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DEVELOPMENT AND STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND TENELIGLIPTIN HBR HYDRATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

[1]Mr.Satish S Mane*, [2]Mr. Mahesh S Mhaske

[1]Aditya Pharmacy College Beed,Email id: satishmane5032@gmail.com

[2] Associate Professor, Dept. of Pharmaceutical Chemistry, Aditya Pharmacy College Beed

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Abstract:

The objective of the current work is to develop a simple, efficient, economical and compatible RP-HPLC method for the analysis of Metformin HCL and Tenegliptin hydrobromide in bulk and pharmaceutical dosage form dosage forms. Samples were separated on Grace C8 (250mm x 4.6 id. particle size: 5 (micron) column with mobile phase composed of OPA and acetonitrile (pH 3.00) The method was carried out on Grace C8 (250mm x 4.6 i.d. particle size: 5 micron) using 0.1% OPA and acetonitrile in the ratio of 70:30 v/v with adjusted pH 3 at flow rate 0.9 mL/min. The wavelength of Metformin HCL and Teneligliptin HBr Hydrate at 246 nm was found to be appropriate. The linearity range was obtained in the concentration of 50-250 µg/mL Metformin HCL and 02-10 µg/mL Teneligliptin HBr Hydrate respectively. The retention time of Metformin HCL and Teneligliptin HBr Hydrate was found to be 2.395 ± 0.2 min and 2.891± 0.2 min respectively. The developed method was found be accurate, robust and sensitive which can be used for estimation of combination of Metformin HCL and Teneligliptin HBr Hydrate in bulk and pharmaceutical dosage form.

Keywords: *Metformin HCL and Tenegliptin hydrobromide Phenomenex Grace C8 Column, Detection, RP HPLC*

Corresponding author:

Mr. Satish S Mane*,

Aditya pharmacy college Beed

Email id: satishmane5032@gmail.com

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INTRODUCTION: [01]

HPLC is a condensing for High Performance Liquid Chromatography (It has similarly been depicted as High-Pressure LC). HPLC has been around for concerning 35 years as well just like the greatest separating strategy utilized. HPLC is a partition technique that involves: The infusion of a minuscule volume of liquid model squarely into a cylinder stacked with small amounts (3 to 5 micron (µm) in size called the decent stage). Where individual pieces of the example are dropped down the stuffed cylinder (section) with a fluid (portable stage) expected through the segment by high strain conveyed by a siphon. These parts are separated from one another by the section pressing that incorporates different synthetic or potentially actual associations between their atoms as well as the pressing pieces. These separated components are identified at the leave of this cylinder (segment) by a course through gadget (indicator) that decides their amount. A result from this locator is known as a "fluid chromatogram In concept, LC and HPLC work similarly except the speed, effectiveness, sensitivity and simplicity of operation of HPLC is significantly exceptional.

HPLC COMPONENTS

1. Pump: The function of the heart is to require a fluid (called the moveable phase) with the runny chromatograph at a exact flow degree, spoken in mils per minutes (mL/min). Regular flow rates in HPLC remain in the 1-to 2-mL/min variety. Characteristic hearts can reach stress in the series of 6000-9000 psi (400-to 600-bar). During the chromatographic experiment, a pump can supply a consistent mobile phase structure (isocratic) or an enhancing mobile stage composition (slope).

2. Injector: The injector helps to present the liquid example into the circulation watercourse of the moveable stage. Common example volumes are 5-to 20-microliters (µL). The injector necessity also be able to by attitude the tall weights of the fluid system. A car sampler is the automatic variation for when the customer has several examples to assess or when hands-on shot is not sensible.

3. Column: Considered the "heart of the chromatograph" the column's stationary phase separates the example elements of interest utilizing various physical as well as chemical criteria. The little bits inside the column are what trigger the high back pressure at typical flow prices. The pump should press tough to relocate the moveable stage finished the column as well as this resistance triggers a high pressure within the chromatograph.

Sorts of columns Analytical [inner size (i.d.) 1.0 -4.6-mm; sizes 15-- 250 mm] Preparative (i.d. > 4.6 mm; sizes 50-- 250 mm). Capillary (i.d. 0.1 -1.0 mm; different sizes). Nano (i.d.< 0.1 mm, or in some cases stated as < 100 µm).

4. Detector: The identifier can see (recognize) the singular particles that come out (elute) from the segment. An indicator effectively gauges how much those atoms by the goalmouth that the scientist can quantitatively investigate the example parts. The indicator gives a result to a recorder or PC those outcomes in the fluid chromatogram (i.e., the diagram of the identifier reaction).

5. Computer: Often called the information framework, the PC not just controls each ace of the modules of the HPLC instrument yet it takes the sign from the locator and utilizations it to decide the hour of elution (maintenance season) of the example parts (subjective investigation) and how much example (quantitative examination).

Advantages of HPLC ¬ It gives explicit, delicate, and exact strategy for examination of various muddled examples. ¬ There is simplicity of test planning and test presentation. ¬ Speed of investigation ¬ Investigation by HPLC is explicit, exact and exact. ¬ Proposals benefit over gas chromatography in investigation of numerous glacial, ionic materials, metabolic items and thermolabile as well as non-unpredictable substances.

1.METHOD DEVELOPMENT:

