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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENELIGLIPTIN AND METFORMIN HCL BY USING RP-HPLC METHOD

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ABSTRACT

A new, simple, precise, accurate and reproducible rp-hplc method for simultaneous estimation of bulk and Pharmaceutical formulations. Separation of Teneligliptin and Metformin Hcl was successfully achieved by using column like thermo, C_{18}, 250 × 4.6mm, 5µm or equivalent in an isocratic mode utilizing 0.1M KH_2PO_4:Methanol (60:40) at a flow rate of 1.0ml/min and eluate was monitored at 280nm with a retention time of 4.421 and 3.421 minutes for teneligliptin and metformin Hcl respectively. The method was validated and their response was found to be linear in the drug concentration range of 50µg/ml to150µg/ml for teneligliptin and 50µg/ml to150µg/ml for metformin Hcl. The values of the correlation coefficient were found to 0.999 for teneligliptin and 1 for metformin Hcl. The LOD and LOQ for teneligliptin were found to be 0.2725 and 0.9085 respectively. The LOD and LOQ for metformin Hcl were found to be 0.801 and 2.671 respectively. This method was found to be good percentage recovery which indicates that the proposed method is highly accurate. This method was extensively validated according to ICH guidelines for accuracy, precision, linearity, robustness and system suitability.

INTRODUCTION

Metformin (dimethyl biguanide) is an orally administered drug used to lower blood glucose concentrations in patients with non-insulin-dependent diabetes mellitus (NIDDM).[1] Metformin hydrochloride chemically, N,Ndimethylimidodicarbonimidic diamide hydrochloride is an antidiabetic agent[2,3] which acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity.[4]
Teneligliptin is an antidiabetic drug that belongs to dipeptidyl peptidase-4 inhibitors or gliptins.[5] Chemically, it is {(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]-2-pyrrolidinyl}(1,3-thiazolidin-3-yl)methanone. Teneligliptin exerts its activity for 24hrs with elevation of activated glucagon-like peptide-1 (GLP-1) levels by suppressing postprandial hyperglycemia after the meals.[6,7,8] 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.[6] The combination of these two drugs provides good control over diabetic patients.[9] The objective of the present study is to develop a simple, accurate, precise and selective rp-hplc method for simultaneous determination of teneligliptin and metformin Hcl from bulk forms available in the market.

MATERIALS AND METHOD

Chemicals and Reagents

Reference standard of teneligliptin and metformin Hcl were gifted by Hetero laboratory. The formulation used for assay is teneligliptin and metformin Hcl and the solvents used in this method were potassium dihydrogen phosphate, methanol, water (hplc grade).

Instrumentation

HPLC Model: Waters e2695, Photo diode array detector (PDA) with an automated sample injector. The output signal was monitored and integrated using Empower-2 software by using column like thermo, C18 column (150*4.6 * 5µm) was used for separations.

Preparation of mobile phase

Transfer HPLC water into a 1000ml beaker and add 0.1M KH_2PO_4. Transfer the above prepared KH_2PO_4 buffer and methanol in the ratio of 60:40 into a 1000ml beaker. They are thoroughly mixed and sonicated for 20 minutes.

Preparation of standard solution

Accurately weigh and transfer 500mg of Metformin Hcl and 200mg Teneligliptin into 50ml of volumetric flask by adding 10ml of methanol and sonicate for 10min and finally makeup the volume with methanol. Transfer the above solution into 1ml of 10ml volumetric flask and dilute the volume with methanol.

Preparation of sample stock solution

Commercially available 20 tablets were weighed and powdered. The powders is equivalent to 785mg of Metformin Hcl and Teneligliptin of active ingredients and transferred into 50ml of volumetric flask and adds 10ml of Methanol and sonicate for 20min (or) shake for 10min and makeup with methanol. Transfer the above solution into 1ml of 10ml volumetric flask and dilute the volume with methanol. Finally the solution was filtered through 0.45µm filter before injecting into HPLC system.

