect, each independent variable was studied at two levels namely, "High" and "Low" which indicated the upper limit and lower limit of the range covered by each variable. The values of the coded levels of independent variables used in experimental are given in **Table 2.**

**2.9.4 Recovery studies**

It was carried out by standard addition method. An accurately weighed quantity of tablet powder equivalent to 10mg of TGP was transferred to 50mL volumetric flask and to it reference standard pure drug here added at three different levels ranging from 50% to 150% of target level, sonicated for 15 min, with sufficient quantity of diluent then volume was made up to the mark.

The content was filtered through 0.45µm whatmann filter paper. A 2mL portion of the filtrate was further diluted to 10.0mL with diluent and injected into the system**.**

**Table 2. Selected Plackett-Burman design for robustness study**

| Factor | Name | Units | Type | Low actual | High actual | Low coded | High coded | Mean | Std. dev. |
|---|---|---|---|---|---|---|---|---|---|
| A | org phase | v/v | Numeric | 63.00 | 77.00 | -1.00 | 1.000 | 70.000 | 7.000 |
| B | aq phase | v/v | Numeric | 27.00 | 33.00 | -1.00 | 1.000 | 30.000 | 3.000 |
| C | pH of MP | pH | Numeric | 7.00 | 7.40 | -1.00 | 1.000 | 7.200 | 0.200 |
| D | flow rate | mL/min | Numeric | 0.80 | 1.20 | -1.00 | 1.000 | 1.000 | 0.200 |
| E | Wavelength | nm | Numeric | 240.00 | 250.00 | -1.00 | 1.000 | 245.00 | 5.000 |
| F | D1 | | Numeric | -1.00 | 1.00 | -1.00 | 1.000 | 0.000 | 1.000 |
| G | D2 | | Numeric | -1.00 | 1.00 | -1.00 | 1.000 | 0.000 | 1.000 |
| H | D3 | | Numeric | -1.00 | 1.00 | -1.00 | 1.000 | 0.000 | 1.000 |
| I | D4 | | Numeric | -1.00 | 1.00 | -1.00 | 1.000 | 0.000 | 1.000 |
| J | D5 | | Numeric | -1.00 | 1.00 | -1.00 | 1.000 | 0.000 | 1.000 |
| K | D6 | | Numeric | -1.00 | 1.00 | -1.00 | 1.000 | 0.000 | 1.000 |

*D1-D6 is dummy factor.*

**2.9.5 Precision**

Precision of any analytical method was expressed as standard deviation and percent relative standard deviation of series of measurements. Precision of estimation of Teneligliptin by proposed method was ascertained by replicate analysis of homogeneous samples of tablets**.**

**2.9.6 Linearity and range**

An accurately weighed tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim was taken and dilutions were made as described under marketed formulation. Then each solution was injected and chromatograms were recorded**.**

**2.9.7 Ruggedness**

*2.9.7.1 Different analyst*

The ruggedness of the proposed method was verified by analysing the tablet sample used for method precision by two different analysts using same instrument. The ruggedness results were compared with method precision data.

*2.9.7.2 Intraday and Interday variation*

Sample solution was injected separately at 0h, 3h and 5h, and chromatograms were recorded. Similarly, the same solutions were injected on $1^{st}$, $3^{rd}$, $7^{th}$ and $10^{th}$ day. The chromatogram so recorded and results were calculated.

**2.9.8 Limit of detection and limit of quantitation**

*2.9.8.1 Limit of Detection (DL)*

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.

*2.9.8.2 Limit of Quantitation (QL)*

The quantitation 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.

DL and QL are calculated based on standard deviation of response and slope of calibration curve.

## 2.10 Forced Degradation Studies

**2.10.1 General procedure for preparation of standard drug**

An accurately weighed 10mg Teneligliptin was weighed and transferred to 50.0mL dry volumetric flask. To it 10.0 mL of reagent (acid, alkali, 10%hydrogen peroxide and distilled water) were added. The contents of the flask were place in oven at 50$^{o}$C. The samples were withdrawn at a specified time (5h.), and volume was made up to the mark with mobile phase.

