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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TENELIGLIPTIN AND METFORMIN HCL BY RP-HPLC.

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Abstract:
Separation of Teneligliptin and MetforminHcl was successfully achieved THERMO, C18, 250X4.6mm, 5µm,or equivalent in an isocratic mode utilizing 0.1M KH2PO4 : 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 3.421minutes respectively using RP-HPLC method for Simultaneous estimation of bulk and pharmaceutical formulations. The method was validated and there response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Teneligliptin and 50µg/ml to150 µg/ml for MetforminHcl .The values of the correlation coefficient were found to1 for Teneligliptin and 0.999 for MetforminHcl respectively. The LOD and LOQ for Teneligliptin were found to be 0.2725 and 0.9085 respectively. The LOD and LOQ for MetforminHcl were found to be 0.801 and 2.671 respectively. The method was extensively validated according to ICH guidelines [2] for Linearity, Accuracy, Precision, Specificity and Robustness

Keywords: *Teneligliptin and MetforminHcl High performance liquid chromatography [3],Validation*

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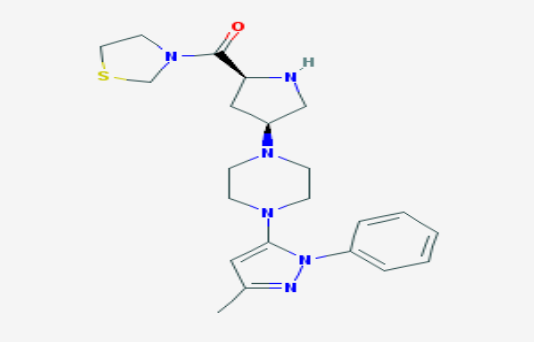
INTRODUCTION:

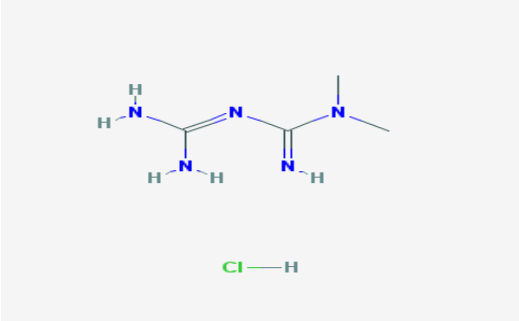
Teneligliptin also known as Teneligliptin hydrobromide hydrate. (TEN) is chemically described as {(2S,4S)-4-[4-(3-methyl-1phenyl-1Hpyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3-thiazolidin-3- yl) methanone hemipentahydrobromide hydrate is a dipeptidyl peptidase inhibitor. TEN slows the inactivation of incretin hormones, thereby increasing bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose-dependant manner in patients with type 2 diabetes mellitus. The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control



Metformin Hydrochloride is the hydrochloride salt of the biguanide metformin with antihyperglycemic& potential antineoplastic activities. Metformin inhibits complex I (NADPH:ubiquinone oxidoreductase) of the mitochondrial respiratory chain, thereby increasing the cellular AMP to ATP ratio and leading to activation of AMP-activated protein kinase (AMPK) and regulating AMPK-mediated transcription of target genes. This eventually prevents hepatic gluconeogenesis, enhances insulin sensitivity and fatty acid oxidation and ultimately leads to a decrease in glucose levels. Metformin may exert antineoplastic effects through AMPK-mediated or AMPK-independent inhibition of mammalian target of rapamycin (mTOR), which is up-regulated in many cancer tissues. Furthermore, this agent also inhibits tumor cell migration and invasion by inhibiting matrix metalloproteinase-9 (MMP-9) expression which is mediated through the suppression of transcription activator protein-1 (AP-1) activation.

Metabolism/Metabolites:

Metformin is not metabolized in the liver or GI tract and is not excreted in bile; no metabolites of the drug have been identified in humans.



EXPERIMENTAL PROCEDURE:

Instruments:

WATERS HPLC, Model: Waters 2695, Photo diode array detector (PDA), with an automated sample injector, Electronic balance , Ultra-sonicator, Heating mantle ,pH meter.