RESULTS AND DISCUSSION

System suitability: Results of system suitability study are summarized in the above table. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis. Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drugs were compared against the recovery amount. Percentage recovery was 100.00% for Metformin Hcl and 100.00% for Teneligliptin. All the results indicate that the method is highly accurate**.** Results of variability were summarized in the above table. % RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves this method is precise. A linear relationship between peak areas versus concentrations was observed for metformin Hcl and teneligliptin in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both metformin Hcl and teneligliptin which proves that the method is linear in the range of 50% to 150%.

Robustness: The results of Robustness of the present method have shown that changes made in the flow rate and temperature did not produce significant changes in analytical results which were presented in the above table. As the changes were not significant we can say that the method is robust.

SUMMARY AND CONCLUSION

The objective of the present work is to develop and validate a HPLC method for teneligliptin and metformin Hcl in tablets. To employ in routine analysis, method development of teneligliptin and metformin Hcl was carried out by incorporating reverse phase high performance liquid chromatography. Then the developed method will be validated according to ICH guidelines for its various parameters. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. The method shows good reproducibility and good percentage recovery. No economic, simple and precise HPLC method was there for simultaneous estimation of teneligliptin and metformin Hcl in bulk and pharmaceutical dosage forms.

Table 1: System suitability data of Teneligliptin and Metformin Hcl

Parameter	Teneligliptin	Metformin Hcl	Acceptance criteria
Retention time	4.421	3.421	+-10
Theoretical plates	4021	3072	>2500
Tailing factor	1.36	1.40	<2.00
% RSD	0.3	0.2	<2.00

Accuracy

Table 2 : Accuracy data for Teneligliptin

S.NO	Accuracy Level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	392.50	19.800	19.84	99	100
		2	392.50	19.800	19.86	100	
		3	392.50	19.800	19.87	100	
2	100%	1	785.00	39.600	39.71	100	100
		2	785.00	39.600	39.71	100	
		3	785.00	39.600	39.61	99	
3	150%	1	1177.50	59.400	59.41	100	100
		2	1177.50	59.400	59.73	101	
		3	1175.50	59.400	59.47	100	

Precision

Table 3(a) : Precision data for Teneligliptin

S.no.	Rt	Area	%Assay
injection1	4.411	1058048	99
injection 2	4.413	1051011	99
injection 3	4.408	1050635	99
injection 4	4.415	1053330	99
injection 5	4.409	1050440	99
injection 6	4.411	1052503	99
Mean			99
Std. Dev.			0.27
%RSD			0.27

Table 3(b) : Precision data for Metformin Hcl

S.no.	Rt	Area	%Assay
injection1	3.419	8143281	99
injection2	3.421	8146100	99
injection3	3.416	8140121	99
injection4	3.422	8141679	99
injection5	3.418	8147232	100
injection6	3.418	8149944	100
Mean			99
Std. Dev.			0.05
% RSD			0.05

Linearity

Table 4(a) :Linearity data for Teneligliptin

S.no	Conc. (µg/ml)	Rt	Area
1	50	4.416	528142
2	75	4.412	792233
3	100	4.405	1059322
4	125	4.393	1326200
5	150	4.390	1585843
Correlation coefficient (r^2)			1.00