**2.10.2 General procedure for preparation of marketed formulation**

Twenty tablets were weighed, powdered and thoroughly mixed. Accurately weighed quantities of tablet powder equivalent to 10.0mg of Teneligliptin were transferred to a series of 5 different 50.0mL dry volumetric flask. To it 10.0mL of reagent (acid, alkali, 10%hydrogen peroxide and distilled water) were added. And the samples were place in oven at 50$^{o}$C as indicated, a-e. The sample solution was withdrawn after specified time and these stress samples were diluted upto volume with mobile phase.

**2.10.3 Alkali hydrolysis studies**

It was performed by placing standard and samples of a marketed formulation with 0.05M sodium hydroxide in the oven at 50$^{o}$C for a period of 5h. The standard was withdrawn at the end of 5h while samples of the marketed formulation were withdrawn at an interval of 1,2,3,4 and 5h. The standard and sample solution was injected and chromatographed separately using optimised chromatographic conditions.

**2.10.4 Acid hydrolysis studies**

It was performed by placing standard and samples of marketed formulation with 1M hydrochloric acid in oven at 50$^{o}$C for a period of 5h. The withdrawal for standard and sample was done similar to that for alkali hydrolysis.

**2.10.5 Neutral hydrolysis studies**

It was performed by placing standard and samples of marketed formulation with double distilled water in the oven at 50$^{o}$C for a period of 5h. The withdrawal for standard and sample was done similar to that for alkali hydrolysis.

**2.10.6 Oxidative studies**

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It was performed by placing standard and samples of marketed formulation with 10% hydrogen peroxide in oven at 50°C for a period of 5h. The withdrawal for standard and sample was done similar to that for alkali hydrolysis.

**2.10.7 Humidity studies (40°C /75%RH)**

TGP Standard drug and tablet powder were spread in two separate petri dishes and kept in stability chamber at 40°C /75%RH. The standard and marketed formulation was withdrawn on the 7th, 15th and 30th day.

**2.10.8 Photochemical studies (UV light)**

TGP Standard drug and tablet powder was spread in two separate petri dishes and kept in stability chamber under UV Light exposure. The standard and marketed formulation was withdrawn on the 7th, 15th and 30th day for analysis.

**2.10.9 Thermal studies**

*2.10.9.1 Dry heat degradation*

An accurately weighed 10mg of Teneligliptin and equivalent weight of marketed formulation was transferred to 50.0mL dry volumetric flask. The contents of the flask were place in oven at 50°C. The samples were withdrawn after 48h. 20µL volume of standard and Marketed sample solution were chromatographed separately.

*2.10.9.2 Wet heat degradation*

An accurately weighed 10mg of Teneligliptin and equivalent weighed of marketed formulation was transferred to 50.0mL dry volumetric flask. To it 10.0mL methanol was added. The contents of the flask were place in oven at 50°C. The samples were withdrawn after 48h. The standard and sample of marketed formulation were prepared on day of analysis by following the general procedure as described earlier. A 20µL volume of standard and Marketed sample solution were chromatographed separately.

## 2.11 Mass Balance Calculation

Mass balance was calculated for degraded standard and samples. Mass balance was calculated using formula

Mass balance = (Assay of degraded sample + total impurities generated)/ (Assay of control sample + total impurities present) x 100

# 3. RESULT AND DISCUSSION

## 3.1 Method Development

The analysis was performed using ACE C18 column (150 X 2.5 mm, 4.6mm), and Methanol : Phosphate buffer (70:30) as mobile phase, at a flow rate 1mL/min, wavelength selected for the analysis was 245.0 nm at which drug show sharp peak and mobile phase used as a diluent for preparation of solutions. The optimised conditions were applied to force degradation studies of TGP.

## 3.2 Assay and Method Validation

The summary of results of system suitability parameter, for assay and method validation parameters, are shown in **Table 3a and 3b** respectively.

