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DEVELOPMENT OF A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND TENELIGLIPTIN HYDROBROMIDE AND ITS VALIDATION AS PER ICH GUIDELINES

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Abstract:

A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Metformin Hydrochloride and Teneligliptin in bulk and dosage forms. The method involves separation on YMC C18column(150mm x 4.6mm x5µm particle size). The optimized mobile phase consists of Phosphate buffer (pH 3) and Acetonitrile (80:20v/v) with a flow rate of 0.8ml/min and UV detection at 220mn. Retention time was 2.138min (Metformin Hydrochloride), 2.943min (Teneligliptin), 5.075 Pioglitazone. Linearity range was 9.98-600ug/ml (Metformin Hydrochloride), 0.51-24ug/ml (Teneligliptin). Accuracy was in the range of 99.41-100.74% for both drugs. Precision was 0.8% and 0.9% for Metformin Hydrochloride and Teneligliptin. LOD and LOQ are 0.72ug/ml and 2.40ug/ml for Metformin Hydrochloride, 0.15ug/ml and 0.51ug/ml for Teneligliptin. The method developed is sensitive, accurate and precise. Retention time and run time were also less and hence the method is economical. When applied for tablet assay, drug content was within 99.89-100.74 % of labeled content. Forced degradation studies indicated the suitability of the method for stability studies.

Key words: *Metformin Hydrochloride, Teneligliptin, RP-HPLC Method, Simultaneous estimation, Validation as per ICH guidelines, Forced degradation studies.*

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INTRODUCTION:

Metformin Hydrochloride is an orally administered biguanide derivative used to lower blood glucose concentration in patients with non-insulin-dependentdiabetes mellitus [1]. Metformin Hydrochloride improves insulin sensitivity and decreases insulin resistance by inhibiting Complex1 of the mitochondrial respiratory chain and inducing AMP activated protein kinase-dependent signaling. Metformin Hydrochloride is chemically known as 1,1-Dimethyl biguanide monohydrochloride [2,3].
Teneligliptin HBr is a novel, potent, peptidomimetic, and long acting DPP-4 inhibitor. Teneligliptin, is chemically known as {(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-yl} (1,3-thiazolidin-3-yl) methanone hemipenta hydrobromide hydrate exhibits a unique structure that is characterized by five consecutive rings[4]. Teneligliptin exerts its activity for 24 h, with elevation of activated glucagon like peptide 1 (GLP1) levels by suppressing postprandial hyperglycemia after the meals. Significant decrease in hemoglobin A1c (HbA1c), fasting blood glucose, and postprandial blood glucose levels was observed in type 2 diabetic patients taking teneligliptin for 12 weeks. This drug showed a promising effect in stabilizing the glycemic fluctuations throughout the day and suppressing the diabetic complications[5,6].
Though several methods are reported in literature for the estimation of Metformin Hydrochloride[7-10] and Teneligliptine [11-16] individually, only one UV spectrophotometric method was reported for the simultaneous estimation of Metformin Hydrochloride and Teneligliptine in combination [17].
The objective of the present study was to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Metformin Hydrochloride and Teneligliptine and validate the method with forced degradation studies according to ICH guidelines [18].

EXPERIMENTAL:

Materials and reagents:

HPLC grade Acetonitrile (Lichrosol^{R}, Merck Lifesciences Pvt. Ltd., Mumbai, India), HPLC water (Lichrosolv^{R}MerckLifesciencesPvt.Ltd., Mumbai, India) Potassium Dihydrogen phosphate (Thermo Fischer Scientific Pvt Ltd., Mumbai, India), and Ortho phosphoric acid (S D Fine –Chem. Ltd., Mumbai, India) were used in the study. The working standards of Metformin Hydrochloride, Teneligliptine and Pioglitazone were generous gift obtained from HiQ Pharma Labs Pvt Ltd.,Hyderabad, India. Afoglip M tablet containing Metformin Hydrochloride 500mg and Teneligliptine 20mg (Torrent pharmaceutical company) was procured from local market.

Instrumentation:

Chromatography was performed on a WATERS 2695 HPLC column (waters corporation, Mildord, USA) with an autosampler and equipped with a 2996 series of PDA detector with a spectral bandpass of 1.2nm. Components were detected using UV and that processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples and a UV crossinker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300nm was selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshniwal), digital P^{H} meter(Adwa – AD 1020), UV/VIS spectrophotometer (Labindia UV 3000) were used in the study.