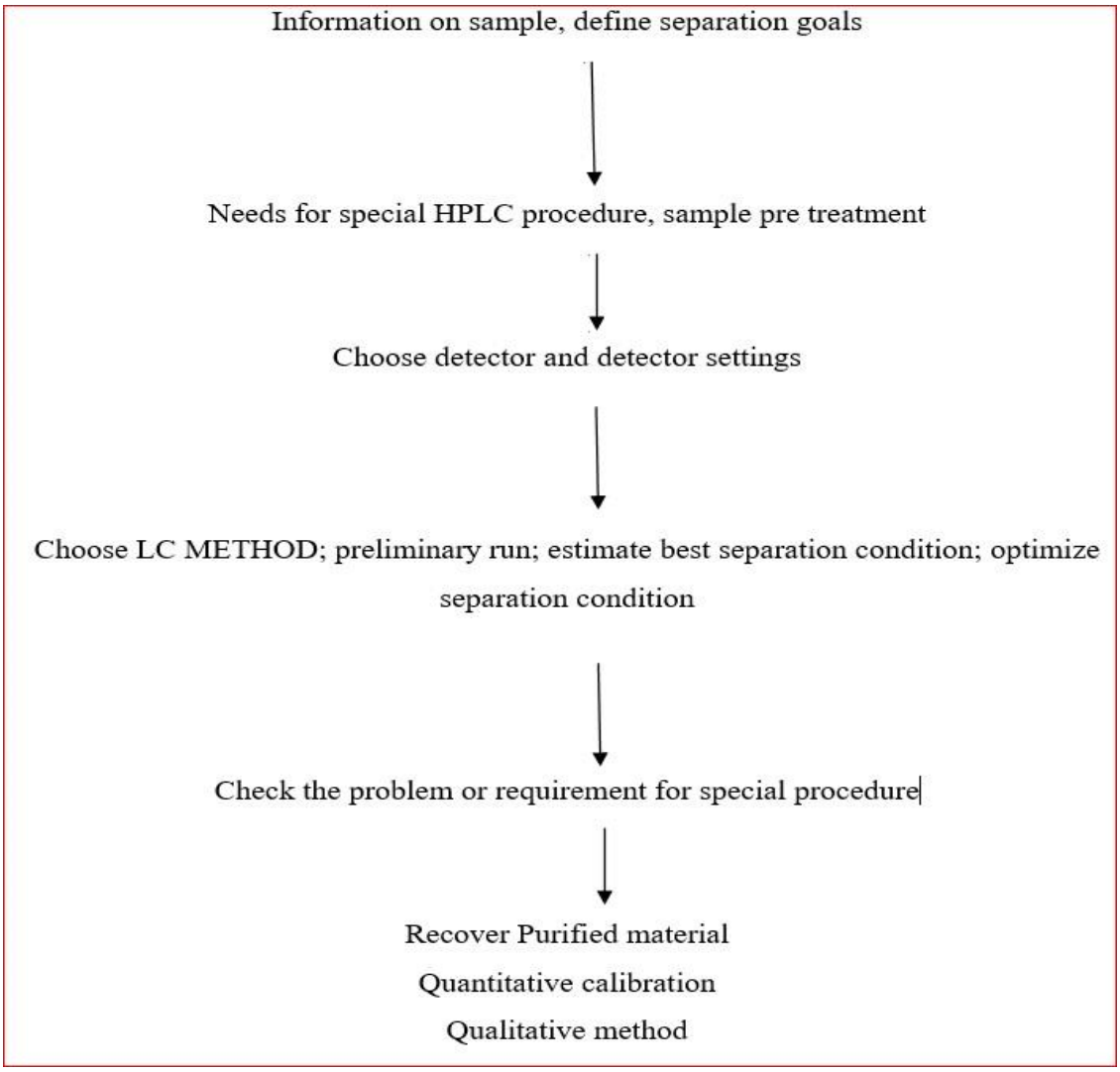


Figure No: 01 method of HPLC

1.2.Method validation :[10]

is the interaction used to affirm that the scientific strategy utilized for a particular test is reasonable for its expected use. Results from technique approval can be utilized to pass judgment on the quality, dependability, and consistency of logical outcomes; it is a rudimentary part of any great insightful practice. As indicated by ICH Guidelines Authentication of a Logical system is to exhibit that it is reasonable for its expected reason. Endorsement of logical techniques is facilitated to the four most ordinary sorts of legitimate system:

• Recognizing evidence tests;
• Quantitative tests for contaminations' substance;
• Limit tests for the control of contaminations;
• Quantitative preliminary of the powerful moiety in instances of prescription substance or drug thing or other picked component(s) in the medicine thing.

Normal approval attributes which ought to be careful are recorded beneath:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

1.2.1 ACCURACY:

The accuracy of an intelligent procedure imparts the closeness of course of action between the value which is recognized either as a standard certifiable worth or

a recognized reference regard and the value found. This is to a great extent named validity.

1.2.2 REPEATABILITY:
The Repeatability conveys the exactness under comparable working conditions all through a short stretch of time. Repeatability is moreover named intra-test precision.

1.2.3 REPRODUCIBILITY:
Reproducibility imparts the exactness between research focuses (agreeable examinations, ordinarily applied to standardization of approach). Recognition LIMIT The ID farthest reaches of an individual logical strategy is the most insignificant proportion of analyte in a model which can be perceived anyway not actually quantitated as an exact worth.

1.2.4 QUANTITATION LIMIT:
The quantitation farthest reaches of an individual legitimate technique is minimal proportion of analyte in a model which can in any case hanging out there with proper exactness and accuracy. Quite far is a limit of quantitative analyzes for low levels of blends in model systems, and is used particularly for the confirmation of contaminations and also corruption things.

1.2.5 LINEARITY:
The linearity of a quick system is its ability (inside a given reach) to get test results which are clearly relating to the obsession (proportion) of analyte in the model.

1.2.5 RANGE:
The extent of a smart procedure is the stretch between the upper and lower center (proportions) of analyte in the model (counting these obsessions) for which it has been displayed that the logical strategy has a healthy level of precision, precision and linearity.

2. REVIEW OF LITERATURE:
There are some analytical methods and stability indicating methods which include UV-visible spectrophotometric, HPTLC and HPLC have been reported in the literature for the determination of Metformin hydrochloride and Teneligliptin HBr hydrate in bulk and pharmaceutical preparation. Yet, our stability indicating HPLC methods have not been reported for estimation of Metformin hydrochloride and Teneligliptin HBr Hydrate. So the objective was to develop and validate stability indicating HPLC method for Metformin hydrochloride and Teneligliptin HBr Hydrate.

3. PLAN OF WORK
3.1 SELECTION OF DRUG AND FORMULATION
By literature and market survey Online Journals, chemical and analytical abstracts were studied to find out drugs for which there were no reported stability indicating methods. Market survey was carried to check the availability of these drugs and their dosage forms.

3.2 PROCUREMENT OF DRUG AND FORMULATION
METFORMIN HYDROCHLORIDE
TENELIGLIPTIN HBr HYDRATE

3.3 SELECTION OF ANALYTICAL TECHNIQUES [1.2]
RP-HPLC METHOD

3.4 VALIDATION OF ABLOVE METHODS AS PER ICH GUIDELINES. [15,21,22,23,35]
- Specificity
- Linearity & Range
- Accuracy
- Precision
- Robustness
- LOD & LOQ

3.5 DETERMINATION OF DRUG BY RP-HPLC METHOD IN BULK AND MARKETED FORMULATION [21,34,9,4,40]
- ✓ Optimization of chromatographic conditions
- ✓ Optimization of mobile phase composition
- ✓ Selection of suitable detection wavelength
- ✓ To determine linearity range
- ✓ To validate the developed method as per ICH guidelines
- ✓ To perform analysis of marketed formulation
- ✓ Force degradation studies

3.6 DEVELOPMENT OF STABILITY INDICATIONGANALYTICAL METHOD OF DRUGS [5,6]
1)Acidic degradation
2)Alkaline degradation
3)Neutral degradation
4)Oxidative degradation
5)Thermal degradation
6)Photolytic degradation.

4. DRUG PROFILE OF METFORMIN HYDROCHLORIDE

Chemical Name	1,1-Dimethylbigunide hydrochloride
Empirical Formula	C4H12ClN5
Molecular weight	165.625 g/mol
CAS NO-	1115-70-4
Appearance:	white powder.
Melting Point	223-226° C
Storage	Store at 4° C
Solubility	Soluble in water (50 mg/ml), ethanol Practically insoluble in acetone, ether and chloroform
Category	Oral anti-hyperglycemic
Official Status	USP 2007, IP 2017

4.1 PHARMACOLOGICAL STUDY [45]
INDICATIONS:
Metformin hydrochloride is the first-medication for treatment of type-2 diabetes, metformin prevents the cardiovascular disease.

4.2 MODE OF ACTION:
Metformin is an anti-hyperglycaemic agent, which improve glucose tolerance in patient with type-2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacological mode of action is different from another oral anti-hyperglycaemic agents [1]. Metformin decreases hepatic glucose production, decrease intestinal absorption of glucose, and improve insulin sensitivity by increasing peripheral uptake and utilization. Unlike sulfonylurea, metformin does not produce hypoglycemia in either patient with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin level and daylong plasma insulin response may actually decrease. [12]

5.DRUG PROFILE OF TENELIGLIPTIN HBr HYDRATE

Chemical Name	((2S,4S)-4-(4-(3-methyl-1-phenyl-1Hpyrazol-5-yl)-1-piperazinyl)-2pyrrolidinyl)-3-thiazolidinyl-, hydrobromide, (2:5) , hydrate
Empirical Formula	C44H67Br5N12O3S2
Molecular Weight	1275.741 g/mol
CAS NO-	1572583-29-9
Appearance:	white powder.
Melting point	>211°C
Storage	Refrigerator
Solubility	water, methanol and Dimethyl sulfoxide
Category	Oral anti-hyperglycemic
Official Status	USP pending monograph

5.1 PHARMACOLOGICAL STUDY
INDICATIONS:
Teneligliptin HBr hydrate used for treatment of Type-2 diabetes mellitus.

5.2 MODE OF ACTION: [18]
Teneligliptin inhibited human dipeptidyl peptidase-4 enzyme activity with the IC_{50}=1 nM, more than 150 fold selectivity against DPP-8 and DPP-9 which suggested little off target skin lesion side effect. By DPP-4 inhibition, teneligliptin prevented the degradation of incretins GIP and promoted insulin release which prevented blood glucose increase after food intake with little hypoglycemia risk during lifetime taken.