## 3.2 Force Degradation Studies

The study of chromatogram **(**Fig. 6a & 6b) revealed that the drug was very labile to alkaline hydrolysis at 0.05M sodium hydroxide at 50°C in 5h leading to degradation around 36.85%% and 34.6% in standard and sample respectively. The two additional peaks was generated were seen in the chromatogram of stressed standard and sample. The major degradant was detected at Peak 1 (Retention time_2.463 min) and peak 2 (Retention time_ 0.912 min) and the Relative retention time (RRT) was found to be 0.430.

In case of acidic hydrolysis (Fig. 6c & 6d) reveals that the drugs was found to be very labile at 1N hydrochloric acid at 50°C in 5h leading to degradation around 9.2% and 8.38% in standard and sample respectively. The major degradant was detected at Retention time _2.5 min and the Relative Retention time was found to be 0.460. The neutral hydrolysis was carried out by using double distilled water. The study of chromatogram (Fig. 6e & 6f) revealed that the drug was very labile to neutral hydrolysis at 50°C in 5h leading to degradation around 11.07% and 11.07% in standard and sample respectively. The major degradant was detected at Retention time _2.632 min and the Relative Retention time was found to be 0.50.

Under oxidative condition, the drug was highly labile to10% hydrogen peroxide at 50°C in 5h leading to degradation around 49.51% and 51.15% in standard and sample respectively (Figs. 6g & 6h). The two additional peaks was generated were seen in the chromatogram of

stressed standard and sample. The major degradant was detected at Peak1 (Retention time _3.310 min) and peak2 (Retention time_2.663 min) and the Relative Retention time was found to be 0.55. It can be said that the drug is intrinsically unstable to alkaline, acidic, neutral and oxidative condition. In case of solid state degradation, the study of chromatogram (Fig. 6i) revealed that the drug was very labile at humidity chamber (40°C/75%RH) for the period of 30 days leading to degradation around 4.14% % and 3.7% in standard and sample respectively. The drug was very labile toUV lightfor the period of 30 days leading to degradation around 11.55% % and 10.68% in standard and sample respectively (Fig. 6j).The drug was labile at 50°C for the period of 48h leading to dry and wet heat degradation around 4.14% % and 3.7% in standard and sample respectively (Figs. 6k and 6l).Thus, it can be said that the drug is intrinsically unstable to humidity, UV light and thermal studies.

The result of solution and solid state force degradation are shown in **Table 4.**

### 3.3 Selectivity (Peak purity analysis and mass balance)

Degradation products were well separated from drug and the peak purity spectra's were recorded**.** From the Peak purity data of the undegraded drug proved the homogeneity of the drug peak. The mass balance of stressed sample and standard were found to be close to 100%. The results of peak purity analysis and mass balance for stressed standard and sample are shown in Table 5a and b.

**Table 3a. Summary of results for system suitability study**

| Parameters | AUC (mV) | Theoretical plate/column | Retention time | Tailing factor |
|---|---|---|---|---|
| Mean (n=6) | 909.517 | 26486 | 5.7 | 1.09 |
| %RSD | 0.71 | 0.49 | 0.05 | 0.32 |

**Table 3b. Summary of results for assay and method validation**

| Linearity and Range (r) | Assay (Mean %label claim) (n=6) | Precision (%RSD) (n=6) | %Recovery (accuracy) | Ruggedness (%RSD) | | Intermediate precision (%RSD) | | DL (µg) | QL (µg) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Analyst 1 | Analyst 2 | Intra day | Inter day | | |
| 0.9988 | 99.93 | 1.27 | 0.70 | 0.33 | 0.62 | 0.21 | 0.75 | 1.61 | 4.88 |

*DL-Limit of Detection, QL-Limit of Quantitation, %RSD-Percent relative standard deviation*