Reagents:

Potassium Dihydrogenphosphate (KH2PO4), Dipotassium hydrogen phosphate (K2HPO4), Water, Methanol, Orthophospsoric acid (OPA), Teneligliptin and MetforminHcl

Standard and sample solutions:

Standard: Accurately weighed quantity of 20mg Teneligliptin and 500mg MetforminHcl was taken in a 100ml volumetric flask and 10 ml of methanol was added and made up with methanol to 100ml .Further dilutions were made with water and methanol to get working standard solutions of 100µg/ml.

Sample : 20 tablets were weighed and crushed, from the powdered tablets, weighed accurately about785.00mg(20mg Teneligliptin and 500mg MetforminHcl) into a 50ml volumetric flask and 10 ml of Methanol was added and made up with methanol to 100ml .Further dilutions were made with water and methanol to get working standard solutions of 100µg/ml.

Separately injected both the standard (5 injections) and sample preparations (1 injection) into the chromatographic system and recorded the peak area responses

RESULTS AND DISCUSSION:

Method Development:

Parameters	Optimized Method
Mobile Phase	K2HPO4: Methanol (60:40)
Column	THERMO, C18, 250X 4.6mm, 5µm
Flow Rate	1.0ml/Min
Temperature	25˚C
Wavelength	280nm
Injection Volume	10µl
Retention Time	Tene:4.435,Met:3.427min

Validation Parameters : System suitability, Accuracy ,Linearity ,Precision ,LOD ,LOQ, Robustness ,Specificity

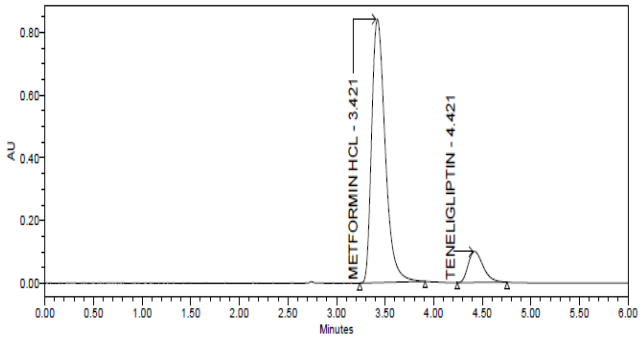
SYSTEM SUITABILITY:
Tailing factor for the peaks due to Teneligliptin and MetforminHcl in standard solution should not be more than 2.0. Theoretical plates for the Teneligliptin and MetforminHcl peaks in standard solution should not be less than 2000.

PRECISION:
% RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

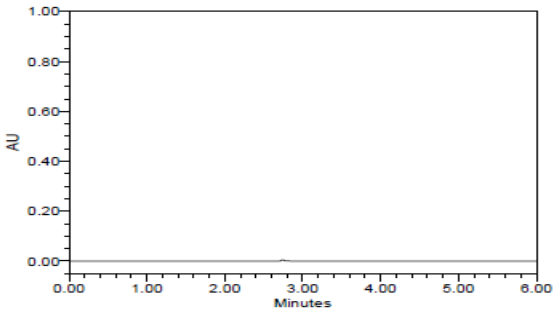
ACCURACY:
The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount.% Recovery was 99% for Teneligliptin and 100.00% for MetforminHcl. All the results indicate that the method is highly accurate [9].

LINEARITY:
The linearity of the method was determined at five concentration levels from 50-150(µg/ml).The calibration curve was constructed by plotting peak area versus concentration the slope and intercept values of Teneligliptin Y= 10585x & R2=1 and MetforminHcl Y=81669x & R2=0.999.

ROBUSTNESS:
The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce significant changes in analytical results.



