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Estimation of Vildagliptin and Metformin HCl in Bulk and Pharmaceutical Dosage Form

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Abstract
A new method was established for simultaneous estimation of Metformin and Vildagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Vildagliptin by using Zodiac sil RPC18 4.5x100mm column 3.0µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol:phosphate buffer($KH_2PO_4$and K_2HPO_4)-pH 3 (pH was adjusted with orthophosphoric acid),detection wave length was 271 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.694mins and 3.334 mins. The % purity of Metformin and Vildagliptin was found to be 100.27% and 99.87% respectively. The system suitability parameters for Metformin-and Vildagliptin such as theoretical plates and tailing factor were found to be 2993, 1.23 and 5735, 1.12, the resolution was found to be 10.69. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Metformin and Vildagliptin was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.18% and 99.38%, %RSD for repeatability was 0.1 and 3.1, % RSD for intermediate precision 1 was 0.7 and 0.8, % RSD for intermediate precision 2 was 0.5 and 0.5 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.17 and 6.60, and LOQ value was 0.032 and 0.1125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Metformin and Vildagliptin in API and Pharmaceutical dosage form.

Keywords
Zodiac sil RPC18, Metformin and Vildagliptin, RP-HPLC

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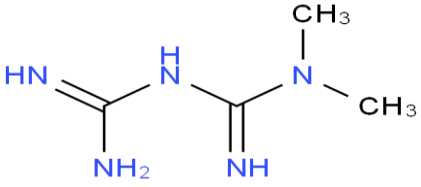
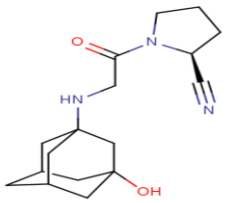
INTRODUCTION
Metformin, sold under the brand name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. It is not associated with weight gain.



Vildagliptin, sold under the brand name Galvus among others, is an oral anti-hyperglycemic agent (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.



MATERIALS AND METHOD AND INSTRUMENTATION

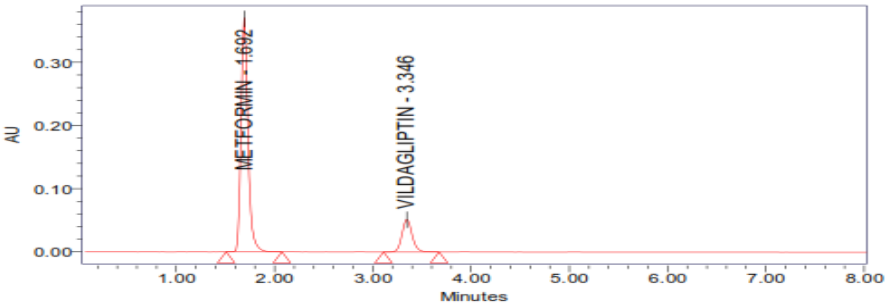
HPLC- Alliance, model No. Waters 2695, Empower 2, U.V double beam spectrometer UV 3000+ U.V win software Lab India Digital weighing balance (sensitivity 5mg) pH meter Sonicator Suction pump. Metformin and Vildagliptin, API, Ortho phosphoric acid, KH_2PO_4, K_2HPO_4, Acetonitrile, Methanol, Water.

Trial -4 (optimized method):

Chromatographic conditions

Column 4.6×250mm 3.0µm	:-Zodiac silRP C18
Mobile phase ratio buffer (70: 30 % v/v)	:-methnol: pH 3
Detection wavelength	:- 271nm
Flow rate	:- 1.0ml/min
Injection volume	:-10µl
Run time	:-10min

Fig.No.1. Chromatogram showing trial-4 injection



Preparation of the Metformin and Vildagliptin standard and sample solution

Sample solution preparation:

10 mg of Metformin and 1mg Vildagliptin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10mg Metformin and 1mg Vildagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

METHOD VALIDATION

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection-
- Limit of Quantification-
- Robustness
- System suitability testing