Chromatography conditions:

The chromatographic separation was performed on YMC C $_{18}$ (4.6 x 150mm, 5µm particle size) at an ambient column temperature. The samples were eluted using Phosphate buffer (pH adjusted to 3 with OPA): Acetonitrile(80:20v/v) as the mobile phase at a flow rate of 0.8ml/min the mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.45µm Nylon (N66) 47mm membrane filter. The measurements were carried out with an injection volume of 20µL, flow rate was set to 0.8 mL/min, and UV detection was carried out at 220 nm. All determinations were done at ambient column temperature (27°C). The chromatograms of the prepared standard stock solutions of Metformin and Teneligliptine and Pioglitazone were recorded under optimized chromatographic conditions.

Preparation of Buffer and Mobile Phase:

Preparation of 0.025M Phosphate buffer:

3.4g of potassium dihydrogen ortho phosphate was weighed and taken in a 1000ml volumetric flask and P^{H} was adjusted to 3 with dilute OPA, finally the solution was filtered by using 0.45 micron membrane filter and sonicated for 10 min.

Preparation of mobile phase:

800 ml (80%) of phosphate buffer and 200 ml of Acetonitrile (20%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent:

Mobile phase was used as diluent

Preparation of Standard Solutions:

Stock solution of Metformin Hydrochloride:

Standard stock solution of Metformin Hydrochloride was prepared by dissolving 400 mg of Metformin Hydrochloride in 100 ml of diluent (Buffer: Acetonitrile, 80:20v/v) in a 100 ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 4000µg/ml of Metformin Hydrochloride. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Metformin Hydrochloride.

Stock solution of Teneligliptine:

Standard stock solution of Teneligliptine was prepared by dissolving 16 mg of Teneligliptine in 100 ml of diluent (Buffer: Acetonitrile, 80:20v/v) in a 100 ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 160µg/ml of Teneligliptine. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Teneligliptine.

Stock solution of Pioglitazone Hydrochloride:

Standard stock solution of Pioglitazone Hydrochloride was prepared by dissolving 100 mg of Pioglitazone Hydrochloride in 100ml of diluent (Buffer: Acetonitrile, 80:20v/v) in a 100ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Pioglitazone Hydrochloride.

Working Standard Solution of Metformin Hydrochloride:

Working standard solution of Metformin Hydrochloride was prepared by taking 0.3 ml of stock solutions of Metformin Hydrochloride in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 360µg/ml of Metformin Hydrochloride.

Working Standard Solution of Teneligliptine:

Working standard solution of Teneligliptine was prepared by taking 0.3 ml of stock
solutions of Teneligliptine in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 4.8µg/ml of Teneligliptine.

Working Standard Solution of Pioglitazone Hydrochloride:

Working standard solution of Pioglitazone Hydrochloride was prepared by taking 1ml of stock solutions of Pioglitazone Hydrochloride in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 100µg/ml of Pioglitazone Hydrochloride.

Preparation of Sample Solutions of Metformin and Teneligliptine:

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 400 mg of Metformin Hydrochloride and 16 mg of Teneligliptine was taken into 100 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution was filtered and suitably diluted to get a concentration of 360 µg/ml of Metformin Hydrochloride and 14.4 µg/ml of Teneligliptine.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:

During the optimization cycle, different columns with different lengths and internal diameters were tried namely, Waters C18 column, hypersil column, lichrosorb, and YMC column but finally satisfactory separation was obtained on YMC C 18 (4.6 x 150mm, 5µm) column. Methanol and acetonitrile were examined individually and simultaneously as organic modifiers and acetonitrile was found to be more suitable, individually, as it allowed better separation of the three analytes under investigation. Isocratic mode of elution with different ratios of organic to aqueous phases was tried in order to achieve proper separation of the cited analytes in a reasonable run time. The use of 0.025M Phosphate buffer was necessary in this method in order to influence the ionization of the analytes and to help in their co-elution. Also, it kept the pH constant as each of MTF, TEN and PGZ is obviously affected by the mobile phase composition and pH. The effect of pH on the separation of the analytes was studied. It was found that pH higher than 4.59 was not suitable as due to improper separation of the analyzed compounds. pH was adjusted at 3 for the best separation of the three analytes in a reasonable run time (<10 min) and with good resolution between all peaks. Different flow rates were studied and flow rate of 0.85 mL min-1 was found to be optimum.

