

Development of stability indicating and robust Rp-HPLC method for determination of Teneligliptin

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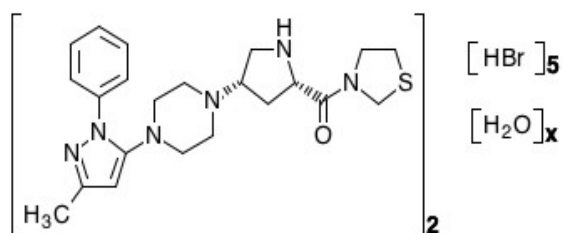
ABSTRACT

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 TGP.

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

1. NTRODUCTION

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] (**Figure 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.



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 TGP 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 Hydrobromide 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 chromatographic conditions

The Shimadzu HPLC system comprising of SPD-20M was used for detection, a manual injector with 20 μ L capacity per injection. Column used was ACE C18 (150 \times 4.6 \times 5 μ). 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 0.45 μ membrane filter and sonicated for 15-20 min.

Injection volume was 20 μ L, 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 0.45 μ 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 0.45 μ m 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 (30 μ g/mL) (S1)

2.6 Preparation of Sample Solution

Twenty tablets were weighed and powdered, average weight was calculated. An accurately weighed quantity equivalent to 10mg of teneligliptin 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 (**Figure 2**) shows the absorption spectrum of TGP.

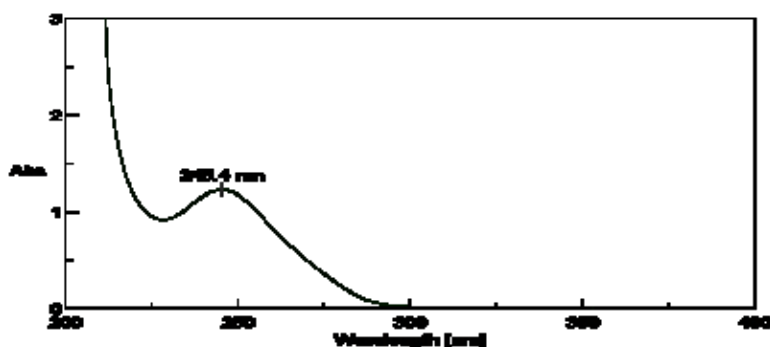


Figure 2: UV spectrum of TGP

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.

Table 1: Selection of mobile 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

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 (**Figure 4**).

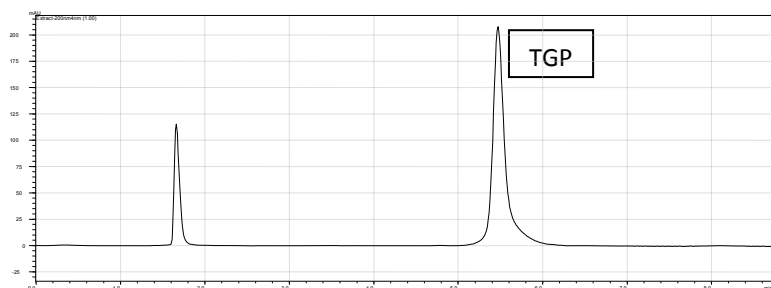


Figure 4: Chromatogram of Formulation of TGP

2.9 Method Validation

2.9.1 Study of system suitability parameter

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

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 10-50 μ g/mL of TGP (Figure 3).

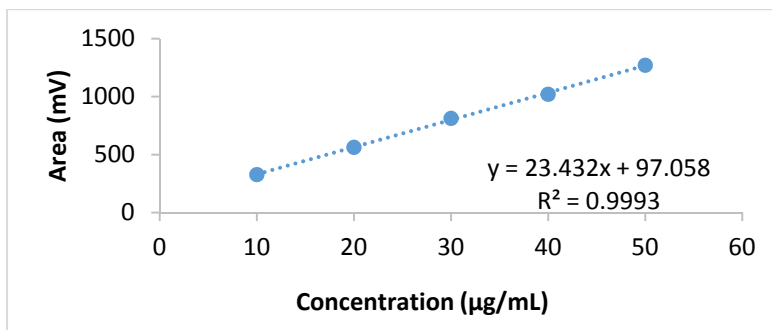


Figure 3: Calibration curve of TGP

2.9.3 Robustness testing (Plackett 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 Plackett-Burman (PB) design because it examines the f selected factors in $N \geq f+1$ 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

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.

volume was made up to the mark. The content was filtered through 0.45µm whatman filter paper. A 2mL portion of the filtrate was further diluted to 10.0mL with diluent and injected into the system.