6. MATERIALS AND METHODS USED:

Materials
Procurement of Working standards:
Drug Sample

1) Metformin hydrochloride
2) Teneligliptin HBr Hydrate

INSTRUMENTS:
Instrument information:
System: HPLC Binary Gradient System
Model No.: HPLC 3000 series
Company: Analytical Technologies Ltd.
Detector: UV-3000-M
Pump: P-3000-m Reciprocating (40MPa)
Column: Grace C8 (250mm x 4.6 id., particle size: 5 micron)
Software: HPLC workstation.
Balance:
Wenser High Precision Balance

7.EXPERIMENTAL WORK:

7.1 CHROMATOGRAPHIC PROCEDURE [1,3,4]
The method was carried out on Grace C8 (250mm x 4.6 i.d., particle size: 5 micron) using 0.1% OPA and acetonitrile in the ratio of 70:30 v/v with adjusted pH 3 at flow rate 0.9 mL/min. The wavelength of

Metformin HCL and Teneligliptin HBr Hydrate at 246 nm was found to be appropriate. The linearity range was obtained in the concentration of 50-250 µg/mL Metformin HCL and 02-10 µg/mL Teneligliptin HBr Hydrate respectively. The retention time of Metformin HCL and Teneligliptin HBr Hydrate was found to be 2.395 ± 0.2 min and 2.891± 0.2 min respectively.

The developed method was found be accurate, robust and sensitive which can be used for estimation of combination of Metformin HCL and Teneligliptin HBr Hydrate in bulk and pharmaceutical dosage form.

8. METHOD DEVELOPMENT [4,18,21,22,23,39]

8.1 PREPARATION OF STANDARED SOULUTION

8.1.1 Stock solution of Metformin Hydrochloride:

Standard stock solution of Metformin Hydrochloride was prepared by dissolving 100 mg of working standard in 100 mL of 0.1% OPA and Acetonitrile (70:30) to get concentration 1000 µg/mL in 100mL clean dry volumetric flask and stock solutions was degassed by sonicated at 25º for 15 min.. Further dilution was made by using (concentration A) (volume A) = (concentration B) (volume B) this calculation formula and the dilution was made in 0.1% OPA and Acetonitrile (70:30) to get final concentration range of Metformin Hydrochloride 50, 100,150, 200, 250 µg/mL.

8.1.2 Stock solution of Teneligliptin HBr Hydrate:

Standard stock solution of Teneligliptin HBr Hydrate was prepared by dissolving 100 mg of working standard in 100 mL of 0.1% OPA and Acetonitrile (70:30) to get concentration 1000 µg/mL in 100mL clean dry volumetric flask and stock solutions was degassed by sonicated at 25º for 15 min.. Further dilution was made by using (concentration A) (volume A) = (concentration B) (volume B) this calculation formula and the dilution was made in 0.1% OPA and Acetonitrile (70:30) to get final concentration range of Metformin Hydrochloride 2,4,6,8,10 µg/mL.

8.1.3 Preparation of Sample Solutions of Metformin Hydrochloride and Teneligliptin HBr Hydrate:

Twenty tablets were weighed and powder equivalent to 150 mg of Metformin HCL and 6 mg of Teneligliptin HBr Hydrate was taken in 100 mL of 0.1% OPA and Acetonitrile (70:30) to get concentration 150 µg/mL of Metformin Hydrochloride and 6 µg/mL Teneligliptin HBr Hydrate in 100 mL clean dry volumetric flask and stock solutions was degassed by sonicated at 25º for 15 min.

8.2 PREPARATION OF MOBILE PHASE AND ANOTHER SOULTION

8.2.1 Preparation of buffer solution

Dissolve 1 mL of OPA in 1000 mL of D.M. Water and pH was adjusted to 6.5 with 0.025 M phosphate buffer and to get 0.1 % OPA(Ortho Phosphoric Acid), and filtered by using 0.45 micron membrane filter, finally this solution was sonicated at 25º for 5 min.

8.2.2 Preparation of mobile phase

Mobile phase was prepared by mixing 0.1 % OPA and acetonitrile in the ratio of 70:30 v/v. and filtered through 0.45 µm membrane filter paper using vacuum filtration assembly, finally this solution was sonicated at 25º for 15 min.

8.2.3 Preparation of 0.1 M Sodium Hydroxide

0.4 gm of odium hydroxide pellets was dissolved in D.M. Water with 100 mL adjusted volume in volumetric flask to get 0.1 M sodium hydroxide solution, finally the solution was sonicated at 25º for 5 min.

8.2.4 Preparation of 0.1 N Hydrochloric acid solutions

0.85 mL of onc. HCL in D.M. Water with 100mL adjusted voume in volumetric flask, to get 0.1 N Hydrochloric acid solutions, finally the solution was sonicated at 25º for 5 min.

8.2.5 Preparation of 3%v/v Hydrogen peroxide solution:

3 mL of Hydrogen peroxide in D.M. Water with 100mL adjusted volume in volumetric flask, to get 3%v/v Hydrogen peroxide solution, finally the solution was sonicated at 25º c for 5 min.

8.3 SELECTION OF ANALYTICAL WAVELENGTH

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm. The spectrum was obtained. It was observed that the both the drugs showed considerable absorbance at 246 nm so it was selected as detection wavelength.

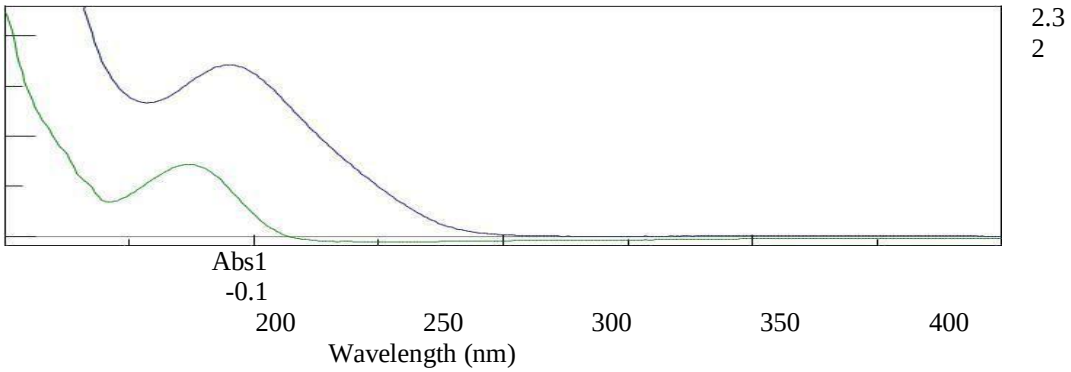


Fig.No: 02 Overlay UV spectrum of Metformin Hydrochloride and Teneligliptin HBr Hydrate

SELECTION OF MOBILE PHASE

The standard solution of Metformin hydrochloride (50µg/ml) and Teneligliptin HBr Hydrate (2 µg/ml) was injected into the HPLC system and run in different solvent systems. Different mobile phases like acetonitrile and water, methanol and water, methanol and phosphate buffer in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Metformin hydrochloride and Teneligliptin HBr Hydrate After several trials, 0.1% OPA and acetonitrile in the ratio of 70:30 v/v was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Summary of chromatographic parameters selected

Sr.no	Parameter	Conditions used for Analysis
1	Column	Grace C8 (250mm x 4.6 i.d., particle size: 5micron)
2	Mobile phase	0.1 % OPA: acetonitrile (70:30 v/v.)
3	Flow rate	0.9 ml/min
4	Detection Wavelength	246 nm
5	Sample Volume	20µl
6	Column temperature	Ambient

9. Validation of Analytical Method

This method was validated according to ICH Q2 (R1) guidelines for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ) under the validation study, the following parameters were studied.