Quantitation was achieved with UV-detection at 220 nm. The column temperature was set at 30 ºC. Optimized method was providing good resolution and peak shape for MTF, TEN and PGZ. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition and flow rates on results were evaluated as a part of testing for methods robustness.

Validation of Method Developed:
The proposed method was validated according to the ICH 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.

System suitability test:
HPLC system was optimized as per the chromatographic conditions. 20 µl of standard solutions of drugs were injected in triplicate into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, and tailing factor were calculated.

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.

Precision:
Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Metformin Hydrochloride (360 µg/mL) and Teneligliptine (4.8 µg/mL),have been analyzed by injecting them into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were taken and standard deviation, % relative standard deviation(RSD),was calculated.

Accuracy
Accuracy is tested by the standard addition method at different levels: 50, 100 and 150%. A known amount of the standard drug was added to the blank sample at each level. The mean recovery of Metformin hydrochloride and Teneligliptine were calculated and accepted with 100±2%.

Linearity:
Appropriate volumes of Metformin hydrochloride and Teneligliptine stock 360µg/ml and working 14.4µg/ml standard solutions were diluted with mobile phase to yield 2.4, 120, 240, 360, 480 and 600µg/mL of Metformin hydrochloride and 0.51, 4.8, 9.6, 14.4, 19.2 and 24µg/ml of Teneligliptine respectively. Six replicates of each concentration were independently prepared and injected in to HPLC system. The linearity was determined by calculating a regression line from plot of peak area ratio of drug and IS versus concentration of the drug. Regression analysis were computed for Metformin hydrochloride and Teneligliptine. The method was evaluated by determination of correlation coefficient and intercept values according to ICH guidelines.

Limit of Detection and Limit of Quantification
Limit of detection (LOD) and limit of quantification (LOQ) of Metformin hydrochloride and Teneligliptine were determined by calibration curve method. Solutions of Metformin hydrochloride and Teneligliptine were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using the following equations:
LOD= 3 x N/ B
LOQ= 10 x N/ B
where N is residual variance due to regression; B is the slope.

Robustness
HPLC conditions were slightly modified to evaluate the analytical method robustness. These changes included the flow rate and Acetonitrile proportion in the mobile phase.

Forced Degradation Study
Alkaline, acidic, oxidative stress, thermal and direct exposure to UV were carried out.
1) ***Alkali Hydrolysis*:** Ten mL of Metformin hydrochloride and Teneligliptine stock solution was mixed in a flask with 1N sodium hydroxide (4mL) for 1hr at 50°C. Before analysis ,the solution was cooled at room temperature and neutralized with 1N hydrochloric acid. The solution was completed with deionised water to reach a targeted concentration.
2) ***Acid Hydrolysis***: Ten mL of Metformin hydrochloride and Teneligliptine stock solution was mixed in a flask with 1N hydrochloric acid (4mL) for 1hr at 50°C .Before analysis, the solution was cooled at room temperature and neutralized with 1N sodium hydroxide. The solution was completed with deionised water to reach a targeted concentration.
(3) ***Oxidative Stress*** : Ten mL of the Metformin hydrochloride and Teneligliptine stock solution was mixed with 1mL of 3% hydrogen peroxide and stored at 50°C for 1hr.The solution was cooled and completed with deionised water until the volumetric flask mark to reach a targeted concentration.
(4) ***Sunlight Degradation*** : Ten mL of the Metformin hydrochloride and Teneligliptine stock solution was transferred in to a 200mLvolumetric flask and

exposed to direct sunlight for 5days at room temperature. The solution was completed to the mark with deionised water.

(5) ***Thermal Degradation*** : Ten mL of Metformin hydrochloride and Teneligliptine stock solution was transferred in to volumetric flask (200mL) and kept in air dry oven at 105°C for 5h.Then, the solution was cooled and completed to the flask mark with deionised water.

