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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ANTIDIABETIC DRUGS (METFORMIN AND LINAGLIPTIN) IN TABLET DOSAGE FORM 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 Metformin and Linagliptin was successfully achieve dona THERMO, C18, 250cmx4.6mm, 5µm or equivalent in an isocratic mode utilizing KH_2PO_4: Methanol (65:35) at a flow rate of 1.0mL/min and eluate was monitored at 226nm, with a retention time of 3.132 and 3.728 minutes for Metformin and Linagliptin respectively. The method was validated and found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Metformin and 50µg/ml to150 µg/ml for Linagliptin. The values of the correlation coefficient were found to 0.999for Metformin and 1 for Linagliptin respectively. The LOD and LOQ for Metformin were found to be 1.909 and 6.362 respectively. The LOD and LOQ for Linagliptin were found to be 0.0349 and 0.1163 respectively. This method was found to be good percentage recovery for Metformin and Linagliptin were found to be 100 and 100 respectively indicates that the proposed method is highly accurate. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precision, Specificity and Robustness.

KEYWORDS: Metformin, Linagliptin, High performance liquid chromatography.

INTRODUCTION

Compound 1: Metformin is a oral tablet available as generic drugs and brand names are Glucophage, fortamet and glumetza. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

Compound 2: Brand name of drug is tradjenta and generic name is linagliptin. It is a DPP-4 inhibitor developed by Boehringer Ingelheim for the treatment of type II diabetes. Two pharmacological characteristics that sets linagliptin apart from other DPP-4 inhibitors is that it has a non-linear pharmacokinetic profile and is not primarily eliminated by the renal system.

MATERIALS AND METHOD

Chemicals and Reagent: PREPARATION OF MOBILE PHASE

Transfer 1000ml of HPLC water into1000ml of beaker and add 0.1M KH 2 PO 4.

Transfer the above prepared KH 2 PO 4 buffer and Methanol is mixed in the proportion of (65:35).

They are mixed and sonicated for 20min.

PREPARATION OF METFORMIN AND LINAGLIPTIN STANDARD AND SAMPLE SOLUTION

PREPARATION OF STANDARD SOLUTION

Accurately weigh and transfer 500mg Metformin and 20mg Linagliptin into 100ml of volumetric flask and add 10ml of methanol and sonicate 10min (or) shake 5min and make with methanol.

Transfers the above solution into 1ml into 10ml volumetric flask dilute to volume with water.

METHODOLOGY

PREPARATION OF SAMPLE STOCK SOLUTION

Commercially available 20 tablets ware weighed, powdered and the powdered equivalent to the 870 mg of Metformin and Linagliptin of active ingredients were transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with methanol. transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Water. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

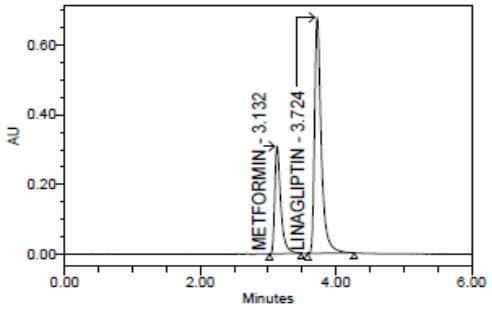
RESULTS AND DISCUSSION

SYSTEM SUITABILITY: System suitability data of Metforminand Linagliptin

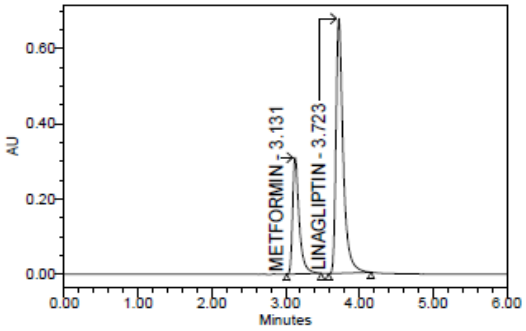
parameter	Metformin	Linagliptin	Acceptance criteria
Retention time	3.132	3.728	+-10
Theoretical plates	4560	7688	>2500
Tailing factor	1.59	1.56	<2.00
% RSD	0.4	0.4	<2.00

Standard Results of Metformin

S.no	Sample name	RT	Area	USPplate count	USP tailing
1.	Injection 1	3.728	4634008	7668	1.59
2.	Injection 2	3.729	4606703	7787	1.57
3.	Injection 3	3.726	4631981	7762	1.60
4.	Injection 4	3.723	4622848	7646	1.59
5.	Injection 5	3.724	4653812	7713	1.59



Typical Chromatogram of Standard-



Typical Chromatogram of Sample-

RESULT

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.