9.1. Specificity:

The specificity of the method was carried out to check whether there is any interference of any impurities with the retention time of analyte peaks. The specificity was performed by the injecting blank, Placebo and standard solutions of drugs. The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 990, indicating the non-interference of any other peak of degradation product or impurity.

Table No 01: Result of Specificity

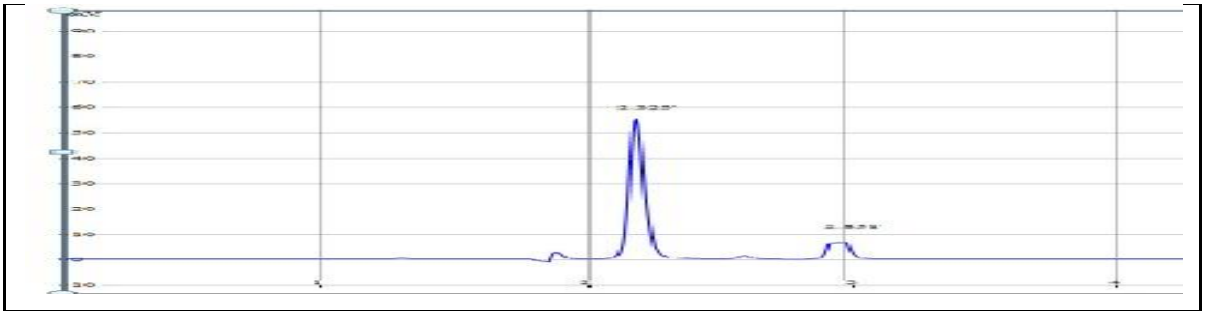
Drug	Purity tail	Purity front
Metformin HCL	993.421	996.464
Teneligliptin HBr Hydrate	992.652	995.965

9.2. Linearity and Range:

Linearity was tested for the range of concentrations Met= 50-250µg/ml and 210µg/ml. Each sample in five replicates was analysed and peak areas were recorded. The response factors were plotted against the corresponding concentrations of Metformin Hydrochloride and Teneligliptin HBr Hydrate obtain in the calibration curve for Metformin Hydrochloride and Teneligliptin HBr Hydrate respectively. Linearity results are given in table

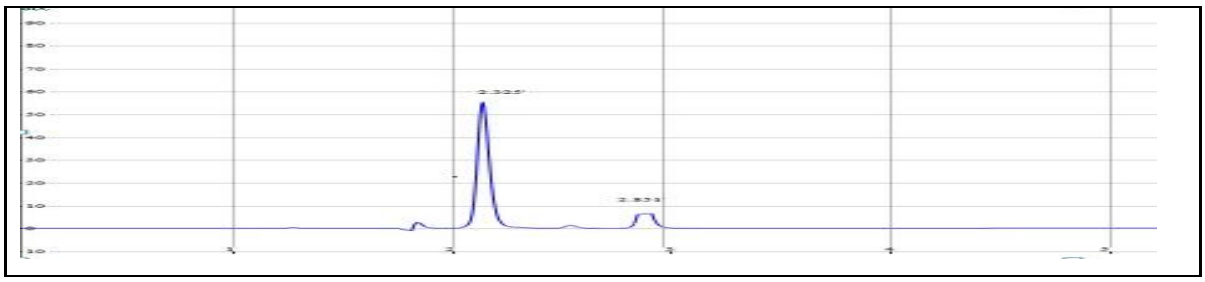
Acceptance Criteria:

The plot should be linear passing through the origin.Correlation Coefficient should not be less than 0.999.



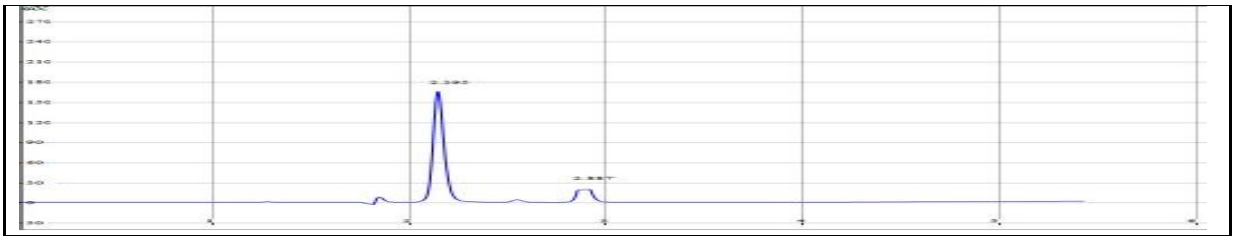
Retention Time	Conc.	Area	Resolution	T. Plate num	Asymmetry
2.325	50ppm	402701	6.95	9685	1.14
2.851	2ppm	39523	0.00	6698	1.85

Fig. No :03 chromatogram of Metformin HCL (50ppm) and Teneligliptin HBr Hydrate (2ppm)



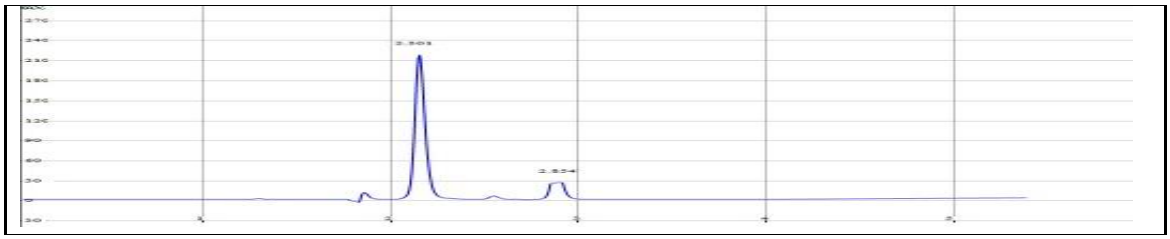
Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.401	100ppm	804327	6.95	9698	1.62
2.885	4ppm	78961	0.00	6745	1.78

Fig. No: 04 chromatogram of Metformin HCL (100ppm) and Teneligliptin HBr Hydrate (4ppm)



Retention Time	Conc.	Area	Resolution	T. Plate num	Asymmetry
2.395	150ppm	1206985	7.32	9685	1.64
2.887	6ppm	117014	0.00	6873	1.69

Fig. No: 05 chromatogram of Metformin HCL (150ppm) and Teneligliptin HBr Hydrate (6ppm)



Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.301	200ppm	1610544	7.74	9645	1.61
2.854	8ppm	156831	0.00	6838	1.74

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.401	250ppm	2011586	7.46	9675	1.21
2.887	10ppm	198561	0.00	6873	1.58

Fig. No: 06 chromatogram of Metformin HCL (200ppm) and Teneligliptin HBr Hydrate (8ppm)