RESULTS AND DISCUSSION:

The Retention time of Metformin hydrochloride and Teneligliptine using optimum conditions was 2.14min and 2.95min respectively. For two of them, the peak symmetries were < 2 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Metformin hydrochloride and Teneligliptine was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in **Table 1.**

System suitability:

Table 1: System suitability results of Metformin Hydrochloride and Teneligliptine

Parameter	Metformin Hydrochloride	Teneligliptine	Pioglitazone
Peak area	1252610±0.91*	246069±0.55*	132489±0.82*
Theoretical plates	3601±0.59*	3348±0.62*	3616±0.87*
Retention time	2.14±0.10*	2.95±0.17*	5.09±0.23*
Tailing factor	1.43±0.40*	1.46±0.30*	1.30±0.20*

*RSD (%)

Specificity:

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Metformin Hydrochloride, Teneligliptine and pioglitazone is shown in Fig. 1 clearly shows the ability of the method to assess the analyte in the presence of other excipients.

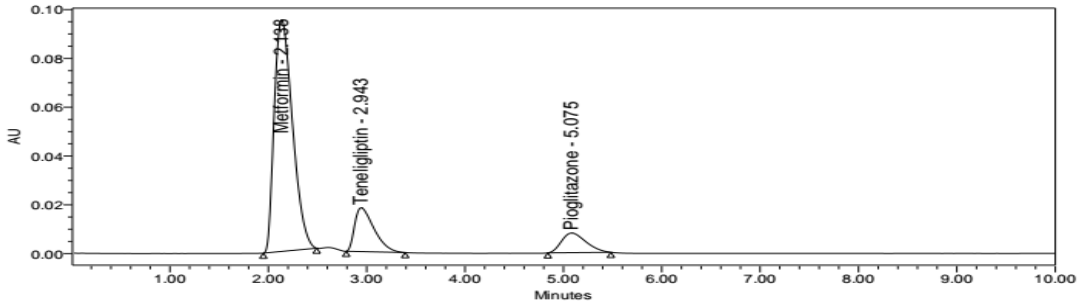

Fig. 1: Optimized Chromatogram of Metformin Hydrochloride and Teneligliptine

Precision:

System Precision: One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2) as shown in the **Table 2** below.

Table 2: System Precision data for Metformin Hydrochloride and Teneligliptine

	Metformin Hydrochloride (360µg/mL)			**Teneligliptine (** 14.4µg/mL)		
S. No	**Retention time (min)**	**Peak Area**	**P/A Ratio**	**Retention time (min)**	**Peak Area**	**P/A Ratio**
1	2.138	1259895	9.214	2.943	245767	1.797
2	2.141	1258504	9.204	2.948	247537	1.810
3	2.142	1239432	9.065	2.953	244903	1.791
4	2.136	1254922	9.178	2.945	245248	1.794
5	2.141	1238504	9.058	2.948	247537	1.810
6	2.148	1249688	9.140	2.959	249083	1.822
Avg	2.14	1250157	9.14	2.95	246679	1.80
SD	0.004	9362.10	0.07	0.01	1630.04	0.01
%RSD	0.19	0.75	0.75	0.20	0.66	0.66

Table 3: Method Precision data for Metformin Hydrochloride and Teneligliptine

Metformin Hydrochloride (360µg/mL)				**Teneligliptine (** 14.4µg/mL)		
S No	Peak Area	P/ A Ratio	% Assay	Peak Area	P/ A Ratio	% Assay
1	1240301	9.07	98.82	247408	1.81	100.34
2	1263614	9.24	100.68	244462	1.79	99.15
3	1249432	9.14	99.55	245903	1.80	99.73
4	1255394	9.18	100.02	249425	1.82	101.16
5	1268091	9.27	101.03	248937	1.82	100.96
6	1251451	9.15	99.71	244110	1.79	99.01
Avg	1254714	9.18	99.97	246708	1.80	100.06
SD	10048.9	0.07	0.80	2248.7	0.02	0.91
%RSD	0.80	0.80	0.80	0.91	0.91	0.91

Method Precision(Repeatability):

Six replicate injections of a known concentration of sample preparation of Metformin Hydrochloride (360 µg/mL) and Teneligliptine (14.4 µg/mL) have been analyzed by injecting them into a HPLC column on the same day . From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in Table 3.

Ruggedness:

Intermediate precision was accessed injecting sample preparation of Metformin Hydrochloride (360µg/mL) and Teneligliptine (14.4 µg/mL) in six replicates in to HPLC column on the same day and on consecutive days and in different laboratories by different analysts . Results were found within the acceptance limits (RSD<2) as shown in the **Tables 4,5** below.