SPECIFICITY

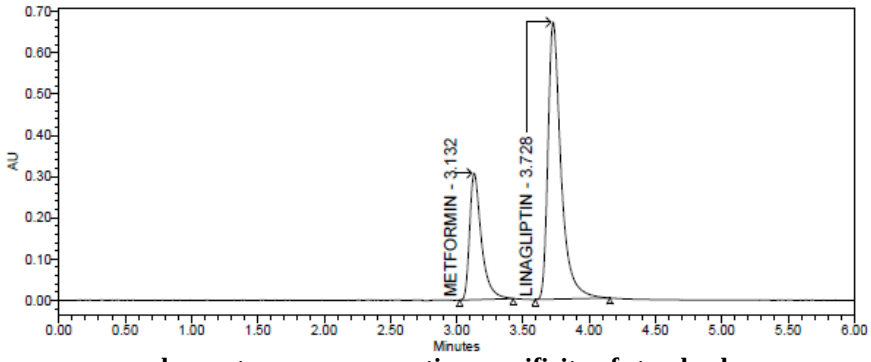
S no	Sample name	Metforminarea	Rt	Linagliptin Area	Rt
1	Standard	1892041	1.132	4606966	3.728
2	Sample	1904192	3.131	4627816	3.723
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Results of forced degradation study for Metformin

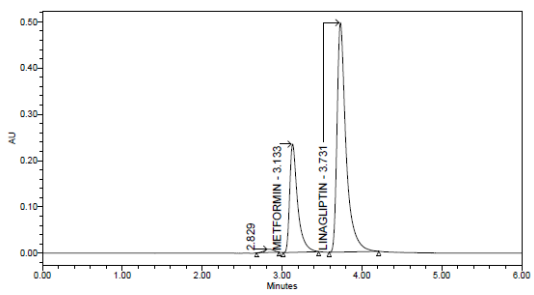
Type of stress	Degradation products/Drug	Retention time	% Area	Peak purity	Result	% Assay	%Amount Degraded
AcidicHydrolysis (mg/mL in 1N HCl) at 70ºC for 2 days	-	3.130	1653385	0.999	Passed	86	14
BasicHydrolysis (mg/mL in 1N NaOH) at 70ºC for 2 days	-	3.130	1634097	0.999	Passed	85	15
Oxidative Hydrolysis (mg/mL in 3% v/v H_2O_2) at 70 ºC for 2 days	-	3.133	1643883	0.999	Passed	86	14
Photo Degradation (to UV light) for 14 days	-	3.131	1617526	0.999	Passed	84	16
Thermal Degradation at 70ºC for 14 days	-	3.134	1608175	0.999	Passed	84	16

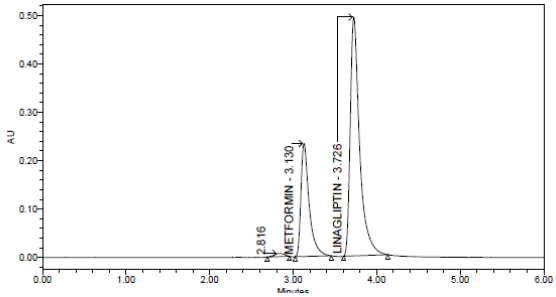
Results of forced degradation study for Linagliptin

Type of stress	Degradation products/ Drug (D)	Retention time	% Area	Peak purity	Result	% Assay	%Amount Degraded
Acidic Hydrolysis (mg/mL in 1N HCl) at 70ºC for 2 days	-	3.726	3891067	0.999	passed	84	16
Basic Hydrolysis (mg/mL in 1N NaOH) at 70ºC for 2 days	-	3.729	3911416	0.999	passed	84	16
Oxidative Hydrolysis (mg/mL in 3% v/v) at 70 ºc for 2 days	-	3.731	3870137	0.999	passed	83	17
Photo Degradation (to UV light) for 14 days	-	3.730	3909913	0.999	passed	84	16
Thermal Degradation at 70ºC for 14 days	-	3.733	3920769	0.999	passed	84	16



chromatogram representing specificity of standard





Chromatograms of Acid stress treated Metformin and Chromatograms of Base stress treated Metformin and Linagliptin mixture