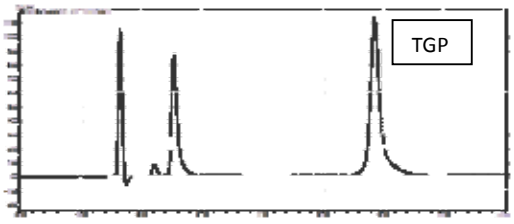



**Fig. 6a. Chromatogram of standard TGP**

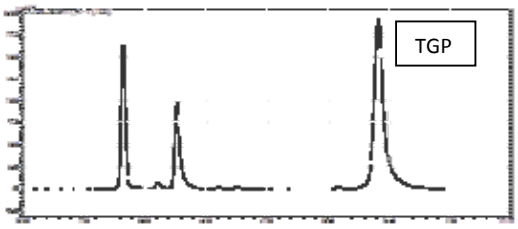
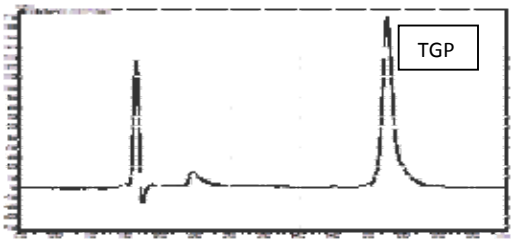



**Fig. 6b. Chromatogram of sample**

**(Alkaline hydrolysis)**



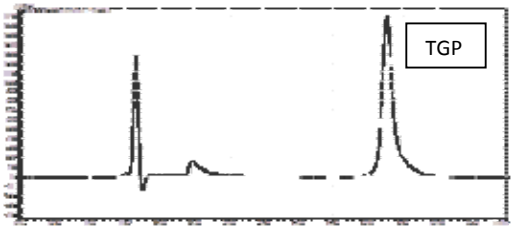
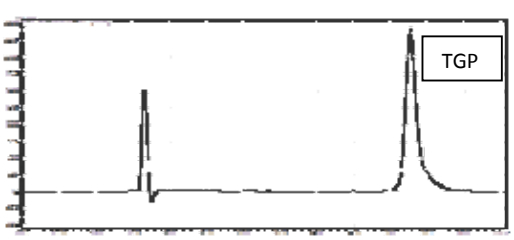



Fig. 6c. Chromatogram of standard TGP

Fig. 6d. Chromatogram of sample

(Acid hydrolysis)



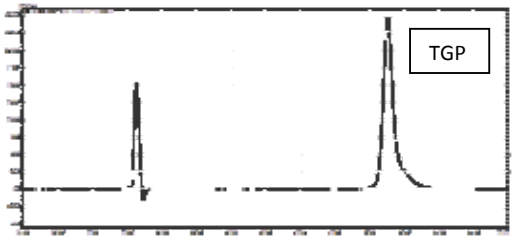
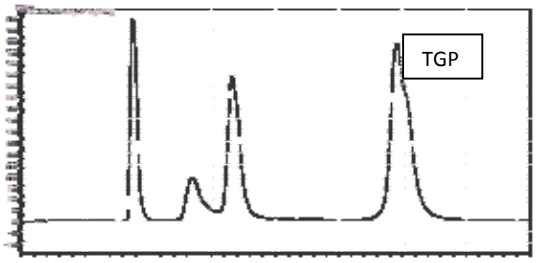




Fig. 6e. Chromatogram of standard TGP

Fig. 6f. Chromatogram of sample

(Neutral hydrolysis)



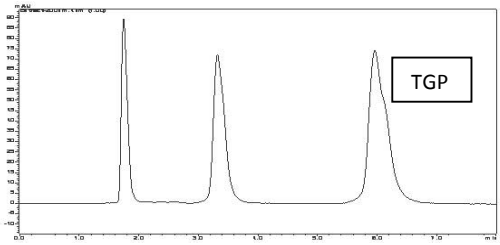
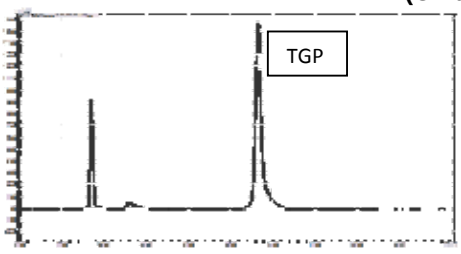




Fig. 6g. Chromatogram of standard TGP

Fig. 6h. Chromatogram of sample

(Oxidative hydrolysis)