Table 4: Ruggedness Data for Metformin Hydrochloride

Laboratory-1 (% Assay)-HPLC-1					**Laboratory-2 (% Assay)-HPLC-2**			
Concentration (µg/ml)	**Analyst-1**		**Analyst-2**		**Analyst-1**		**Analyst-2**	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
360	99.14	99.48	99.76	99.54	99.34	99.46	100.02	99.68
360	100.12	100.02	99.14	99.43	99.45	99.34	99.24	100.12
360	99.23	99.56	99.64	99.68	99.82	99.1	99.72	99.25
360	99.68	99.64	100.41	100.32	99.54	99.54	99.84	99.45
360	100.42	99.48	100.17	100.41	100.32	100.18	99.95	100.15
360	99.13	100.41	100.12	99.87	100.3	100.21	99.67	99.29
Avg	99.62	99.77	99.87	99.88	99.80	99.64	99.74	99.66
SD	0.55	0.37	0.46	0.41	0.43	0.46	0.28	0.40
%RSD	0.55	0.38	0.46	0.41	0.43	0.46	0.28	0.40

Table 5 :Ruggedness Data for Teneligliptine

Laboratory-1 (% Assay)-HPLC-1					**Laboratory-2 (% Assay)-HPLC-2**			
Concentration (µg/ml)	**Analyst-1**		**Analyst-2**		**Analyst-1**		**Analyst-2**	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
14.4	99.24	99.42	99.24	99.34	99.48	99.92	99.14	99.12
14.4	100.1	99.64	99.48	99.68	99.94	99.54	99.46	99.84
14.4	99.87	100.11	99.65	99.54	99.43	99.18	99.2	99.24
14.4	99.38	99.89	100.14	99.26	99.98	99.24	99.98	99.16
14.4	99.56	100.32	99.84	100.32	99.49	99.91	100.12	99.94
14.4	100.04	100.34	99.64	100.24	99.14	99.21	99.73	100.12
Avg	99.70	99.95	99.67	99.73	99.58	99.50	99.61	99.57
SD	0.36	0.37	0.31	0.45	0.32	0.35	0.41	0.45
%RSD	0.36	0.37	0.31	0.45	0.33	0.35	0.41	0.45

Table 6: Recovery data of Metformin hydrochloride

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	200	200.75	100.37	Mean = 100.25%(n=3) SD =0.164 %RSD = 0.163
S2:50%	200	200.13	100.06	
S3:50%	200	200.63	100.31	
S4:100%	400	399.94	99.98	Mean = 99.41%(n=3) SD =0.668 %RSD = 0.672
S5:100%	400	394.70	98.68	
S6:100%	400	398.27	99.57	
S7:150%	600	595.71	99.28	Mean = 99.53%(n=3) SD =0.319 %RSD = 0.321
S8:150%	600	599.33	99.89	
S9:150%	600	596.43	99.41	

Table 7: Recovery data of Teneligliptine

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	8	8.07	100.82	Mean = 100.74%(n=3) SD =0.520 %RSD =0.516
S2:50%	8	8.01	100.19	
S3:50%	8	8.10	101.22	
S4:100%	16	15.91	99.47	Mean =100.29%(n=3) SD =0.782 %RSD =0.780
S5:100%	16	16.06	100.40	
S6:100%	16	16.16	101.02	
S7:150%	24	23.88	99.51	Mean = 99.83%(n=3) SD =0.613 %RSD =0.614
S8:150%	24	23.87	99.44	
S9:150%	24	24.13	100.54	

Accuracy:
A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Metformin hydrochloride and Teneligliptine was achieved between 99.41–100.25 and 99.83-100.74 respectively. The results are given in **Tables 6,7**.