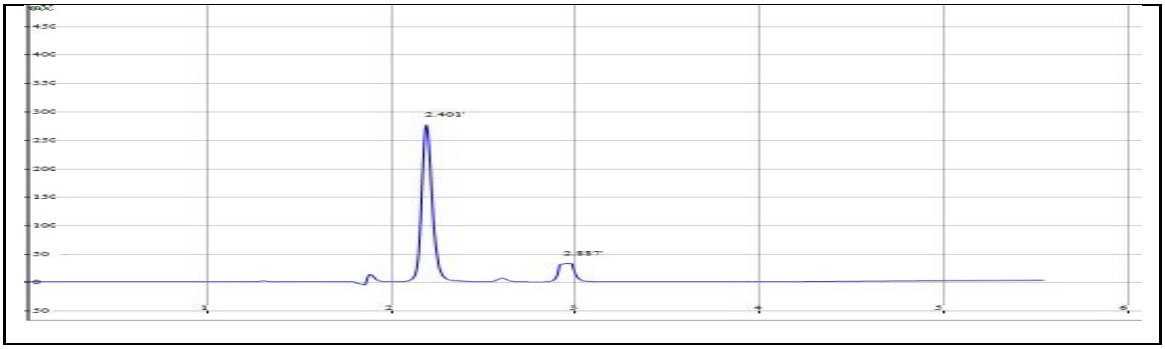


Fig. No: 07 chromatogram of Metformin HCL (200ppm) and Teneligliptin HBr Hydrate (8ppm)

Table No: 02 Result of Linearity data of Metformin Hydrochloride and Teneligliptin HBr Hydrate

Conc. Of MET (µg/ml)	Peak Area	Conc. Of (TEN µg/ml)	Peak Area
50	402701	02	39523
100	804327	04	78961
150	1206985	06	117014
200	1610544	08	156831
250	2011586	10	198561

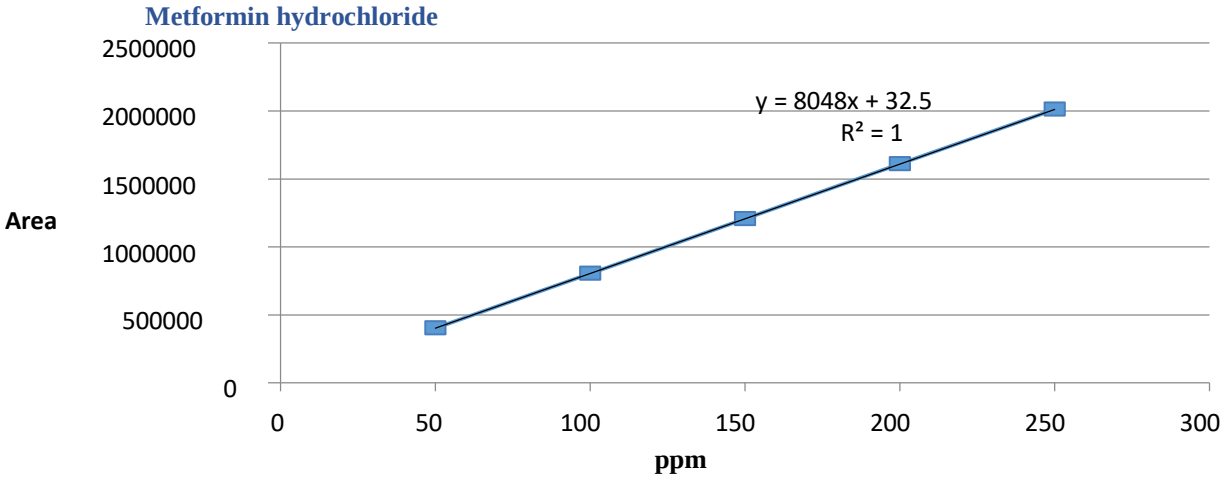


Fig. No: 08. Linearity graph of Metformin Hydrochloride

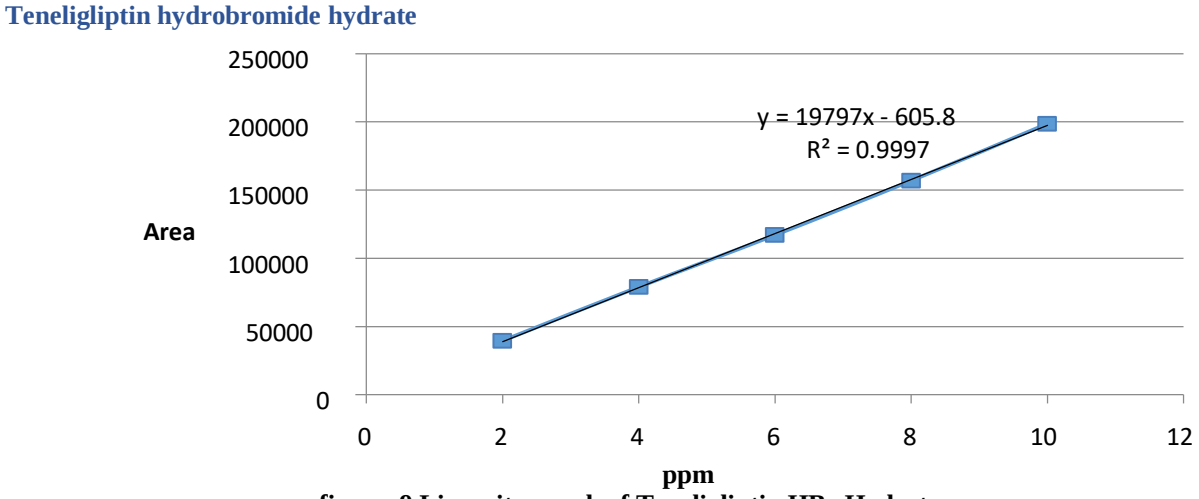


fig no :9 Linearity graph of Teneligliptin HBr Hydrate

9.3 Assay (%):

Assay was carried out by dissolving tablet sample equivalent to 150ppm of Metformin HCL and 6ppm of Teneligliptin HBr Hydrate. The sample solution was injected and area was recorded. Concentration and purity was determined from linearity equation. The result was found to Metformin HCL 100.03% and Teneligliptin HBr Hydrate 100.19%.

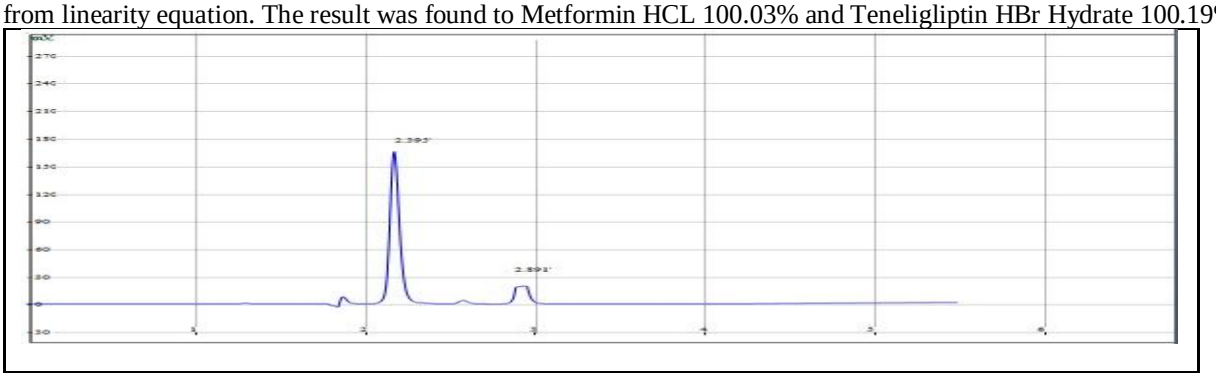


fig no :10 Chromatogram of marketed formulation

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.393	150ppm	1207297	7.32	9675	1.41
2.891	6ppm	118402	0.00	6848	1.74

Table No :07 Assay of Marketed formulation.