2.9.5 Precision

Precision of any analytical method was expressed as SD and %RSD of series of measurements. Precision of estimation of TGP 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 analyzing 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 1st, 3rd, 7th and 10th 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 TGP 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°C. The samples were withdrawn at 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 10.0 mg of TGP 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°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 marketed formulation with 0.05M NaOH in oven at 50°C for a period of 5h. The standard was withdrawn at the end of 5h while samples of 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 optimized chromatographic conditions.

2.10.4 Acid Hydrolysis Studies

It was performed by placing standard and samples of marketed formulation with 1M HCL in oven at 50°C for a period of 5h. The withdrawal for standard and sample was done similar to that for alkali hydrolysis.

2.10.5 Neutral Hydrolysis Studies

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

2.10.6 Oxidative studies

It was performed by placing standard and samples of marketed formulation with 10% H₂O₂ 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 TGP and equivalent weight of marketed formulation was transferred to 50.0 mL 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 TGP 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

$$\text{Mass balance} = \frac{(\text{Assay of degraded sample} + \text{total impurities generated})}{(\text{Assay of control sample} + \text{total impurities present})} \times 100$$

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 optimized conditions were applied to force degradation studies of TGP.

3.2 Assay and Method Validation

The summary of results for assay and method validation parameters is shown in **Table 3**.

3.2 Force degradation studies

The study of chromatogram (**Figure 6a & 6b**) revealed that the drug was very labile to alkaline hydrolysis at 0.05M NaOH 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 (Rt_2.463 min) and peak 2 (Rt 0.912 min) and the **Relative retention time** (RRT) was found to be 0.430.

Table 3: Summary of results for assay and method validation

System suitability (%RSD)	Linearity and Range	Assay (%label claim)	Precision (%RSD)#	%Recovery (accuracy)	Ruggedness (%RSD)		Intermediate precision (%RSD)		DL (µg)	QL (µg)
					Analyst 1	Analyst 2	Intra day	Inter day		
0.71	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

In case of acidic hydrolysis (**Figure 6c & 6d**) reveals that the drugs was found to be very labile at 1N HCL 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 Rt_2.5 min and the RRT was found to be 0.460. The neutral hydrolysis was carried out by using double distilled water. The study of chromatogram (**Figure 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 $Rt_{2.632}$ min and the RRT was found to be 0.50.

Under oxidative condition the drug was highly labile to 10% H_2O_2 at $50^\circ C$ in 5h leading to degradation around 49.51% and 51.15% in standard and sample respectively (**Figure 6g & 6h**). The two additional peaks were generated were seen in the chromatogram of stressed standard and sample. The major degradant was detected at Peak1 ($Rt_{3.310}$ min) and peak2 ($Rt_{2.663}$ min) and the RRT 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 (**Figure 6i**) revealed that the drug was very labile at humidity chamber ($40^\circ C/75\%RH$) 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 to UV light for the period of 30 days leading to degradation around 11.55% and 10.68% in standard and sample respectively (**Figure 6j**). The drug was labile at $50^\circ C$ for the period of 48h leading to dry and wet heat degradation around 4.14% and 3.7% in standard and sample respectively (**Figure 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**.

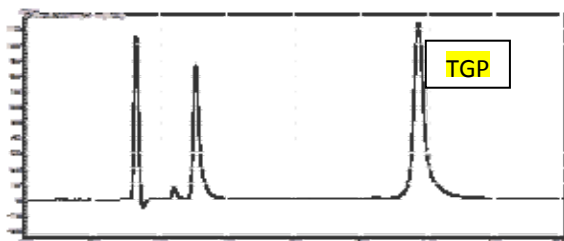


Figure 6a: Chromatogram of standard TGP

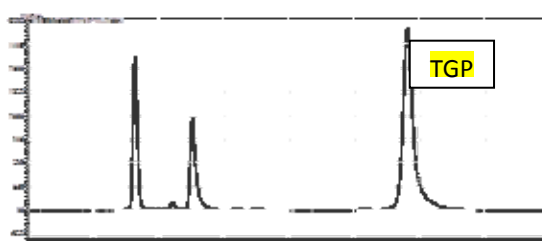


Figure 6b: Chromatogram of sample

(Alkaline hydrolysis)