Standard chromatogram



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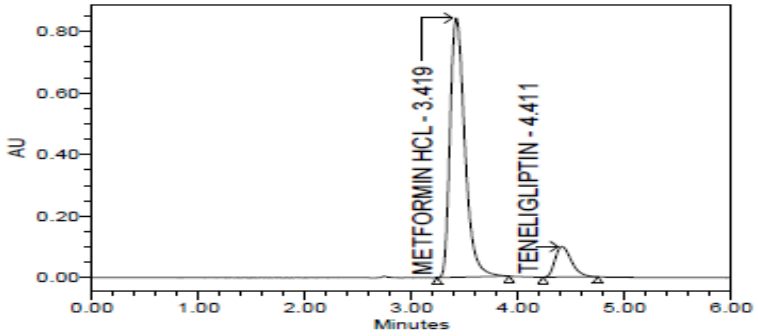


Sample chromatogram

SYSTEM SUITABILITY

Tabel 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

SPECIFICITY

Table 2: Specificity data for Metformin Hcl and Teneligliptin

S no	Sample name	Teneligliptin area	Rt	Metformin Hcl area	Rt
1	Standard	1057589	4.421	8142838	3.421
2	Sample	1058048	4.411	8143281	3.419
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

PRECISION

Table 3 :Precision data for Teneligliptin

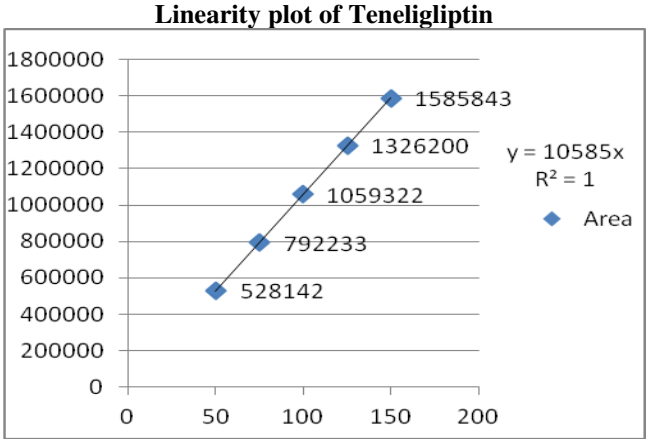
S.no	RT	Area
injection1	4.411	1058048
injection 2	4.413	1051011
injection 3	4.408	1050635
injection 4	4.415	1053330
injection 5	4.409	1050440
injection 6	4.411	1052503
Mean		1052661
Std. Dev.		2872.362
%RSD		0.2728

Tabel 4: Precision data for Teneligliptin

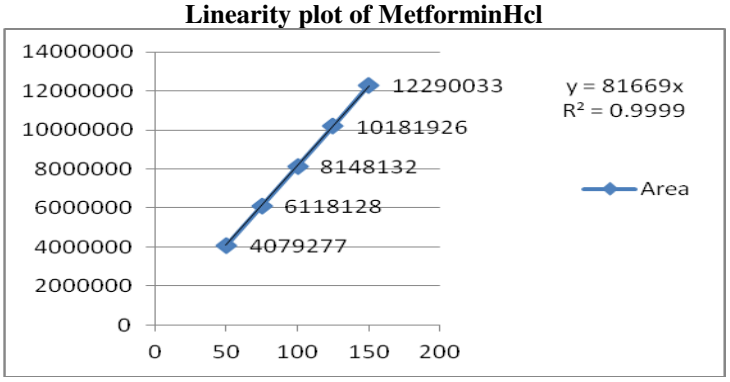
S.no	RT	Area
injection1	4.411	1058048
injection 2	4.413	1051011
injection 3	4.408	1050635
injection 4	4.415	1053330
injection 5	4.409	1050440
injection 6	4.411	1052503
Mean		1052661
Std. Dev.		2872.362
%RSD		0.2728

LINEARITY:

Linearity plot of Teneligliptin



Linearity plot of MetforminHcl



Tabel 5: 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 (r2)			1.00

Tabel 6: 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 (r2)			0.999

ACCURACY

Tabel 7: Accuracy (%recovery) results of 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	100.2	100.2
		2	392.50	19.800	19.86	100.3	
		3	392.50	19.800	19.87	100.3	
2	100%	1	785.00	39.600	39.71	100.2	100.1
		2	785.00	39.600	39.71	100.2	
		3	785.00	39.600	39.61	100	
3	150%	1	1177.50	59.400	59.41	100	100.2
		2	1177.50	59.400	59.73	10.5	
		3	1175.50	59.400	59.47	100.1	

