RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TENELIGLIPTINE HYDROBROMIDE HYDRATE (TEN) AND METFORMIN HYDROCHLORIDE (MET) IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, reproducible and economic method developed and validated for the simultaneous estimation of teneligliptine hydrobromide hydrate (TENE) and metformin hydrochloride (MET HCl) in pharmaceutical dosage form. TENE and MET HCl were estimated on Thermoscientific C_{18} column using mobile phase 0.01M PDP: methanol (45:55 % V/V) (pH 3.5 adjusted with 5% acetic acid) at flow rate 1.0 mL/min. Detection was carried out at 254 nm. The retention time of teneligliptine hydrobromide hydrate and metformin hydrochloride were 7.77 min and 2.64 min, respectively. The linearity was found to be 4-12 µg/mL and 100-300 µg/mL for TENE and MET HCl respectively. R^2 value was found to be 0.998 and 0.995. For the assay method % recovery was found in the range of 98.16 – 101 for TENE and MET HCl. The LOD and LOQ were found to be 0.3527 and 1.0690 for TENE and 0.5077 and 1.538 for MET HCl respectively. Method was validated as per ICH guidelines.

INTRODUCTION

Diabetes is a group of metabolic diseas in which a person suffers with high blood glucose (blood sugar), either because of low insulin production or because of body's cells inability to produce insulin properly, or both. A combination of teneligliptin hydrobromide hydrate (**Scheme 1a**) and metformin hydrochloride (**Scheme 1b)** produces synergistic effect in diabetes[1,5,6]. Teneligliptin hydrobromide hydrate is a dipeptidyl peptidase 4 (DPP-4) inhibitor. It is highly potent, competitive and long lasting DPP-4 inhibitor[2]. Metformin hydrochloride is an antihyperglycemic agent which lowers both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production and intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization[3].

MATERIALS AND METHODS

Teneligliptine hydrobromide hydrate was kindly gifted by Glenmark Pharmaceuticals Ltd., Mumbai, Metformin hydrochloride procured from Norris Medicines Ltd., Ankleshwar. Methanol (HPLC Grade), acetic acid
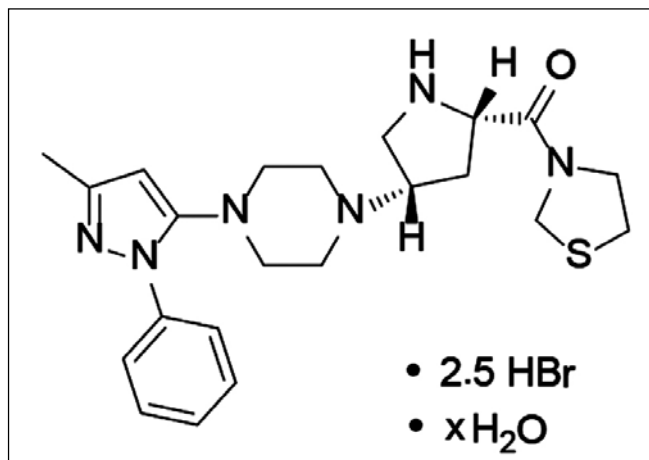
Scheme 1a: Structure of Teneligliptin hydrobromide hydrate[2]
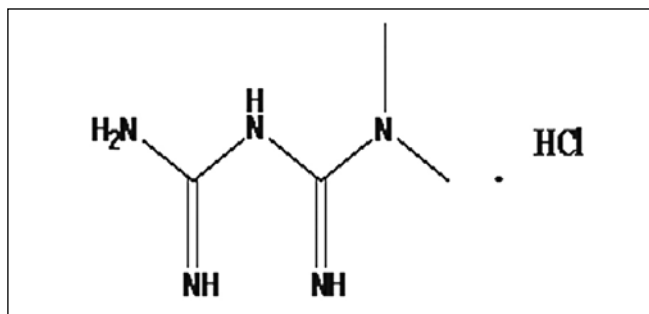
Scheme 1b: Structure of Metformin hydrochloride[4]

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(HPLC Grade) and water (HPLC Grade) were procured from Fischer Scientific, Mumbai. Potassium dihydrogen phosphate procured from Merck, Mumbai

METHOD DEVELOPMENT[7]

Selection of Wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. Sample solutions of metformin hydrochloride (100 μg/mL) and (4 μg/mL) were scanned separately in the range of 200-400 nm. Data was obtained by overlay spectra of both drugs. Detection wavelength was 254 nm, at which both drugs gives absorbance.