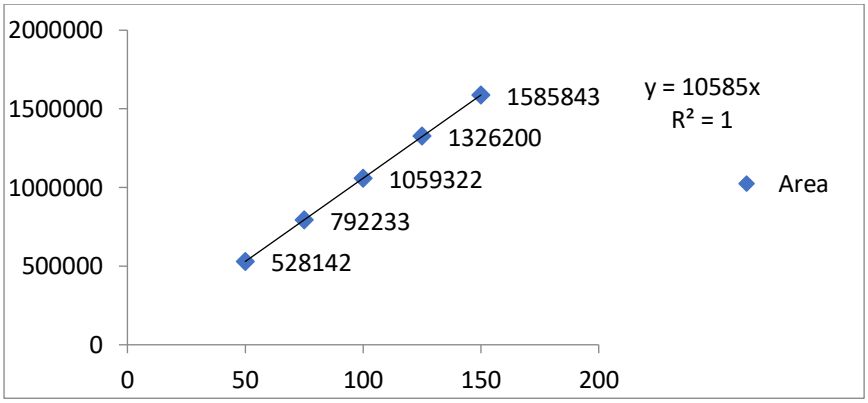

Fig.1(a) : Linearity plot of Teneligliptin

Table 4(b) :Linearity data for Metformin Hcl

S.no	Conc. (µg/ml)	Rt	Area
1	50	3.424	4079277
2	75	3.424	6118128
3	100	3.419	8148132
4	125	3.409	10181926
5	150	3.409	12290033
Correlation coefficient (r^2)			0.999

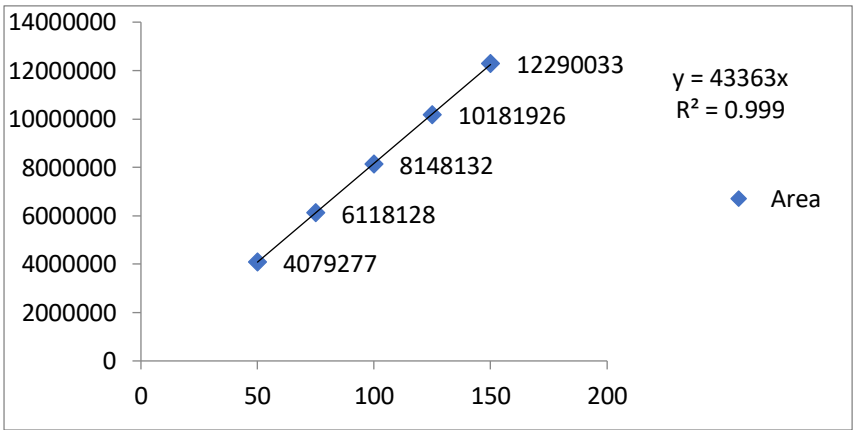

Fig.1(b): Linearity plot of Metformin Hcl

Table 5(a) : Robustness data for Teneligliptin

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	5.451	4144	1.36
Increased flow rate (1.2ml/min)	3.656	3983	1.30
Decreased temperature(20^0c)	5.461	4082	1.36
Increased temperature(30^0c)	3.674	3998	3.68

Table 5(b) :Robustness data for Metformin Hcl

Parameter	Rt	Theoretical plates	Tailing Factor
Decreased flow rate (0.8ml/min)	4.242	3296	1.42
Increased flow rate (1.2ml/min)	2.837	2955	1.42
Decreased temperature (20^0c)	4.248	3271	1.43
Increased temperature (30^0c)	2.845	2932	1.42