Tabel 8: Accuracy (%recovery) results of MetforminHcl

S.NO	Accuracy level	Sample name	Sample Weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	392.50	500.000	497.53	99.5	99.5
		2	392.50	500.000	498.04	99.6	
		3	392.50	500.000	497.23	99.4	
2	100%	1	785.00	1000.000	994.50	99.4	99.4
		2	785.00	1000.000	995.12	99.5	
		3	785.00	1000.000	994.91	99.4	
3	150%	1	1177.50	1500.000	1498.50	99.9	99.8
		2	1177.50	1500.000	1499.60	99.9	
		3	1177.50	1500.000	1495.32	99.6	

ROBUSTNESS:

Tabel 9: 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(200c)	5.461	4082	1.36
Increasedtemperature(300c)	3.674	3998	3.68

Tabel 10: 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(200c)	4.248	3271	1.43
Increased temperature(300c)	2.845	2932	1.42

LIMIT OF DETECTION VALUES

LOD for Teneligliptin and Metformin Hcl = 0.2725 and 0.801

LIMIT OF QUANTIFICATION VALUES

LOQ for Teneligliptin and Metformin Hcl =0.9085 and2.671

SUMMARY

Tabel 11: Summary of validation data for Teneligliptin and MetforminHcl

S.NO	PARAMETER	RESULT Teneligliptin	RESULT MetforminHcl	ACCEPTENCE CRITERIA
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 4021 1.36 4.421 0.3	 3072 1.40 3.421 0.2	 Not less than 2000 Not more than 2 Not more than 2
2	Specificity Blank interference Placebo interference	 Specific	 Specific	 Specific
3	Method precision(%RSD)	0.27	0.05	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r2)	50-150 mcg/ml 1	50-150 mcg/ml 0.999	 Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	 100 100 100	 100% 99% 100%	 97 - 103%
6	Robustness Flow rate variation Temperature variation	All the system suitability parameters are within the limits.	All the system suitability parameters are within the limits.	

CONCLUSION:

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 hence the suggested method is more reliable using Rp-HPLC.

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REFERENCES:

1. Clifford J. Bailey, M.R.C.Path and Robert C. Turner. New England Journal of Medicine 1996; 334:574-579.
2. Goda M and Kadowaki T. Teneligliptin for the treatment of type 2 diabetes. Drug today (Barc) 2013;49,615–29.
3. Ideta T, Shirakami Y, Miyazaki T, Kochi T, Sakai H, Moriwaki H and Shimizu M. The dipeptidyl peptidase4 inhibitor teneligliptin attenuates hepatic lipogenesis via AMPK activation in nonalcoholic fatty liver diseasemodel mice. Int J Mol Sci 2015;16, 29207–18.
4. MousumiKar and P. K. Choudhury. HPLC Method for Estimation of Metformin Hydrochloride in Formulated Microspheres and Tablet Dosage Form. Indian Journal of Pharmaceutical Sciences 2009;71(3), 318–320.
5. Madhukar, A. Prince, Vijay Kumar. R, Sanjeeva. Y, Jagadeeshwar. K and D. Raghupratap. Simple And Sensitive Analytical Method Development And Validation of Metformin Hydrochloride by Rp-Hplc. International Journal of Pharmacy and Pharmaceutical Sciences 2011;3(3),117-120.
6. Gadipally Saikiran. Method Development of Metformin Hydrochloride by Rp-Hplc. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(3),1149-1159.
7. HimalPaudelChhetri, PannaThapa and Ann Van Schepdael. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharmaceutical Journal 2014; 22(5),483–487.
8. Sohan S. Chitlange, Diptee G. Rawat and Sneha Chandani. Estimation of anti- diabetic teneligliptin hydrobromide hydrate by rp-hplc and derivative spectroscopic method. Indo American Journal of Pharmaceutical Research 2016; 6 (7), 6144-6153.
9. M.Chandana, Dr.M.Prasad Rao, B.Samrajyam, K.S.K.D.Sireesha, V.V.Naga premi. Analytical method development and validation of teniligliptin in pharmaceutical dosage form by rp-hplc method. Journal of Health Sciences and Nursing 2016; 1(12), 1-12.
10. Validation of Analytical Proceudures , ICH HarmonisedTripatite Guidelines ,Q2 B 1997.