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Research Article

A SIMPLE AND VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF VILDAGLIPTIN AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Vildagliptin and Metformin in bulk and pharmaceutical dosage forms. The determination was performed by using Kromacil C_{18} column (150 x 4.6mm 5µ) as stationary phase and 0.1M DiPotassium mono hydro phosphate and Acetonitrile in the ratio of (70:30%v/v) adjust the pH 7.03 by using Ortho phosphoric acid as mobile phase. The flow rate of mobile phase was optimized as 1mL/min and effluents were monitored at 250nm. The retention time of Vildagliptin and Metformin were found as 2.58min and 11.69min respectively. The method shows linearity in the concentration range of 150-450 µg/mL and 15-45 µg/mL respectively. The developed method was validated for specificity, precision, linearity, accuracy, LOD, LOQ and robustness. Recovery of Vildagliptin and Metformin in formulations was found to be in the range of 98.0-103.0% and 98-99% respectively conforms the non-interferences of the excepients in the formulation. Due to its simplicity, rapidness and high precision, the proposed RP- HPLC method can be used for the simultaneous determination of these two drugs in Quality control department for regular analysis.

Keywords: RP-HPLC, Vildagliptin and Metformin.

INTRODUCTION

Vildagliptin belongs to a new class of oral anti-diabetic drugs and is a selective and reversible inhibitor of dipeptidyl peptidase 4 (DPP-4), the enzyme which inactivates the in cretin hormones, Glucagon-like peptide-1 (GLP-1), and glucose-dependent insulin tropic polypeptide (GIP), hormones which significantly contribute to the maintenance of glucose homeostasis. Vildagliptin is chemically known as (-)-(2S)-1-[[(3-Hydroxytricyclo [3.3.1.1[3, 7]] dec-1-yl) amino] acetyl] pyrrolidine-2-carbonitrile. The Molecular Formula is $C_{17}H_{25}N_3O_2$, and Molecular Weight is 303.40.

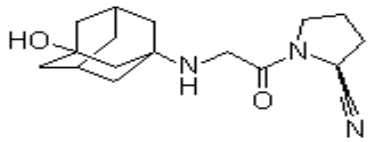

Fig. 1: Structure of vildagliptine

METFORMIN

Metformin is an iguanid antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control l by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. When used alone, Metformin does not cause hypoglycaemia; however, it may potentiate the hypoglycaemic effects of sulfonylurea's and insulin. Metformin is available in market various combinations. Metformin is chemically known as 1-carbamimidamido-N, N-dimethylmethanimidamide. The Molecular Formula is $C_4H_{11}N_5$, and Molecular Weight is 129.16.

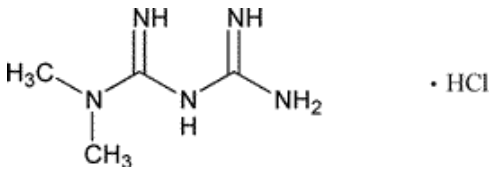

Fig. 2: Structure of metformin

MATERIAL SAND METHODS

A Waters e2695 Alliance HPLC system, connected with PDA Detector 2998 and Empower2 Software was used for method development a gift sample from Novartis Europharm limited company (Hyd). Acetonitrile (HPLC grade) and methanol (HPLC grade) collected from E. Merck. Di Potassium mono hydrogen phosphate analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from inhouse Milli-QR water purification system. Other Apparatus like Electronic balance, Sonicator, 0.45µ membrane filter were used.

Commercial Formulation

Vildagliptin and Metformin Tablets are available in the market as Galvusmet and Eucreas . Those are Fixed-dose combinations of 50/500 mg, 50/850 mg and 50/1000 mg with enteric coated. The authority required (streamlined) listing is for the treatment of people with type 2 diabetes. The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Preparation and Selection of mobile phase

The preliminary isocratic studies on a reverse phase C_{18} column with different mobile phases like 0.1M di Potassium mono hydro phosphate and Acetonitrile at the ratio (70:30 %v/v) adjust the pH: 7.03 by using Ortho phosphoric acid and filtered through the 0.45µ membrane filter.

Preparation of standard solution

Weigh accurately 150mg of Vildagliptin and1500mg of Metformin and dissolve in 50 mL of mobile phase and was further diluted to get stock solution of Vildagliptin and Metformin concentration. Solution containing mixture of Vildagliptin and Metformin of five different concentrations (50%, 75%, 100% 125%, and 150% of target concentration) were prepared in the same way.

Preparation of Sample Solution

Weigh the individual weight of Ten tablets (Galvusmet-50/500 mg) and Their average weights were determined. Powder of tablets, weigh powder equivalent to three tablets and transfer into 100 mL volumetric flask, dissolved with diluent, shaken and sonicated for about 10 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted with diluent to make the final concentration of working sample equivalent to 100%.