Drug Name	Composition in ppm	Area of Standard	Area of Sample	% Assay
Metformin HCL	**150**	1206985	1207297	100.03
Teneligliptin HBr Hydrate	**6**	117014	118402	100.19

9.4 Accuracy: A known amount of the standard drug was added to the blank sample at different level 50%, 100%, 150%. The mean % recovery of metformin HCL and Teneligliptin HBr hydrate were calculated and acceptance criteria 100%±2%.

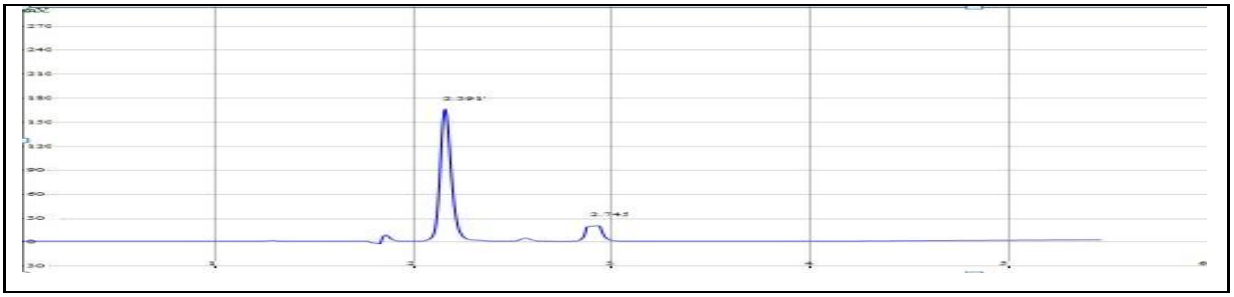


Fig. No: 11 chromatogram of Metformin HCL and Teneligliptin HBr Hydrate at 50% Recovery

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.391	150ppm	1207147	7.46	9668	1.25
2.742	6ppm	117231	0.00	6848	1.48

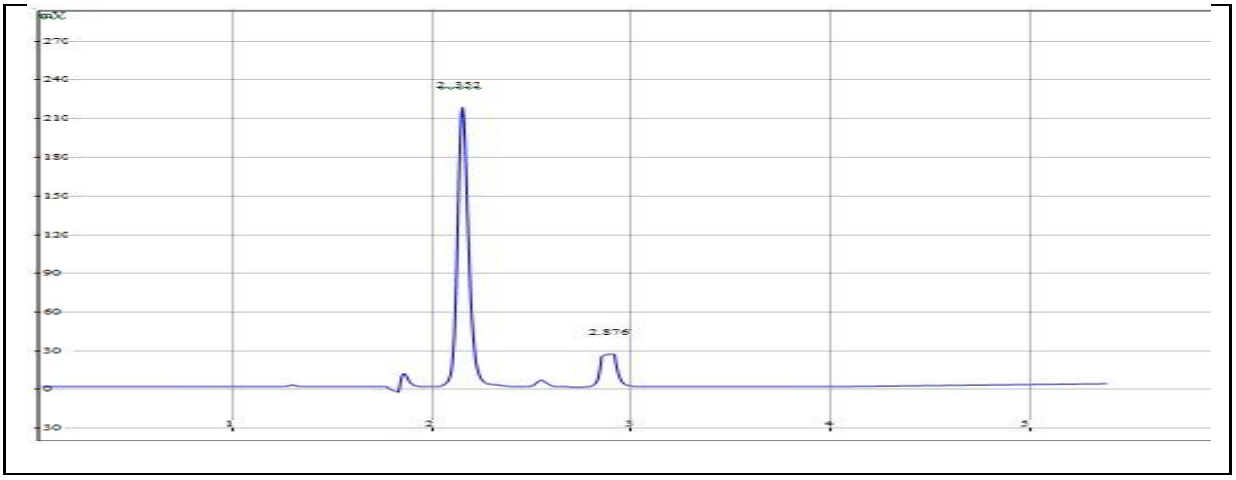


Fig. No:12 chromatogram of Metformin HCL and Teneligliptin HBr Hydrate at 100% Recovery

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.332	200ppm	1610804	7.51	9672	1.12
2.876	8pm	156985	0.00	6858	1.58

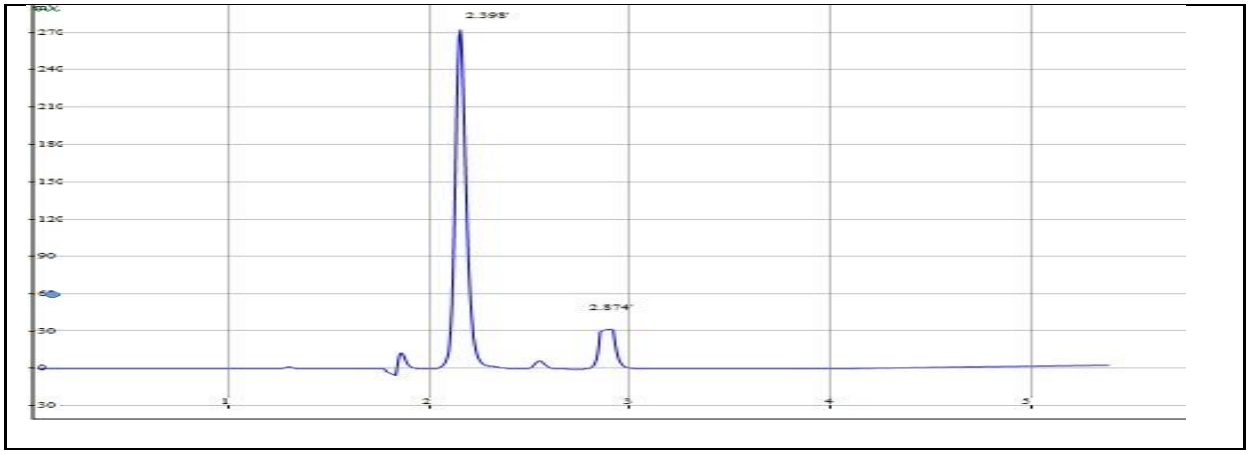


Fig. No: 13 chromatogram of Metformin HCL and Teneligliptin HBr Hydrate at 150% Recovery

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.398	250ppm	2012870	7.48	9672	1.31
2.874	10pm	197482	0.00	6985	1.58

Table No: 03 Result of Recovery studies.
% Recovery of Metformin HCL

Conc. (%)	Sample amount (ppm)	Amount added (ppm)	Amount recovered (Area)	Amount recovered (ppm)	% recovery	%mean recovery
50%	100	50	1207147	149.9894	99.99293	99.99273
	100	50	1207102	149.9838	99.9892	
	100	50	1207185	149.9941	99.99607	
100%	100	100	1610804	200.1456	100.0728	100.0723
	100	100	1610758	200.1398	100.0699	
	100	100	1610825	200.1482	100.0741	
150%	100	150	2012870	250.1041	100.0416	100.0415
	100	150	2012895	250.1072	100.0429	
	100	150	2012835	250.0997	100.0399	

%Recovery of Teneligliptin HBr hydrate

Conc. (%)	Sample amount (ppm)	Amount added (ppm)	Amount recovered (Area)	Amount recovered (ppm)	% Recovery	%Mean recovery
50%	4	2	117231	5.982866	99.71443	99.40911
	4	2	117301	5.955791	99.26318	
	4	2	117285	5.954983	99.24972	
100%	4	4	156985	7.960337	99.50421	99.49538
	4	4	156924	7.957256	99.4657	
	4	4	157004	7.961297	99.51621	
150%	4	6	197482	10.00595	100.0595	100.0683
	4	6	197514	10.00757	100.0757	
	4	6	197502	10.00696	100.0696	