RESULTS AND DISCUSSIONS

Linearity

Table.No.1. Showing Linearity results for Metformin and Vildagliptin

	Peak Name	RT	Area (µv sec)	Height(µv)	USP Plate Count	USP Tailing
1	Metformin	1.689	1274954	264679	3109.6	1.2
2	Metformin	1.691	1548753	325904	3151.3	1.2
3	Metformin	1.692	1796583	381389	3111.1	1.2
4	Metformin	1.689	2045498	402853	3060.7	1.2
5	Metformin	1.688	2272948	466405	3034.3	1.3
Mean			1773747.3		3093.4	1.2
Std. Dev.			381086.8			
%RSD			21.5			

	Peak Name	RT	Area (µv sec)	Height(µv)	USP Plate Count	USP Tailing
1	Vildagliptin	3.303	257359	38680	5873.9	1.1
2	Vildagliptin	3.299	321497	48431	5780.7	1.1
3	Vildagliptin	3.294	380389	57171	5898.2	1.1
4	Vildagliptin	3.290	418105	62980	5931.1	1.1
5	Vildagliptin	3.288	470352	69863	5631.3	1.1
Mean			369540.4		5823.0	1.1
Std.Dev.			82964.0			
%RSD			22.5			

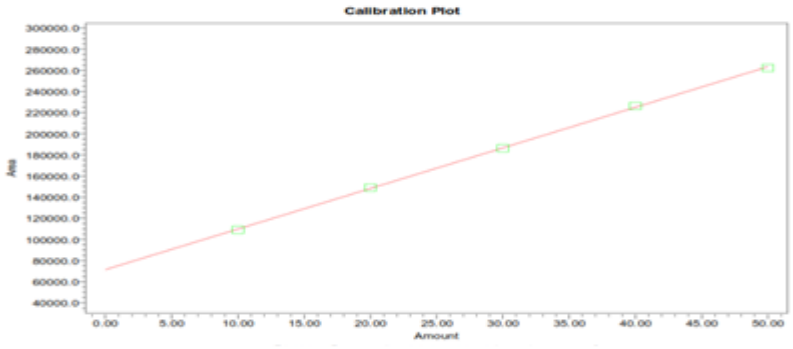


Fig.No.2. Showing calibration graph for Metformin

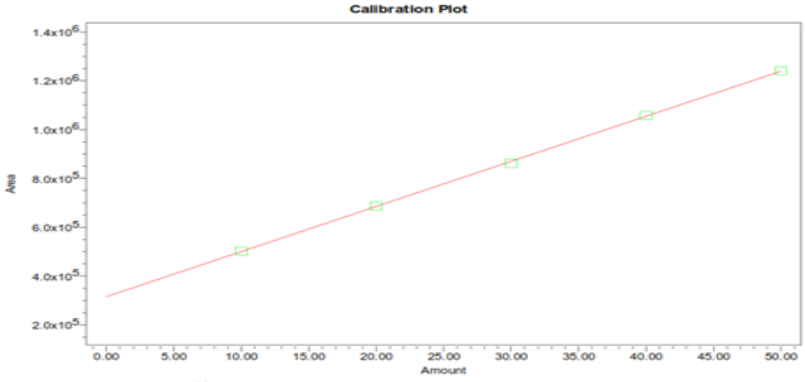


Fig.No .3. Showing calibration graph for Vildagliptin

Accuracy-

Table.No.2. Showing accuracy results for Metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7371253	5	4.96	99.91%	
100%	14634226.7	10	9.98	99.18%	99.56%
150%	2243270.7	15	15.02	99.60%	

Table.No.3. Showing accuracy results for Vildagliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	
100%	967998	1.0	1.05	99.38%	99.47%
150%	145437	1.5	1.495	99.52%	