Selection of Mobile Phase

Depending upon solubility of the drugs, various solvents were tried as mobile phase for separation of teneligliptine hydrobromide hydrate and metformin hydrochloride.

PREPARATION OF SOLUTIONS

Preparation of Stock Solution for Teneligliptine hydrobromide hydrate and Metformin hydrochloride

Accurately weighed 25 mg of teneligliptine hydrobromide hydrate and 25 mg metformin hydrochloride was transferred separately into 25 mL volumetric flask, dissolved and diluted up to mark with methanol. It gives a stock solution having concentration of 1000μg/mL teneligliptine hydrobromide hydrate and 1000 μg/mL of metformin hydrochloride respectively.

Preparation of Working Standard Solution for Teneligliptine hydrobromide hydrate and Metformin hydrochloride

From the stock solution of TENE (1000 μg/mL), 0.4, 0.6, 0.8, 1.0 and 1.2 mL of aliquots were transferred in five different 10 mL volumetric flask and from the stock solution of MET HCl (1000 μg/mL), 1.0, 1.5, 2.0, 2.5 and 2.5 mL aliquots were transferred in five different 10 mL volumetric flask and volume was made up to mark with the methanol to prepare 4, 6, 8, 10 and 12 μg/mL of the TENE and 100, 150, 200, 250 and 300 μg/mL of the MET HCl.

METHOD VALIDATION

System suitability

System suitability parameters like theoretical plates, tailing factor and retention time were studied by injecting 6 replicates of standard concentration (8 μg/mL of TENE and 200 μg/mL of MET HCl) and then % RSD for retention time was calculated and other parameter measured.

Specificity

Specificity of the method was observed by blank, standard solution and sample solution. There was no interference of blank, sample and other impurities at retention time of each standard.

Linearity and Range

The calibration curves were potted over the con-centration range and the linear response was observed over a range of 4-12 μg/mL for TENE and 100-300 μg/mL for MET HCl. The calibration curves of peak area against concentration were plotted. Correlation coefficient and regression line equations for TENE and MET HCl were calculated.

PRECISION

A) Repeatability

The data for repeatability of area measurement for TENE (8 μg/mL) and MET HCl (200 μg/mL) based on six measurements of same solution of TENE and MET HCl % RSD was calculated.

B) Intraday Precision

Three replicates of three concentrations of standard solution of TENE (6, 8 and 10 μg/mL) and MET HCl (150, 200 and 250 μg/mL), making a total of nine determinations were analyzed at three consecutive times on the same day and peak area was measured at 254 nm for TENE and MET HCl and % RSD was calculated.

INTERMEDIATE PRECISION

Interday Precision

Three replicates of three concentration of standard solution of TENE (6, 8 and 10 μg/mL) and MET HCl (150, 200 and 250 μg/mL), making a total of nine determination were analyzed at three consecutive days and peak area was measured at 254 nm for TENE and MET HCl and % RSD was calculated.

Robustness

➢ The robustness of the analytical method was established by few parameters being deliberately varied. The parameters included change in flow rate, pH and change in wavelength. The change was made at 3 levels and replicates for three times and % RSD was calculated.

Limit of Detection

➢ The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

$$LOD = 3.3(SD/Slope)$$

where, SD = standard deviation of the Y- intercepts of the 5 calibration curves

Slope= Mean slope of the 5 calibration curves.

Limit of Quantification

➢ The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

$$LOQ = 10 (SD/Slope)$$

where, SD= standard deviation of the Y- intercepts of the 5 calibration curves

Slope= Mean slope of the 5 calibration curves

Accuracy

➢ The accuracy of the method was determined by calculating % recovery of Tenegliptine hydrobromide hydrate and Metformin hydrochloride by standard addition method. Known amount of standard solutions of TENE and MET HCl corresponding to 50, 100 and 150% of target concentration were spiked with preanalyzed sample solution. The amounts of TENE and MET HCl were estimated by applying obtained values to regression equation of calibration curve.