9.5 Precision:
Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. The precision method was demonstrated by inter-day and intra-day studies. Three replicate injections of a known concentration of Metformin HCL (150ppm) and Teneligliptin HBr Hydrate (6ppm), has been injecting into HPLC and analysed.[31,21,22,39,34]

Table No: 04 Result of Inter-day studies

Metformin HCL		Teneligliptin HBr Hydrate	
Day 1	Area	Day1	Area
	1207012		117085
	1207151		117214
	1207742		117287
Day2	1207001	Day2	117245
	1207754		117301
	1206974		117014
Mean	1206974	Mean	117014
%RSD	0.03 %	%RSD	0.10 %

Table No: 5 Result of Intra-day studies

Metformin HCL		Teneligliptin HBr Hydrate	
Morning	Area	Morning	Area
	1206142		117021
	1207241		117301
	1208521		116001
Evening	1207145	Evening	117188
	1206985		117014
	1207286		117256
Mean	1207220	Mean	116964
%RSD	0.06 %	%RSD	0.42 %

9.6 Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, wavelength, mobile phase ratio were altered and the effects on the peak area were noted

Table No-6 Result of % RSD Found For Robustness Study (peak area)

Drug	% RSD Found For Robustness Study(peak area)					
	Flow Rate (0.9 ml/min)		Wavelength(nm)		Mobile phase ratio(70:30)	
	0.7	1.2	244	248	68:32	73:27
Metformin HCL	0.18	0.93	0.45	0.87	0.98	0.95
Teneligliptin HBr Hydrate	0.09	0.12	0.34	0.74	1.43	1.62

9.7 Limit of Detection (LOD) and Limit of Quantification (LOQ)
LOD was calculated by using this formula

$$LOD = \frac{3.3 \times \text{Std. Deviation}}{\text{Slope}}$$

$$LOQ = \frac{10 \times \text{Std. Deviation}}{\text{Slope}}$$

Where,
Std. Deviation calculated from accuracy,
And slope from linearity

Table No: 7 Data for LOD and LOQ

Sr.No.	Drug	LOD	LOQ
1	METFORMIN HCL	1.032 µg/ ml	3.127 µg/ ml
2	TENELIGLIPTIN HBr HYDRATE	1.67055 µg/ ml	5.0623 µg/ ml

Table No: 8 Summary of validation parameter

Sr. No	Validation parameter	Metformin HCL	Teneligliptin HBr Hydrate
01	Specificity	Complies	Complies
02	Linearity Equation	y = 8048.x + 15.47	y = 19754x - 288.4
	(R^2)	1	0.999
	Range (ppm)	50-250	2-10
03	Accuracy (%)	%Recovery	%Recovery
	50	99.99273	99.40911
	100	100.0723	99.49538
	150	100.0415	100.0683
04	%Assay	100.03%	100.19%
05	Precision	%RSD	%RSD
	Inter-day	0.03	0.10
	Intra-day	0.06	0.42
06	Robustness	Complies	Complies
07	LOD (µg/ ml)	1.032	1.67055
	LOQ (µg/ ml)	1.67055	5.0623

10. STRESS DEGRADATION STUDIES OF BULK DRUG[6,8]

Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis as per ICH Q1A R2 and Q1B. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and working standard solution of Metformin Hydrochloride and Teneligliptin HBr Hydrate subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state.

10.1 Acid hydrolysis:

Acid induced degradation was performed by adding 1 ml of 0.1N Hydrochloric acid (HCl) to volumetric flask containing 9ml of sample solutions of Metformin Hydrochloride and Teneligliptin HBr Hydrate standard solution (150 and 6µg/ml) kept for 24 hrs in dark place at 70 ^{0}c. Final solution was injected to HPLC.

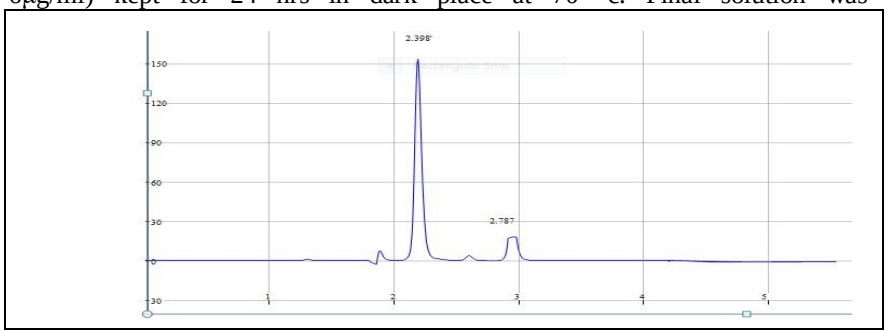


Fig. 14: Chromatogram obtained from sample subjected to acid degradation for overnight RT- Met= 2.398min, Ten= 2.787 min.

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.398	150ppm	1129617	7.45	18548	1.03
2.787	6ppm	112427	0.00	12347	1.58

10.2 Alkaline hydrolysis:

Alkali induced degradation was performed by adding 1 ml of 0.1 M Sodium Hydroxide (NaOH) to volumetric flask containing 9ml of sample solutions of Metformin Hydrochloride and Teneligliptin HBr Hydrate standard solution (150 and 6µg/ml) kept for 24 hrs in dark place at 70 ^{0}c. Final solution was injected to HPLC.

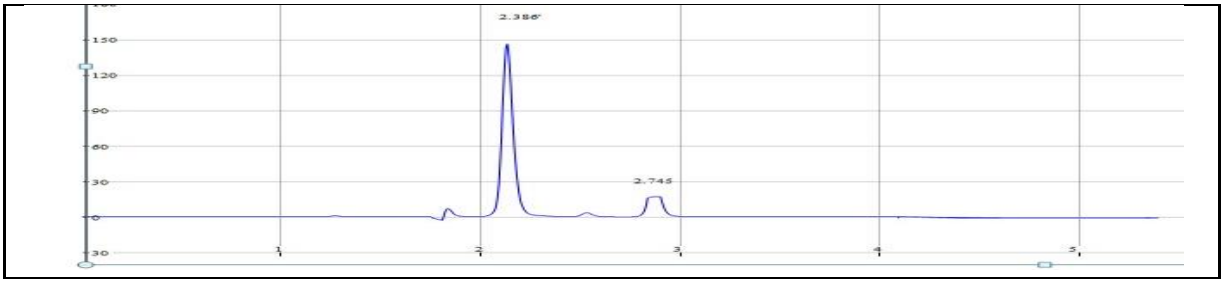
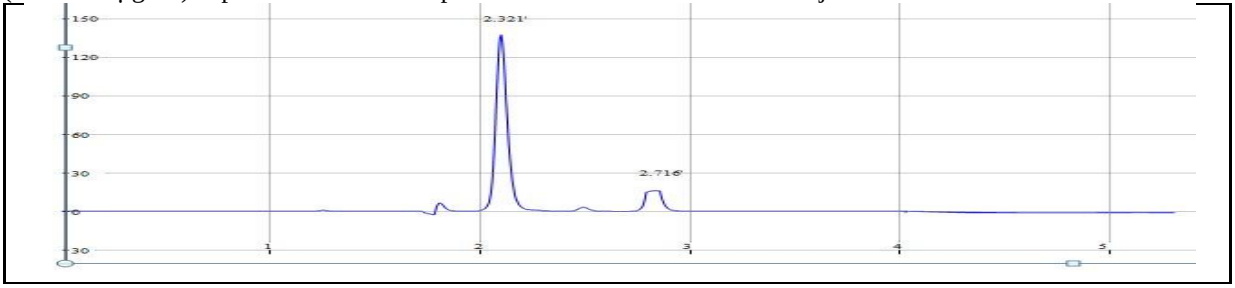


Fig. 15: Chromatogram obtained from sample subjected to alkaline degradation for overnight RT- Met= 2.386min, Ten= 2.745 min.