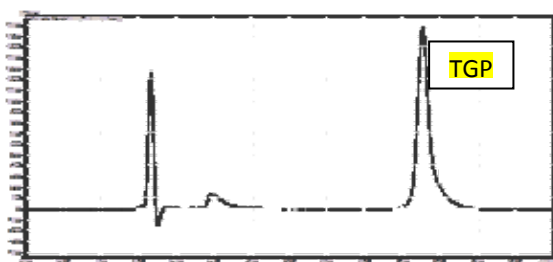


Figure 6c: Chromatogram of standard TGP

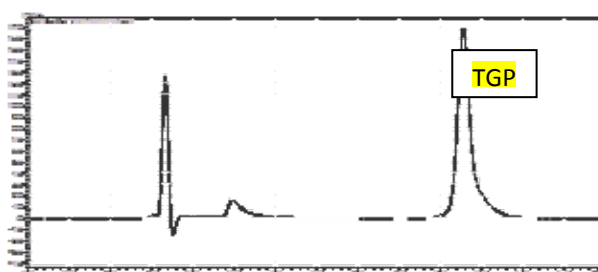


Figure 6d: Chromatogram of sample

(Acid hydrolysis)



Figure 6e: Chromatogram of standard TGP

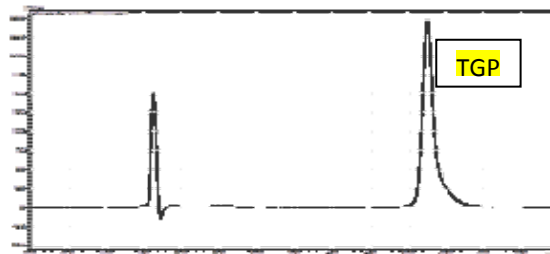


Figure 6f: Chromatogram of sample

(Neutral hydrolysis)

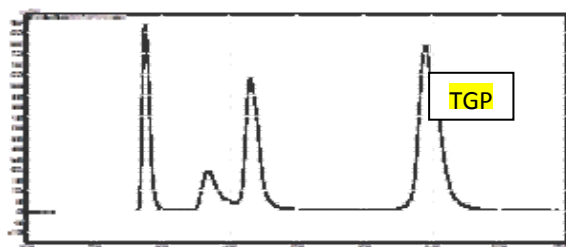


Figure 6g: Chromatogram of standard TGP

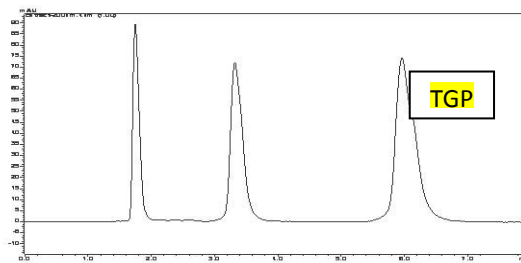


Figure 6h: Chromatogram of sample

(Oxidative hydrolysis)

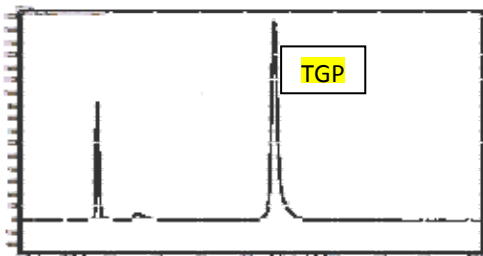


Figure 6i: Chromatogram of sample

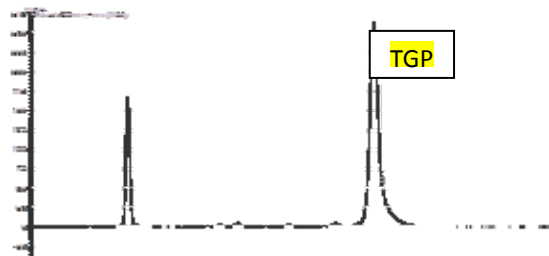


Figure 6j: Chromatogram of sample

(Humidity conditions)

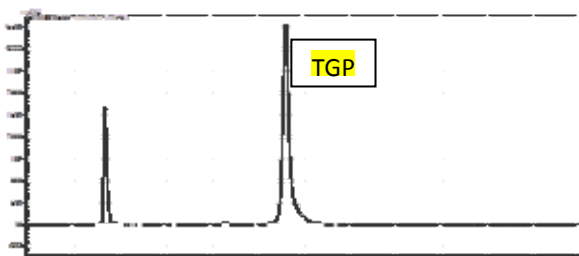


Figure 6k: Chromatogram of sample

(Photochemical exposure)

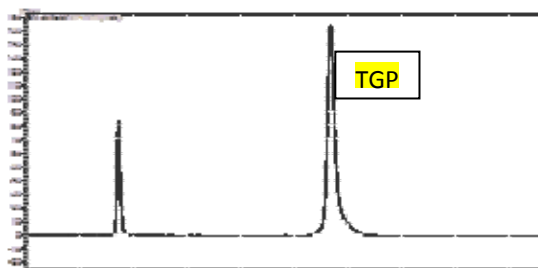


Figure 6l: Chromatogram of sample

(Dry thermal condition)

(Wet thermal condition)

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°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**

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 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
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Table 5 b: 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

3.4 Robustness

The Pareto charts were prepared to examine the relationship in the Independent parameters which are shown in **Figure 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 TGP.