Analysis of marketed formulation[9]

➢ The sample solution of drug product was measured at 254 nm under the optimized chromatographic conditions for quantitation of TENE and MET HCl. The total amount of TENE and MET present in sample solution were calculated.

Preparation of sample solution[4,9]

The average weight of 20 tablets was calculated and the tablets powdered. Weight equivalent to 1mg of tenegliptine hydrobromide hydrate and 25mg of metformin hydrochloride was transferred in 100mL volumetric flask. The volume was adjusted with methanol up to the mark. The solution then filtered through Whatman filter paper. Resulting solution was having concentration 10μg/mL and 250μg/mL of tenegliptine hydrobromide hydrate and metformin hydrochloride, respectively. 6mL from above solution was pipette out to get the final concentration 6μg/mL of TENE and 150μg/ml of MET HCl and measure the area was measured at 254nm. % recovery was calculated.

RESULTS AND DISCUSSION

METHOD VALIDATION[7,8]

Validation of the Proposed Method

The proposed method was validated according to the International Council on Harmonization (ICH) guideline Q2 (R1).

Linearity and Range

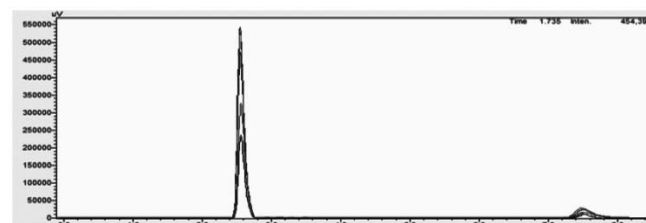The linearity for TENE and MET HCl was found to be in the range of 4 to 12μg/mL and 100 to 300μg/mL, respectively.

Fig.1: Overlay chromatogram of TENE and MET HCl

Table I: Linearity data for TENE

Concentration of TENE (μg/mL)	Peak area
4	201935
6	278508
8	347055
10	422273
12	480140
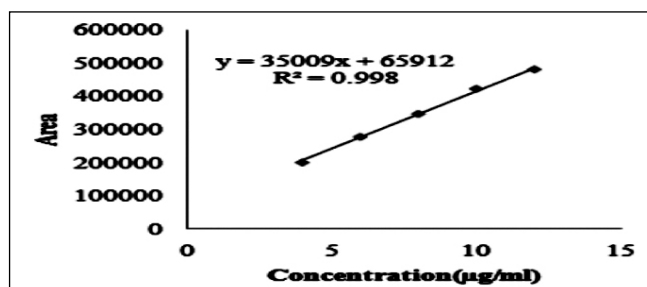
Fig. 2: Calibration curve of TENE

Table II: Linearity data for MET HCl

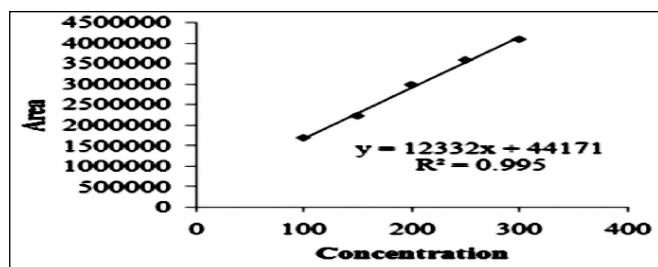| Concentration of MET HCl (μg/mL)	Peak area
100	1682358
150	2216188
200	2978033
250	3580848
300	4082985

Fig. 3: Calibration curve of MET HCl

PRECISION

Repeatability

The data for repeatability of area measurement for TENE (8µg/mL) and MET HCl (200µg/mL) based on six measurements of same solution of TENE and MET HCl. % RSD was calculated and data given in Table III.