Precision

Table.No.4. Showing% RSD results for Metformin

	Peak Name	RT	Area (µv sec)	Height(µv)	USP Plate Count	USP Tailing
1	Metformin	1.691	1819456	377420	3038.7	1.2
2	Metformin	1.691	1822446	374222	3019.0	1.3
3	Metformin	1.691	1824679	376000	2997.8	1.3
4	Metformin	1.693	1825211	371345	2932.8	1.2
5	Metformin	1.690	1826102	384153	3162.5	1.2
Mean			1823578.7		3030.2	1.3
Std.Dev.			2670.1			
%RSD			0.1			

	Peak Name	RT	Area (µv sec)	Height(µv)	USP Plate Count	USP Tailing
1	Vildagliptin	3.308	339557	54848	6445.0	1.2
2	Vildagliptin	3.319	351364	54315	6047.3	1.2
3	Vildagliptin	3.314	359377	55298	5992.5	1.1
4	Vildagliptin	3.328	361817	54713	5795.0	1.1
5	Vildagliptin	3.335	368227	54247	5554.4	1.1
Mean			356068.6		5966.9	1.1
Std.Dev.			11029.2			
%RSD			3.1			

Table.No.5. Showing %RSD results for Vildagliptin

Intermediate precision/Ruggedness

	Peak Name	RT	Area (µv sec)	Height(µv)	USP Plate Count	USP Tailing
1	Metformin	1.688	1817589	368060	3001.1	1.2
2	Metformin	1.690	1834970	371075	2862.2	1.3
3	Metformin	1.689	1840643	373296	2945.5	1.3
4	Metformin	1.693	1825211	371345	2936.2	1.3
5	Metformin	1.690	1826102	384153	3162.5	1.2
Mean			1831067.5		3030.2	1.3
Std.Dev.			12012.5			
%RSD			0.7			

	Peak Name	RT	Area (µv sec)	Height(µv)	USP Plate Count	USP Tailing
1	Vildagliptin	3.282	376633	54987	5537.9	1.1
2	Vildagliptin	3.277	380765	5345.1	5345.1	1.1
3	Vildagliptin	3.277	382506	56202	5453.2	1.1
4	Vildagliptin	3.328	361817	54713	5445.4	1.1
5	Vildagliptin	3.335	368227	54247	5554.4	1.1
Mean			379967.9		5966.9	1.1
Std.Dev.			3016.1			
%RSD			0.8			

Detection limit

Table.No.6. Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Vildagliptin-	5401.60	479884400	0.0372

Quantitation limit

Table.No.7. Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	371827.90	563365963	6.60
Vildagliptin-	5401.60	479884400	0.112

Robustness

Table.No.8. Showing system suitability results for Metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	**1**	**2294**	**1.27**
3	1.2	2146	1.26

Table.No.9. Showing system suitability results for Vildagliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

Table.No.10. Showing system suitability results for Metformin

S. No	Change in organic compoVildaion in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2347	1.44
2	***Actual**	**2294**	**1.27**
3	5 % more	2239	1.13

Table.No.11. Showing system suitability results for Vildagliptin

S. No	Change in organic compoVildaion in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	0.99
2	***Actual**	**4891**	**1.03**
3	5 % more	4817	1.05

The results showed that the method provided adequate accuracy, precision, sensitivity, reproducibility with better resolution for the simultaneous analysis of VIDA and MET. The advantages of proposed method are its short analysis time and a simple procedure for sample preparation. The results showed that the method provided adequate accuracy, precision, sensitivity, reproducibility with better resolution for the simultaneous analysis of VIDA and MET. The advantages of proposed method are its short analysis time and a simple procedure for sample preparation.

SUMMARY AND CONCLUSION

The-results showed that the method provided adequate accuracy, precision, sensitivity, reproducibility with-better resolution for the simultaneous analysis of VIDA and MET The advantages of proposed method are its short analysis time and a simple procedure for sample preparation. The developed method is stability indicating where well resolved peaks were observed for analyte-and-degradation. Hence the suggested RP-HPLC method can be used for routine analysis of Metformin and Vildagliptin in API and Pharmaceutical dosage form.

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