RESULT

The forced degradation study showed the method was highly specific, the chromatographic peaks does not interfere with any other impurities**.** This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

ACCURACY

Accuracy data for Metformin

S.NO	Accuracy Level	Injection	Sample area	RT
1	50%	1	953677	3.122
		2	953428	3.124
		3	953033	3.122
2	100%	1	1901769	3.131
		2	1901974	3.134
		3	1902392	3.136
3	150%	1	2868938	3.141
		2	2865114	3.152
		3	2860981	3.150

Accuracy (%recovery) results of Metformin

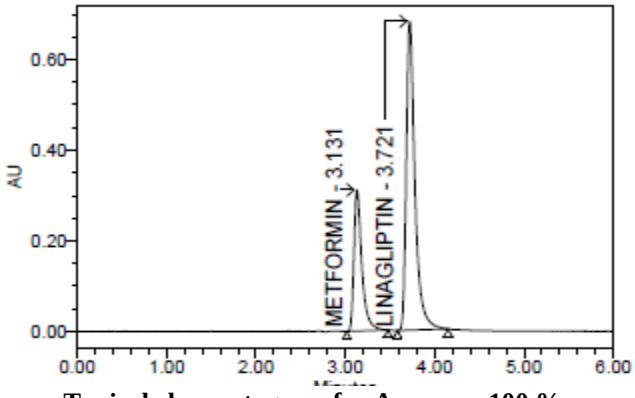
S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	435.00	247.500	245.91	99	99
		2	435.00	247.500	246.08	99	
		3	435.00	247.500	246.06	99	
2	100%	1	870.00	495.000	494.09	100	100
		2	870.00	495.000	492.55	100	
		3	870.00	495.000	493.48	100	
3	150%	1	1305.00	742.500	738.64	99	99
		2	1305.00	742.500	737.88	99	
		3	1305.00	742.500	736.69	99	

Accuracy data for Linagliptin

S.NO	Accuracy Level	Injection	Sample area	RT
1	50%	1	2325183	3.716
		2	2317701	3.716
		3	2317648	3.713
2	100%	1	4620300	3.721
		2	4626991	3.725
		3	4622070	3.726
3	150%	1	6948428	3.732
		2	6949946	3.744
		3	4940474	3.739

Accuracy (%recovery) results of Linagliptin

S.NO	Accuracy Level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	435.00	10.000	9.90	99	99
		2	435.00	10.000	9.88	99	
		3	435.00	10.000	9.90	99	
2	100%	1	870.00	20.000	19.73	99	99
		2	870.00	20.000	19.74	99	
		3	870.00	20.000	19.75	99	
3	150%	1	1305.00	30.000	29.66	99	99
		2	1305.00	30.000	29.64	99	
		3	1305.00	30.000	29.64	99	



Typical chromatogram for Accuracy 100 %

RESULT

Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 100.00% for Metformin and 100.00% for Linagliptin. All the results indicate that the method is highly accurate**.**

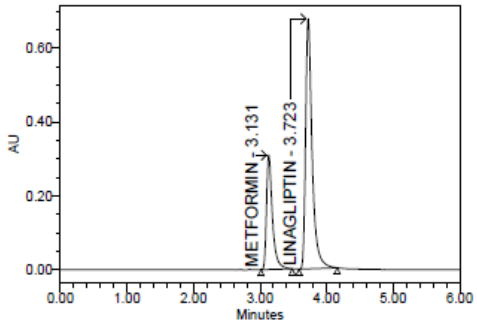
4. PRECISION

Precision data for Metformin

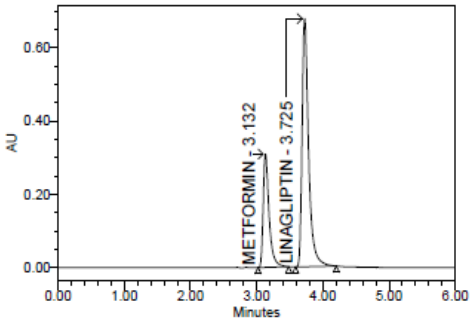
S.no	RT	Area	%Assay
injection1	3.131	1904192	99
injection2	3.132	19000711	99
injection3	3.137	1907020	99
injection4	3.134	1908231	99
injection5	3.127	1909733	99
injection6	3.131	1906386	99
Mean			99
Std. Dev.			0.17
% RSD			0.17