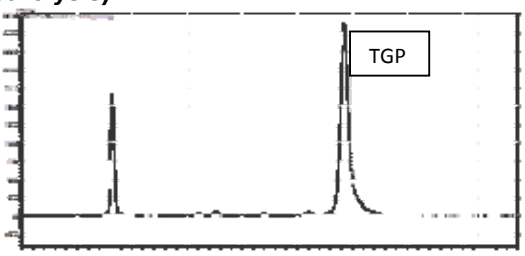
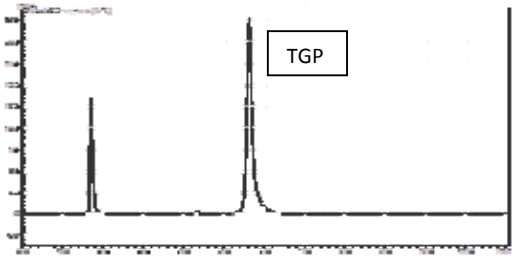




Fig. 6i. Chromatogram of sample (Humidity conditions)

Fig. 6j. Chromatogram of sample (Photochemical exposure)



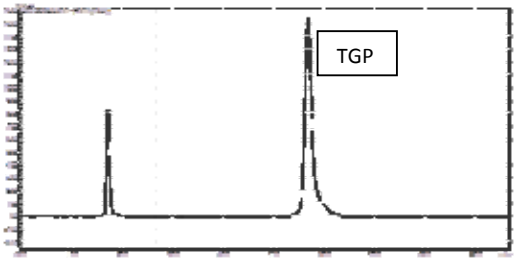




Fig. 6k. Chromatogram of sample (Dry thermal condition)

Fig. 6l. Chromatogram of sample (Wet thermal condition)

### 3.4 Robustness

The Pareto charts were prepared to examine the relationship in the Independent parameters which are shown in Fig. 5a-d respectively. From the Pareto charts it was observed that the statistical t-test at 0.05 significance level.

The calculated t-value was found to be less than theoretical t-value 2.20. The factor effect on critical factors like retention time and theoretical plate was found to be non-significant while factor A was found to be significant on asymmetry. Hence the method was found to be robust for the evaluation of Teneligliptin.