Linearity and Range:
Linearity was assessed for the two oral anti diabetic drugs at concentration ranges 2.4- 600µg/ml for Metformin Hydrochloride and 0.51-24µg/ml for Teneligliptine. The overlay of Chromatograms of all concentrations are shown in **Fig.2**. The peak area ratio of drug and internal standard was found to be linear in the above concentration range for both the drugs. Good linearity was proved by high values of coefficient of determinations (**Fig.3 and Fig.4**). The results were tabulated in **Table 8**

Table 8: Linearity data of Metformin Hydrochloride and Teneligliptine

Concentration of Metformin (µg/ml)	Peak area	P/ A Ratio	Concentration of Teneligliptine (µg/ml)	Peak area	P/ A Ratio
2.4	8393	0.061	0.51	5751	0.042
120	429471	3.141	4.8	81909	0.599
240	850557	6.221	9.6	162295	1.187
360	1288733	9.425	14.4	243466	1.781
480	1719868	12.578	19.2	322320	2.357
600	2178273	15.931	24.0	407262	2.979

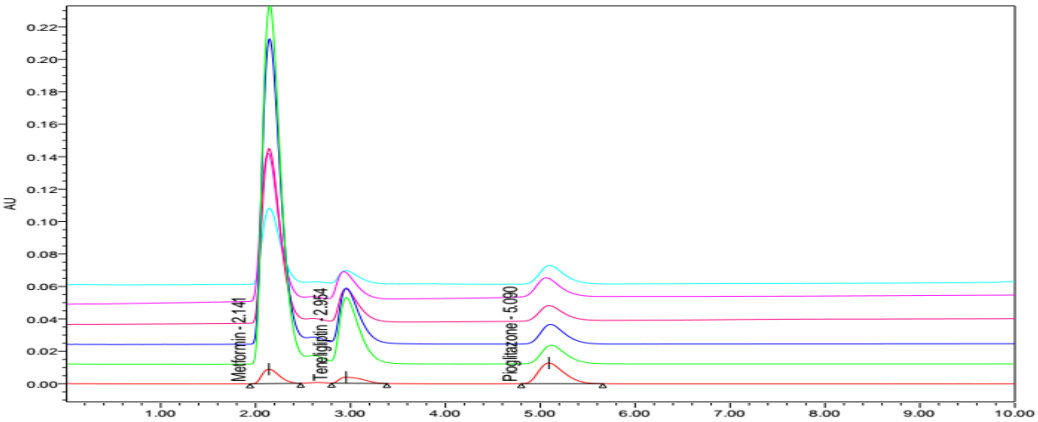

Fig.2. Overlay of Linearity Chromatograms

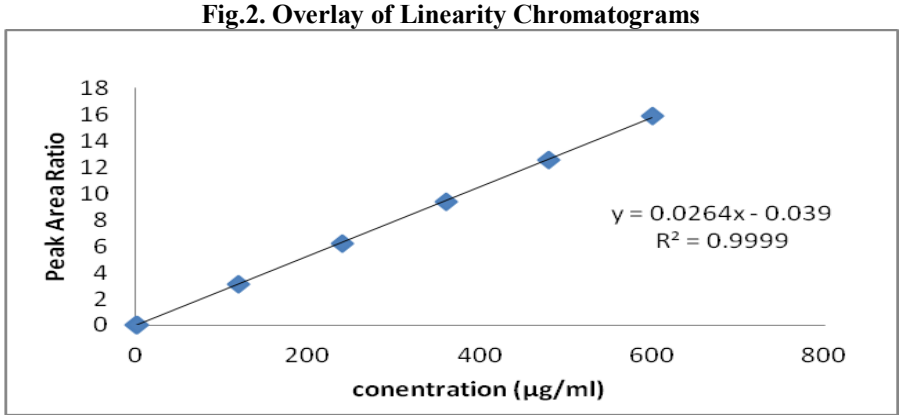

Fig.4. Linearity graph of Metformin Hydrochloride

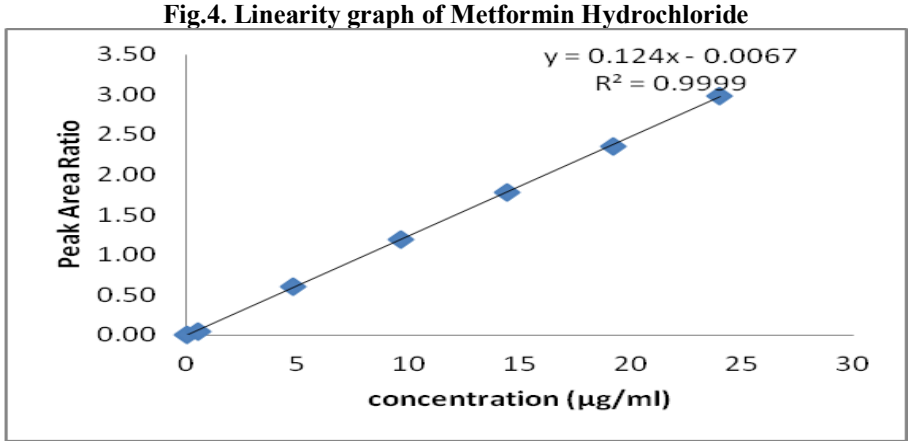

Fig.5. Linearity graph of Teneligliptine

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The limit of detection and limit of quantification were evaluated by serial dilutions of Metformin Hydrochloride and Teneligliptine stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Metformin Hydrochloride and Teneligliptine was found to be 0.72 µg/mL and 0.15 µg/mL, respectively, and the LOQ value 2.40 µg/mL and 0.51µg/mL, respectively.

Robustness:

The result of robustness study of the developed assay method was established in **Table 9**. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Table 9: **Robustness for Metformin Hydrochloride and Teneligliptine**

Drug	Change in flow rate	% Assay	Change in organic composition in mobile phase	% Assay
Metformin Hydrochloride	0.6	100.30	10% less	99.58
	0.8	99.41	Actual	99.41
	1.0	101.11	10% More	100.38
Teneligliptine	0.6	100.90	10% less	99.05
	0.8	100.29	Actual	100.29
	1.0	100.07	10% More	100.40

Table 10: Forced Degradation studies of Metformin Hydrochloride

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	-	0.624	0.967
Thermal Stress Sample	4.03	0.379	1.146
Photolytic Stress Sample	4.81	0.726	2.545
Acid Degradation	5.44	0.589	1.619
Alkali Degradation	4.58	0.865	3.529
Peroxide Degradation	7.24	0.426	0.964

Table 11: Forced Degradation studies of Teneligliptine

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	-	0.592	0.710
Thermal Stress Sample	7.23	0.746	0.979
Photolytic Stress Sample	3.43	0.714	1.268
Acid Degradation	3.62	0.428	0.574