Chromatographic Conditions

The mobile phase pumped at a flow rate of 1 mL /min through the column (C_{18}; 4.6 X 150 mm, 5µ, Kromacil column) at 45ºC.The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed high absorbance values at 250 nm, which was selected as wavelength for further analysis.

Development and validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC simultaneous determination of Vildagliptin and Metformin in tablet dosage form. The experiment was carried out according to the official specifications of ICH- 1996 and Global Quality Guidelines-2002.The method was validated for the parameters like system suitability , selectivity, linearity, accuracy, precision ,LOD, LOQ, and robustness.

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Vildagliptin and Metformin. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

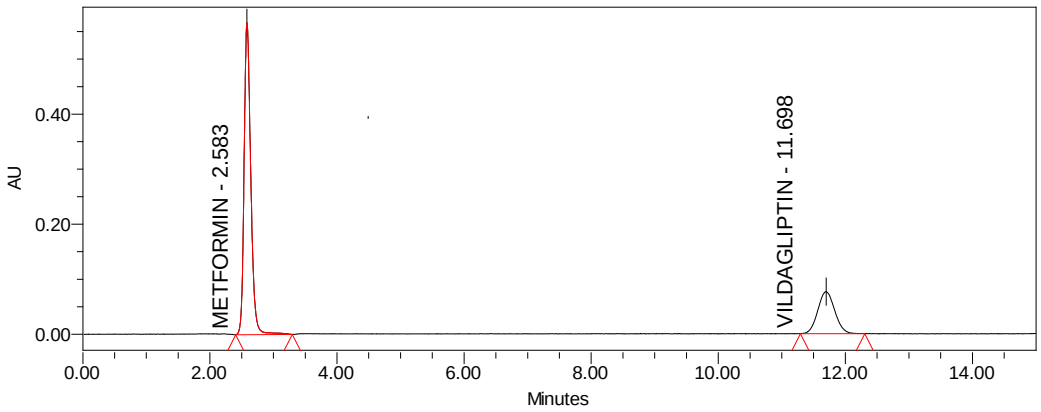

Fig. 3: Typical chromatogram of standard 1 vildagliptin and metformin

Table 1: For STD1 values

Name	Retention time	Area	USP resolution	S/N	USP tailing	USP plate count
Metformin	2.583	1473904		56.563872	1.324	2720
Vildagliptin	11.698	1802727	26.093	17.348185	1.04	9224

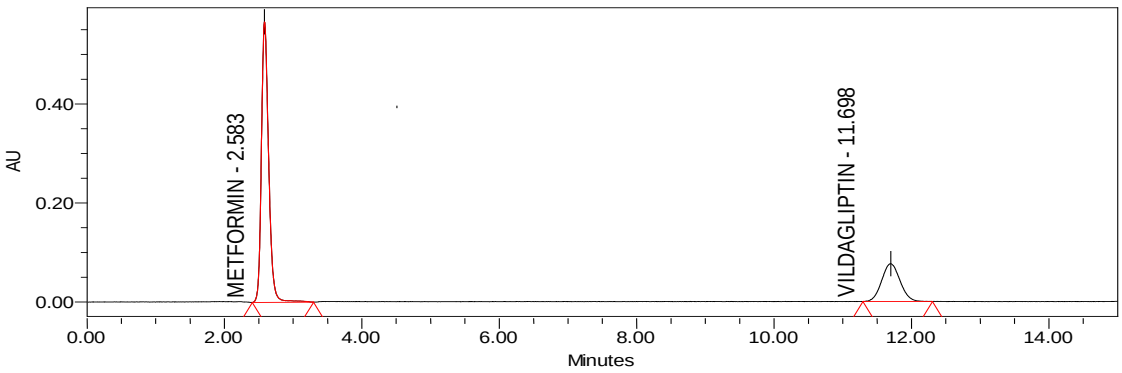

Fig. 4: Typical chromatogram of standard 2 vildagliptin and metformin

Table 2: For STD2 values metformin

S. No.	Sample name	Name	RT	Area	USP tailing	USP plate count
1	STD2	Metformin	2.582	15055341	1.277	2741
2	STD2	Metformin	2.578	15054558	1.325	2691
3	STD2	Metformin	2.589	15075745	1.202	2705
4	STD2	Metformin	2.578	15027594	1.327	2682
5	STD2	Metformin	2.579	15078035	1.286	2667
Mean				15058255		
Std.Dev				20368		
%RSD				0.1		

Table 3: For STD2 values vildagliptin

S. No.	Sample name	Name	RT	Area	USP resolution	USP tailing	USP plate count
1	STD2	Vildagliptin	11.690	1903986	25..91	1.078	9122
2	STD2	Vildagliptin	11.628	1906989	26.077	1.078	9009
3	STD2	Vildagliptin	11.636	1901327	26.18	1.056	9126
4	STD2	Vildagliptin	11.641	1906532	26.13	1.046	9308
5	STD2	Vildagliptin	11.651	1902605	26.12	1.049	9518
Mean				1904288			
Std.Dev				2450			
%RSD				0.1			

Standerds six replicate injections are showed uniformity of %RSD, retention time , thereotical plates, tailing and those values are given above tables 1&2..System suitabilty parameters and values are given below table 3 so system is suitable for the method devlopment and validation.