Table III: Repeatability Data of TENE (6 µg/mL) and MET HCl (150 µg/mL)

Sr. No.	Peak Area	
	TENE	MET HCl
1	347055	2978033
2	346089	2960203
3	345290	2975564
4	347560	2982560
5	345922	2985634
6	346033	2965234
Mean peak area	346324.8333	2974538
SD	828.5793	9927.7191
% RSD	0.2392	0.3337

Table IV: Intraday precision data of TENE and MET HCl

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		I	II	III			
TENE	6	278508	277095	275398	277000.333	1557.159	0.562
	8	347055	345545	346212	346270.666	756.707	0.218
	10	422273	421280	419820	421124.333	1233.886	0.292
MET HCl	150	2216188	2192980	2205289	2204819	11611.136	0.526
	200	2978033	2899123	2940154	2939103.333	39465.490	1.342
	250	3580848	3512924	3490928	3528233.333	46874.122	1.328

Table V: Inter day precision data of TENE and MET HCl

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		Day 1	Day 2	Day 3			
TENE	6	278508	279922	276252	278227.333	1851.028	0.665
	8	347055	346012	347562	346876.333	790.295	0.227
	10	422273	419820	423572	421888.333	1905.348	0.451
MET HCl	150	2216118	2210250	2198990	2208452.667	8704.303	0.394
	200	2978033	2999987	2988038	2988686	10991.335	0.367
	250	3580848	3610329	3570288	3587155	20752.207	0.578

Table VI: Different wavelength data for TENE and MET HCl

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		253 nm	254 nm	255 nm			
TENE	6	275320	278508	276052	276626.666	1669.885	0.603
	8	349625	347055	346020	347566.666	1856.167	0.534
	10	421280	422273	425037	422863.333	1946.826	0.460
MET HCl	150	2199938	2216188	2210238	2208788	8221.465	0.372
	200	2953140	2978033	2892960	2941377.667	43739.206	1.487
	250	3598734	3580848	3560952	3580178	18899.908	0.527

Table VII: Different flow rates data for TENE and MET HCl

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		0.9 mL/min	1.0 mL/min	1.1 mL/min			
TENE	6	281011	278508	279035	279518	1319.552	0.472
	8	345012	347055	351028	347698.333	3059.162	0.879
	10	419920	422273	425089	422427.333	2587.953	0.612
MET HCl	150	2190988	2216188	2250238	2219138	29734.954	1.339
	200	2950013	2978033	2990132	2972726	20579.279	0.692
	250	3540328	3580848	3510526	3543900.667	35296.867	0.995

Table VIII: Different pH data for TENE and MET HCl

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		pH 3.4	pH 3.5	pH 3.6			
TENE	6	275013	278508	272050	275190.3333	3232.650	1.174
	8	342088	347055	351020	346721	4475.357	1.290
	10	429076	422273	427088	426145.6667	3498.027	0.820
MET HCl	150	2199920	2216188	2250268	2222125.333	25693.758	1.156
	200	2930123	2978033	2980327	2962827.667	28346.287	0.956
	250	3520624	3580848	3590602	3564024.667	37901.167	1.063

Intraday precision

The data for intraday precision of area measurement for standard solution of TENE (6, 8 and 10 µg/mL) and MET HCl (150, 200 and 250 µg/mL), total nine determinations, were analyzed at three consecutive times on same day and %RSD was calculated and depicted in Table IV..

INTERMEDIATE PRECISION

Inter day precision

The data for inter day precision of standard solution of TENE (6, 8 and 10 µg/mL) and MET HCl (150, 200 and 250 µg/mL), total nine determinations, were analyzed on three consecutive days and %RSD was calculated and depicted in Table V.

ROBUSTNESS

Different wavelength

Robustness carried out by changing wavelength, flow rate and pH. %RSD was calculated for TENE and MET HCl. Robustness data given in Table VI, VII and VIII.

LOD and LOQ

Calibration curves were repeated for five times and standard deviation of intercept was calculated, then LOD and LOQ were calculated and depicted in Table IX.

Table IX: Data of LOD and LOQ

Parameter	TENE	MET HCl
SD of the Y-Intercepts of 5 Calibration curve	2611.221	6542.4512
Mean slope of 5 calibration curve	24426	42519
LOD(µg/mL)	0.3527	0.5077
LOQ(µg/mL)	1.0690	1.5387

ACCURACY

From marketed formulation at three levels of standard addition accuracy of the method was confirmed by recovery study. % Recovery of TENE and MET HCl were found to be between 98% to 102%. Accuracy data given in Table X.