Alkali Degradation	6.19	0.785	1.563
Peroxide Degradation	4.38	0.825	1.396

Forced degradation studies:
The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out for acid hydrolysis (1M HCl heated for 30 min at 60°C), alkali hydrolysis (2 N NaOH heated for 30 min at 60°C), oxidative degradation (20%H2O2 heated at 60°C for 30 min) and thermal degradation (samples placed in an oven at 105°C for 6 h). For photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 7 days. Results are shown in **Tables 10,11**.

The retention time of Metformin Hydrochloride and Teneligliptine was found to be 2.138 min and 2.943 min respectively with resolution of 2.28. Linearity was established for Metformin Hydrochloride and Teneligliptine in the range of 9.98-600µg/ml for Metformin Hydrochloride and 0.51-24µg/ml for Teneligliptine with correlation coefficients (r^2=0.999) and the percentage recoveries were between 99.41 % to 100.25% and 99.83% to 101.74% for Metformin Hydrochloride and Teneligliptine respectively, which indicate accuracy of the proposed method. The % RSD values of accuracy for Metformin Hydrochloride and Teneligliptine were found to be < 2 %. The % RSD values of method precision are 0.80% and 0.91% for Metformin Hydrochloride and Teneligliptine respectively and % RSD values of system precision are 0.75% and 0.66% for Metformin Hydrochloride and Teneligliptine. The % RSD values of reproducibility for Metformin Hydrochloride and Teneligliptine were found to be < 2 %, reveal that the proposed method is precise. LOD values for Metformin Hydrochloride and Teneligliptine were found to be 0.72µg/ml and

0.15µg/ml respectively and LOQ values for Metformin Hydrochloride and Teneligliptine were found to be 2.40µg/ml and 0.51µg/ml respectively. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough was shown in (Table 9). These data show that the proposed method is specific and sensitive for the determination of Metformin Hydrochloride and Teneligliptine.

CONCLUSIONS:

1. RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Teneligliptine in their combine dosage form was developed and validated as per the ICH guidelines.
2. Linearity was observed in the range of 9.98-600µg/ml for Metformin Hydrochloride and 0.51-24µg/ml for Teneligliptine with correlation coefficients (r^2=0.999).
3. The percentage recoveries of Metformin Hydrochloride and Teneligliptine were in the range of 99.41-100.74% which was with in the acceptance criteria.
4. The percentage RSD was NMT 2% which proved the precision of the developed method.
5. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

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