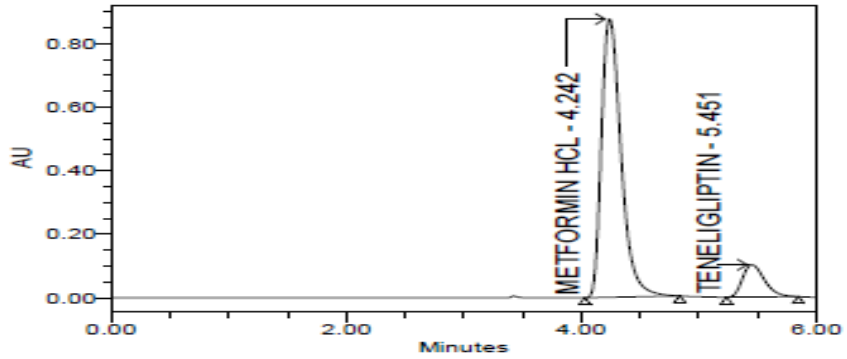

Fig.2(a): Chromatogram for decreased flowrate

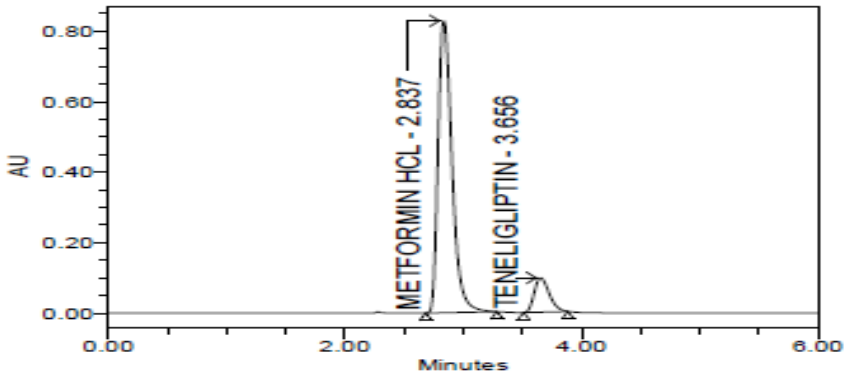

Fig.2(b): Chromatogram for increased flowrate

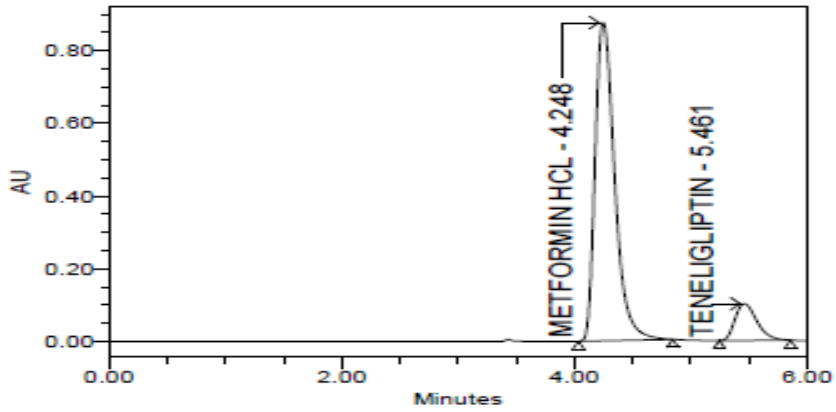

Fig.2(c) : Chromatogram for decreased temperature

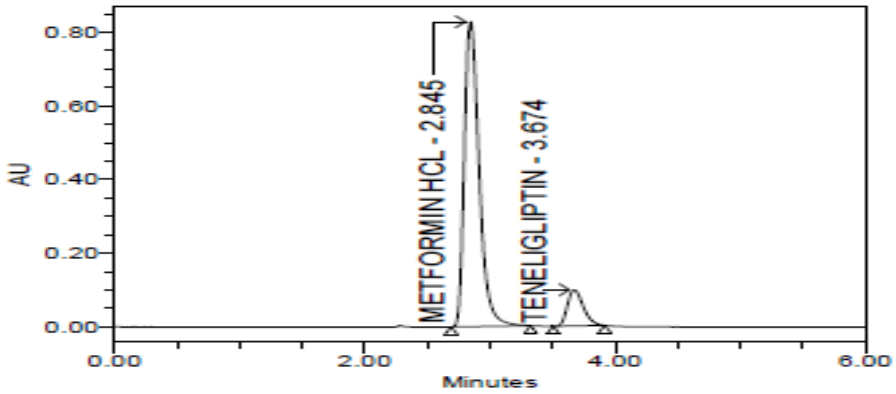

Fig.2(d) : Chromatogram for increased temperature

Therefore in proposed method, successful attempt has been made to develop a simple, accurate and economic method for analysis of teneligliptin and metformin Hcl.

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