%Assay

Analysis of proposed method was carried out and % assay was calculated.
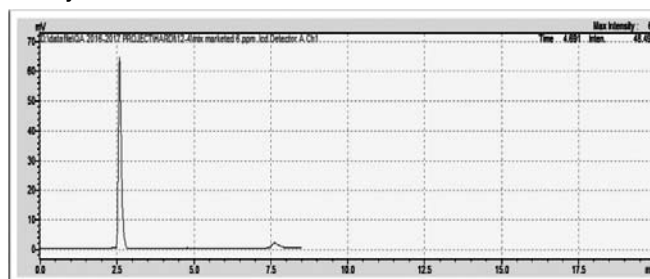
Fig. 4: Chromatogram of formulation TENE (6µg/mL) and MET HCl (150µg/mL)

Table X: Accuracy data for TENE and MET HCl

Drug	% Level	Amt. of Sample taken (µg/mL)	Amt. of Standard spiking (µg/mL)	Total Amt. (µg/mL)	Conc. Found (µg/mL)	% Recovery
TENE	I (50%)	4	2	6	5.92	98.66
		4	2	6	5.89	98.16
		4	2	6	6.02	100.33
TENE	II (100%)	4	4	8	8.10	101.25
		4	4	8	7.99	99.87
		4	4	8	8.03	100.37
TENE	III (150%)	4	6	10	9.93	99.30
		4	6	10	9.95	99.50
		4	6	10	10.10	101
MET HCl	I (50%)	100	50	150	150.10	100.06
		100	50	150	149.98	99.98
		100	50	150	149.85	99.90
MET HCl	II (100%)	100	100	200	200.10	100.05
		100	100	200	200.01	100
		100	100	200	199.89	99.94
MET HCl	III (150%)	100	150	250	249.78	99.91
		100	150	250	249.89	99.95
		100	150	250	250.10	100.40

Table XI: Analysis of TENIVA-M tablet

Sample	Label Claim%		Amt. Found		%Assay	
	TENE (mg/tab)	MET HCl (mg/tab)	TENE (mg/tab)	MET HCl (mg/tab)	TENE	MET HCl
1	20	500	19.65	496.95	98.25	99.39
2	20	500	19.62	495.60	98.10	99.12
3	20	500	19.79	500.85	98.95	100.17
4	20	500	20.01	495.05	100.05	99.01
5	20	500	19.93	497.82	99.65	99.56
6	20	500	20.30	498.99	101.50	99.79

RESULTS AND DISCUSSION

Teneligliptine hydrobromide hydrate and Metformin hydrochloride were estimated on Thermoscientific, C_{18} column using 0.01M PDP:Methanol (45:55 V/V) (pH 3.5) adjusted with 5% acetic acid as mobile phase, flow rate 1.0 mL/min. Detection was carried out at 254 nm. The retention time of teneligliptine hydrobromide hydrate and metformin hydrochloride was 7.77 min and 2.64 min, respectively.

The linearity range was found to be 4-12 µg/mL for teneligliptine hydrobromide hydrate and 100-300 µg/mL for Metformin hydrochloride. The correlation coefficient was found to be 0.998 and 0.995 for TENE and MET HCl. % RSD of Repeatability, intraday and intermediate precision was Found to be less than 2%. % RSD for robustness parameters (flow rate change, detection wavelength change and pH change) was found to be less than 2%. So, the developed method was precise and robust. For the method % recovery was found in the range of 98.16 – 101 for TENE and MET HCl 99.98-100.40 % in tablet dosage form.

The LOD and LOQ for TENE were found to be 0.3527 and 1.0690 and for MET HCl were found to be 0.5077 and 1.538, respectively.

CONCLUSIONS

Both the drugs showed better solubility and stability in methanol. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed method is new, simple, accurate, sensitive, economic and precise and can be adopted for routine analysis and in tablet dosage form.

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