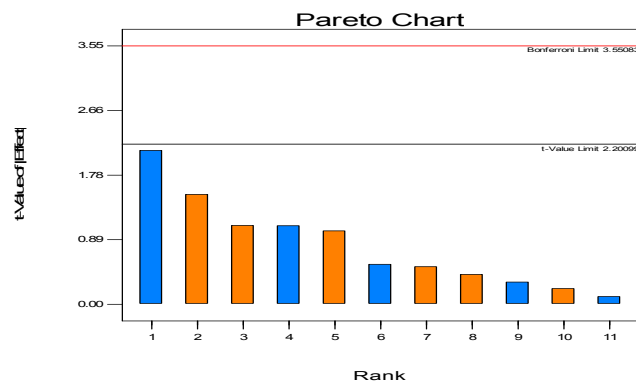


Figure 5a: Pareto chart for Retention time

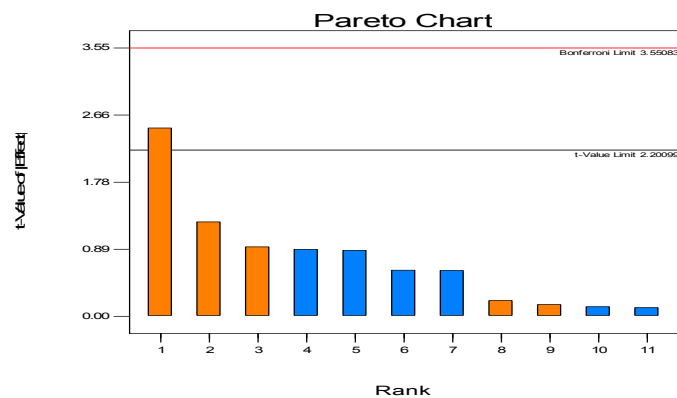


Figure 5b: Pareto chart for Asymmetry

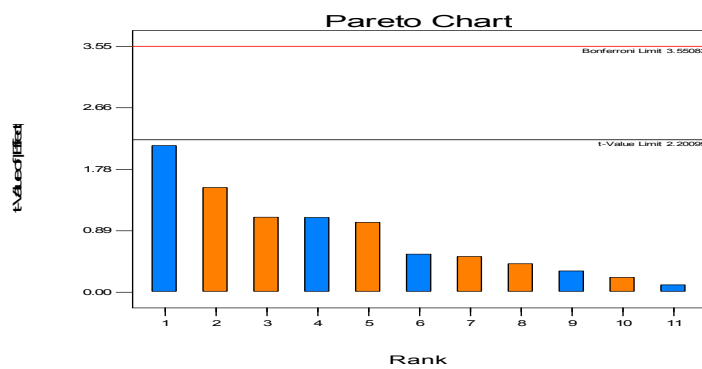


Figure 5c: Pareto chart for theoretical plate

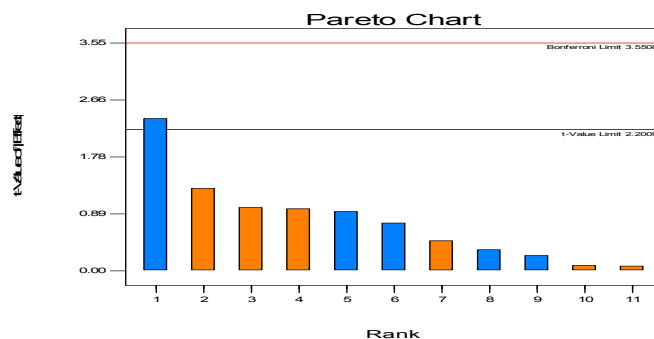


Figure 5d: Pareto chart for Area

3. 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 TGP in formulation.

4. References

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6. Authors contribution

Dr Krishna R Gupta designed the study, performed the statistical analysis and finalized the draft; Anvesha V Ganorkar wrote the first draft of the manuscript; Rekha S Jibhkate managed the literature searches and carried the experimental work designed. All authors read and approved the final manuscript.”