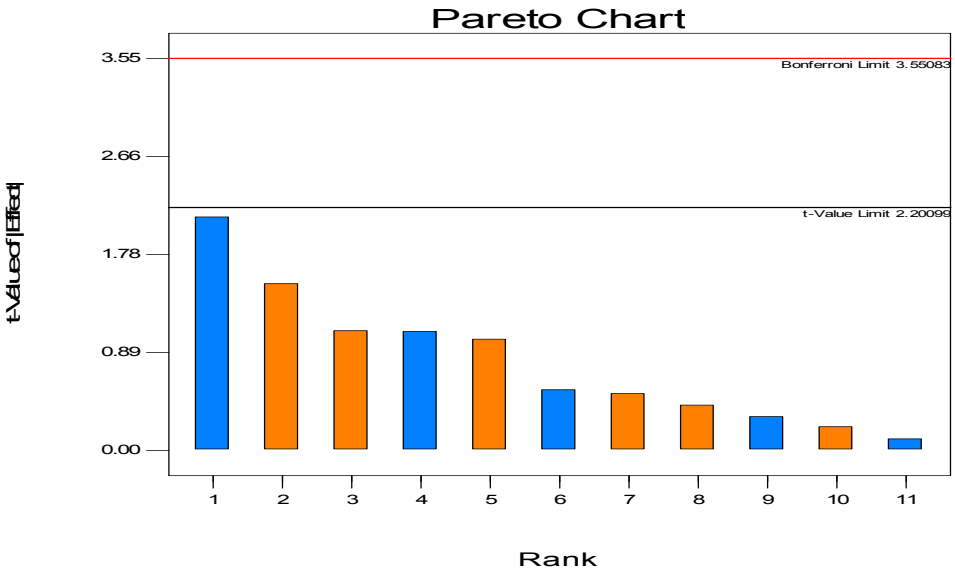



**Fig. 5a. Pareto chart for retention time**

**Table 4. Summary of force degradation study for TGP**

| Sr.No. | Stress degradation | Stress parameters | Time (h/d) | Drug peak | | Degradation peaks | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Rt (min) | % un-degraded | Peak 1(Rt) | % assay | Peak 2 (Rt) | % Assay |
| 1 | Acid | 1N | 5h | 5.264 | 91.62 | 2.430 | 6.210 | -- | -- |
| 2 | Base | 0.05N | 5h | 5.757 | 65.40 | 2.463 | 29.329 | 0.912 | 3.895 |
| 3 | Oxidative | 10%$H_2O_2$ | 5h | 5.904 | 51.15 | 3.310 | 47.014 | 0.306 | 3.082 |
| 4 | Humidity | (40$^{o}$C/75%RH) | 30d | 5.260 | 88.15 | 2.431 | 8.230 | 1.883 | 3.381 |
| 5 | UV light | 254nm | 30d | 5.676 | 89.32 | 2.438 | 9.035 | -- | -- |
| 6 | Thermal | 50°C | 48h | 5.619 | 96.30 | 3.198 | 4.643 | -- | -- |

*h- hours; d- days; Rt-retention time*

**Table 5a. Results of Peak purity analysis and mass balance for TGP**

| Condition | Max unknown impurity | Total impurity | RRT | Purity threshold | Peak purity | % Assay | % mass balance |
|---|---|---|---|---|---|---|---|
| Control | ND | ND | NA | NA | NA | 100.51 | NA |
| Acid 1N HCL 50°C | 5.137 | 1.392 | 0.46 | 0.999530 | 0.998780 | 90.80 | 97.32 |
| Base 0.05N NaOH 50°C | 37.957 | 0.925 | 0.43 | 0.944360 | 0.923897 | 63.61 | 101.97 |
| Peroxide 10%,50°C | 42.343 | 8.230 | 0.55 | 0.999210 | 1.00000 | 49.51 | 99.57 |
| Neutral 50°C | 6.147 | 1.243 | 0.35 | 0.999449 | 0.998717 | 88.93 | 95.83 |
| Humidity study | 5.250 | 2.231 | 0.46 | 0.999446 | 0.998706 | 89.72 | 96.70 |
| Photolytic study | 7.351 | 1.399 | 0.34 | 0.999551 | 0.998834 | 88.45 | 96.70 |
| Thermal study | 3.211 | 1.590 | 0.48 | 0.999568 | 0.998999 | 95.86 | 100.15 |

**Table 5b. Results of Peak purity analysis and mass balance for Tablet formulation**

| Condition | Max unknown impurity | Total impurity | RRT | Purity threshold | Peak purity | % Assay | % mass balance |
|---|---|---|---|---|---|---|---|
| Control | ND | ND | NA | NA | NA | 100.51 | NA |
| Acid 1N HCL 50°C | 6.210 | 1.391 | 0.46 | 0.999425 | 0.998868 | 91.62 | 98.71 |
| Base 0.05N NaOH 50°C | 29.329 | 3.895 | 0.43 | 0.999560 | 0.998709 | 65.40 | 98.12 |
| Peroxide 10%, 50°C | 47.014 | 3.082 | 0.55 | 0.999279 | 1.00000 | 51.15 | 100.74 |
| Neutral, 50°C | 5.311 | 1.674 | 0.50 | 0.99440 | 0.998692 | 89.44 | 95.93 |
| Humidity study | 8.230 | 3.381 | 0.09 | 0.999428 | 0.998659 | 88.15 | 99.25 |
| Photolytic study | 9.035 | 1.125 | 0.10 | 0.998659 | 0.998659 | 89.32 | 98.97 |
| Thermal study | 4.643 | 1.670 | 0.76 | 0.998659 | 0.998659 | 96.30 | 102.08 |