Precision data for Linagliptin

S.no	RT	Area	%Assay
injection1	3.723	4627816	100
injection 2	3.725	4624364	100
injection 3	3.730	4628747	100
injection 4	3.725	4626814	100
injection 5	3.719	4626237	100
injection 6	3.721	4623058	100
Mean			100
Std. Dev.			0.05
%RSD			0.05



Chromatogram for precision injection



Chromatogram for precision injection

RESULTS

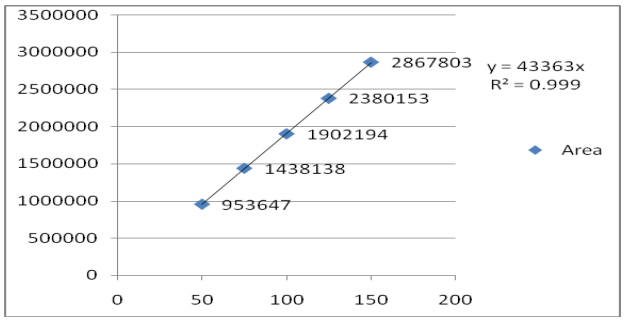
Results of variability were summarized in the above table. % RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

5. LINEARITY

Linearity data for Metformin

S.no	Conc (µg/ml)	RT	Area
1.	50	3.125	953647
2.	75	3.137	1438138
3.	100	3.140	1902194
4.	125	3.145	2380153
5.	150	3.153	2867803
Correlation coefficient (r^2)			0.999

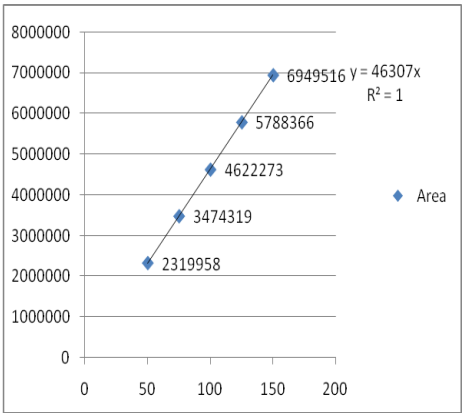
Linearity plot of Metformin

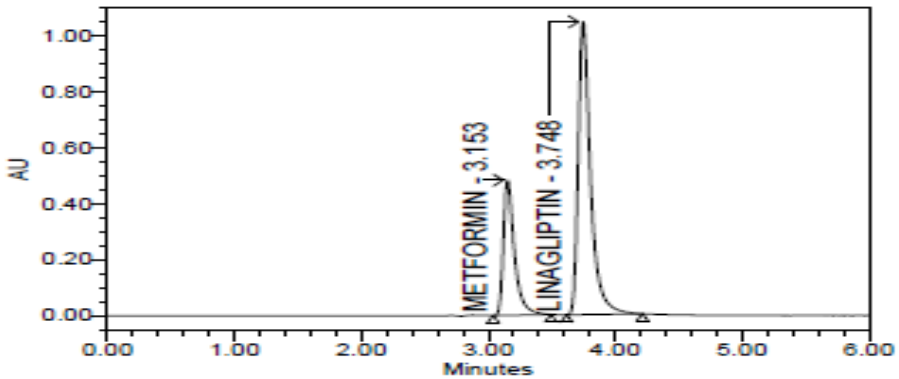


Linearity data for Linagliptin

s.no	Conc(µg/ml)	RT	Area
1.	50	3.720	2319958
2.	75	3.731	3474319
3.	100	3.733	4622273
4.	125	3.741	5788366
5.	150	3.748	6949516
Correlation coefficient (r^2)			1

Linearity plot of Linagliptin





Chromatogram representing linearity

RESULT

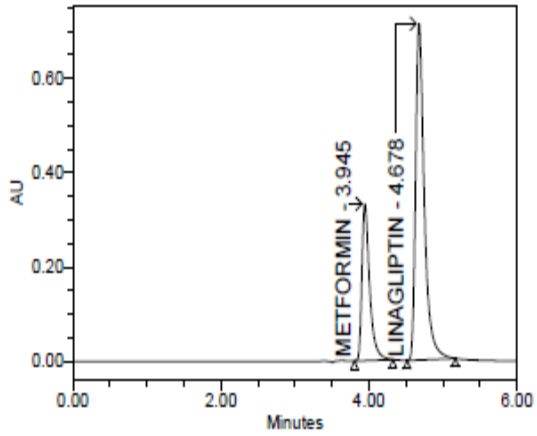
A linear relationship between peak areas versus concentrations was observed for Glecaprevir and Linagliptin in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both Metformin and Linagliptin which prove that the method is linear in the range of 50% to 150%.