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.386	150ppm	1143738	6.85	19124	1.12
2.745	6ppm	109010	0.00	13845	1.45

10.3 **Peroxide Degradation:**

Oxidative degradation was performed by adding 1 ml of Hydrogen peroxide (H_2O_2, 30%v/v) to volumetric flask containing 9ml of sample solutions of Metformin Hydrochloride and Teneligliptin HBr Hydrate standard solution (150 and 6µg/ml) kept for 24 hrs in dark place at 70 ^{0}c. Final solution was injected to HPLC.



Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.321	150ppm	1093528	7.25	17642	1.08
2.716	6ppm	107091	0.00	12845	1.59

Fig.16: Chromatogram obtained from sample subjected to oxidative degradation for overnight RT- Met= 2.321 min, Ten= 2.716 min.

10.4 Thermal Stress degradation:

Thermal stress degradation was performed to volumetric flask containing 10ml of sample solutions of Metformin Hydrochloride and Teneligliptin HBr Hydrate standard solution (150 and 6µg/ml) kept for 24 hrs in dark place at 70 ^{0}c. Final solution was injected to HPLC.

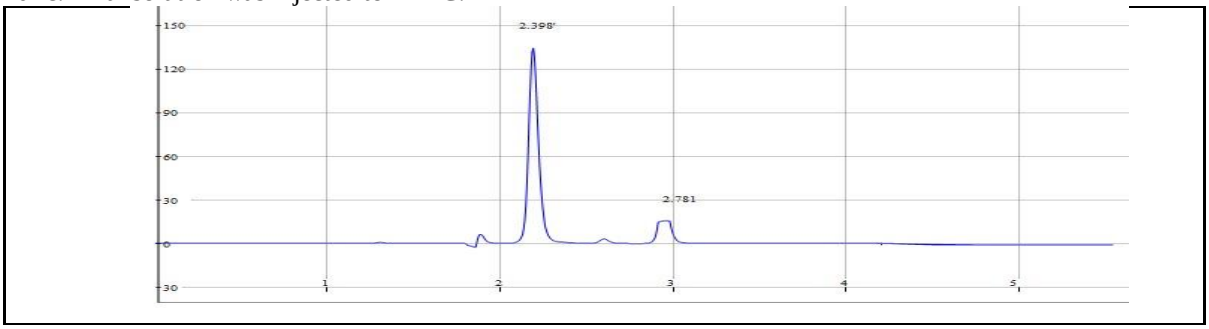


Fig.no:17 Chromatogram obtained from sample subjected to thermal stress degradation overnight RT-Met=2.398min, Ten=2.781 min

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.398	150ppm	1146153	7.85	17589	1.04
2.781	6ppm	108893	0.00	13524	1.62

10.5 Photolytic Stress degradation:

Photolytic degradation studies were carried out by exposure of drug to fluorescent light illumination not less than 1.2 million lux hours. A volumetric flask containing 10ml of sample solutions of Metformin Hydrochloride and Teneligliptin HBr Hydrate standard solution (150 and 6µg/ml)

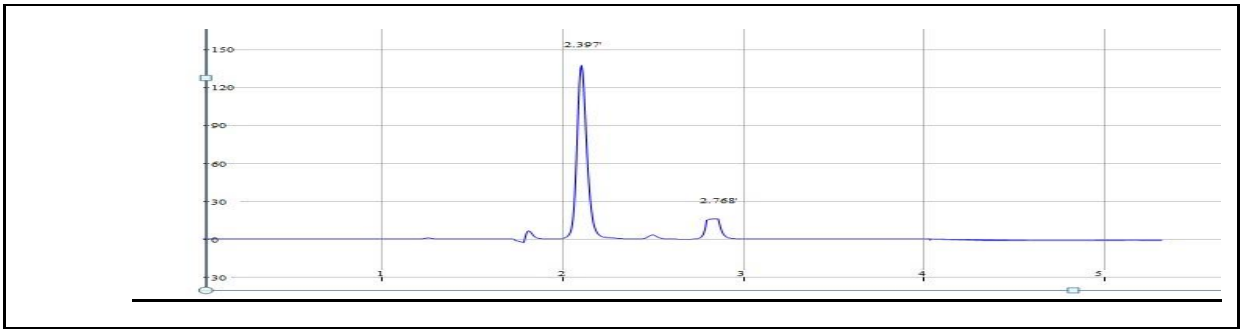


Fig.no:18 Chromatogram obtained from sample subjected to Photolytic stress degradation overnight RT-Met=2.397min, Ten=2.768 min

Retention Time	Conc.	Area	Resolution	T.Plate num	Asymmetry
2.397	150ppm	1155808	6.85	16845	1.07
2.768	6ppm	113339	0.00	11234	1.69

Table 9: Summery of Forced Degradation studies of Metformin Hydrochloride

Sr. No.	Stress condition	%Recovery	Peak front	Peak tail
01	Acid Hydrolysis Degradation	93.59	996.6	993.6
02	Alkaline Hydrolysis Degradation	94.76	997.8	994.3
03	Peroxide Degradation	90.6	993.4	992.1
04	Thermal Stress Degradation	94.96	994.7	996.9
05	Photolytic Stress Degradation	95.76	995.3	994.6

Table N0 10: Summery of Forced Degradation studies of Teneligliptin HBr Hydrate

Sr. No.	Stress condition	%Recovery	Peak front	Peak tail
01	Acid Hydrolysis Degradation	96.08	997.9	996.1
02	Alkaline Hydrolysis Degradation	93.16	996.2	993.6
03	Peroxide Degradation	91.52	992.2	991.9
04	Thermal Stress Degradation	93.06	996.8	995.1
05	Photolytic Stress Degradation	96.86	997.7	996.5

11. SUMMARY AND CONCLUSION:

A simple and rapid stability indicating RP-HPLC method for Metformin hydrochloride and Teneligliptin HBr hydrate was developed and validated. The stationary phase used was Grace C8 (250mm x 4.6 id., particle size: 5 micron) with a mobile phase consisting of mixture of 0.1 % OPA: acetonitrile in the ratio 70:30 v/v at a flow rate of 0.9 ml/min. Detection was carried out at 246 nm. The retention time observed was Metformin hydrochloride = 2.395 ± 0.2 min Teneligliptin HBr Hydrate= 2.891 ± 0.2 min.

The column was maintained at ambient temperature and 20µl of solution were injected.

The eluted compound was detected by using UV-3000-M detector. The linear regression analysis data for calibration plot show good relationship with coefficient of regression value of Metformin hydrochloride was r^2=1 And Teneligliptin HBr Hydrate was r^2=0.999 in the concentration range 50-250 ppm and 2-10ppm respectively.[34,36,37,40]

12. CONCLUSION:

- The method was found to be linear, accurate, precise, specific and robust according to acceptance criteria.
- The coefficient of correlation was obtained in acceptable range.
- The percentage recovery obtained in acceptable range.
- The percentage relative standard deviation of main peak area, tailing factor and theoretical plate is well within the acceptable range. Hence the precision of given method is confirmed.
- Variation in flow rate, wavelength, mobile phase ratio composition does not have any effect on the % RSD of standard and assay value.
- There is no interference of any degradation product with peak of drug.

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