*ND = Not Detected, NA = Not Applicable*

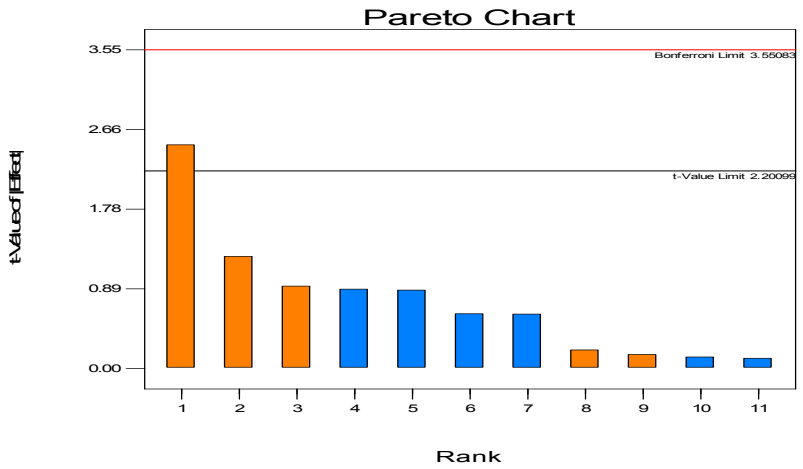



**Fig. 5b. Pareto chart for asymmetry**

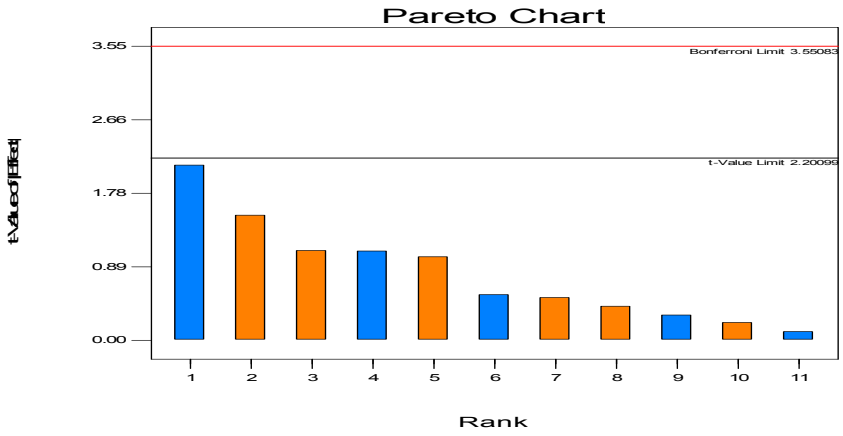



**Fig. 5c. Pareto chart for theoretical plate**

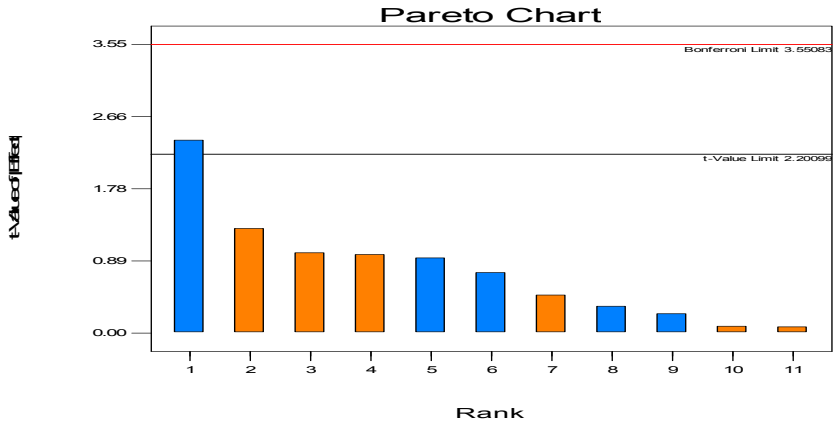



**Fig. 5d. Pareto chart for area**

## 4. CONCLUSION

Moreover, the mass balance calculations carried are consistent with the assay results obtained under normal conditions. The Plackett Burman approach helped in developing more robust method as compared to one variation at one time. It allowed the usage of variation in parameters within a limited number of sampling. Hence, from the results obtained by the proposed method indicates that method is simple, precise, robust, & accurate and can be adopted for routine quality control of Teneligliptin in formulation.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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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