6. ROBUSTNESS: Robustness data for Metformin

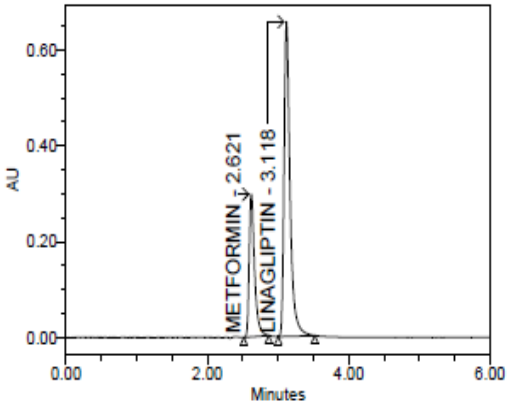
Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	3.945	7450	1.62
Increased flow rate(1.2ml/min)	2.621	6131	1.55
Decreased temperature(20^0c)	3.940	7434	1.61
Increased temperature(30^0c)	2.621	6131	1.52

Robustness data for Linagliptin

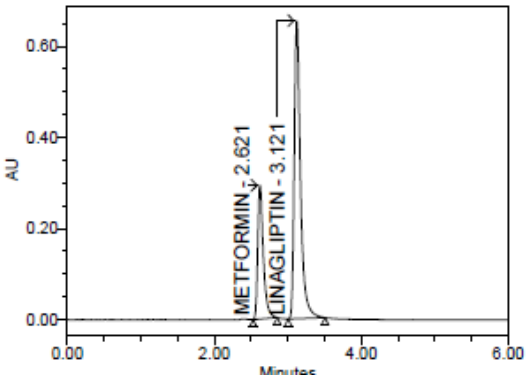
Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	4.678	8484	1.55
Increased flow rate (1.2ml/min)	3.118	7356	1.56
Decreased temperature(20^0c)	4.676	8409	1.55
Increased temperature(30^0c)	3.121	7304	1.55



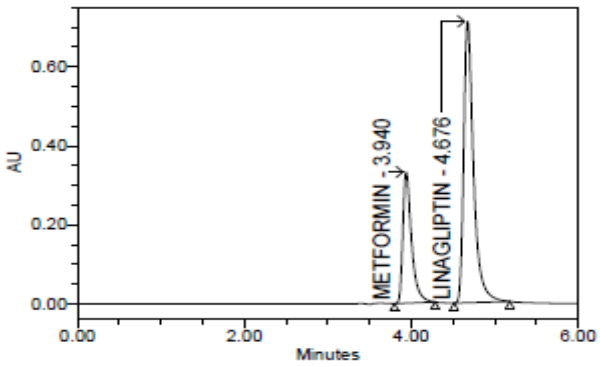
Chromatogram for decreased flow rate



Chromatogram for increased flow rate



Chromatogram for decreased temperature



Chromatogram for increased temperature

RESULT

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 which were presented in the above table. As the changes are not significant we can say that the method is Robust.

LIMIT OF DETECTION: Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD**LOD = 3.3* σ/S**

Where; σ = standard deviation S = slope LOD for Metformin = 1.909 LOD for Linagliptin =0.0349

LOD data for Metformin and Linagliptin

S.No.	Sample name	RT	Area
1	Metformin	3.127	4887
2	Linagliptin	3.724	12240

Chromatrogram for LOD: LIMIT OF QUANTIFICATION

Minimum concentration of standard component in which the peak of the standard gets detected and quantification

LOQ = 10*σ/S

Where; σ = standard deviation S = slope LOQ for Metformin = 6.362

LOQ for Linagliptin =0.1163

LOQ data for Metformin and Linagliptin

S.no	Sample name	RT	Area
1	Metformin	3.134	3255
2	Linagliptin	3.716	9713

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