Table 4: Result of system suitability tests of vildagliptin and metformin

Parameters	Metformin	Vildagliptin
Linearity range	1500-4500 µg/mL	150-450 µg/mL
Correlation coefficient	0.999	0.999
Slope	5132.7x	6481.5x
Retention time	2.583	11.698
Resolution Factor	26.093	
USP plate count	2720	9224
Tailing factor*	1.324	1.04

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of Vildagliptin and Metformin were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another. Chromatograms shown in figure1 and figure 2 explain that retention time for standard sample and commercial product of Vildagliptin and Metformin are same. 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.

Linearity of Metformin

Table 5: Linearity of metformin

S. No.	Conc.	Area
1	1500	7709385
2	2250	11761953
3	3000	15430195
4	3750	19263861
5	4500	23111023

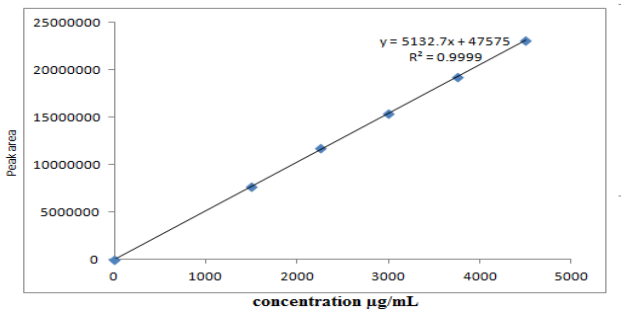

Fig. 3: Linearity of vildagliptin

Table 6: Linearity of vildagliptin

S. No.	Conc.	Area
1	150	976285
2	225	1463515
3	300	1932890
4	375	2447921
5	450	2911423

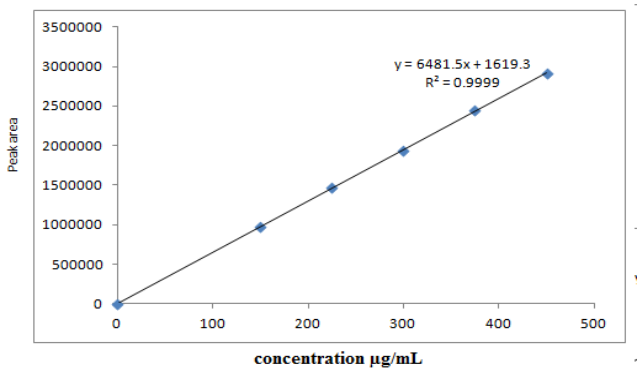

Fig. 4: Accuracy (Recovery Studies)

Linearity of the method was determined by constructing calibration curves. Standard solutions of Vildagliptin and Metformin of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients were 1.000for both the drugs which prove that the method is linear.

Table 7: Accuracy (%recovery) results of metformin

Metformin						
Spiked level	**Sample Weight**	**Sample Area**	**µg/mL added**	**µg/mL found**	**%Recovery**	**Mean**
50%	310.00	8022190	1028.34	1054.83	103	
50%	310.00	8000398	1028.34	1051.60	102	103
50%	310.00	8064517	1028.34	1063.50	103	
50%	310.00	8073662	1028.34	1061.47	103	

50%	310.00	8088116	1028.34	1063.50	103	
50%	620.0	8072646	1028.34	1061.47	103	
100%	620.0	15573611	2056.68	2047.76	100	
100%	620.0	15692663	2073.26	2063.42	100	99
100%	930.00	15382775	2056.68	2022.67	98	
150%	930.00	22534833	2985.50	2963.09	99	
150%	930.00	22554833	3018.68	2966.20	98	98
150%	930.00	22590178	3028.63	2970.37	98	
150%	930.00	22526209	3018.68	2961.96	98	
150%	930.00	22530550	3018.68	2962.53	98	
150%	930.00	22550575	3018.68	2965.16	98	

Table 8: Accuracy (%recovery) results of vildagliptin

Vildagliptin				
Sample area	**µg/mL added**	**µg/mL found**	**%Recovery**	**Mean**
955195	102.83	99.32	98	
963206	102.83	100.15	97	98
978299	102.83	101.72	98	
974158	102.83	101.29	97	
973235	102.83	101.19	98	
979530	102.83	101.85	98	
1957727	205.6	203.56	100	
1973751	207.32	205.22	100	98
1916562	205.66	199.28	99	
2875891	298.55	299.02	101	
2899247	301.86	301.45	101	99
2878908	302.86	299.34	102	
2888952	301.86	300.38	101	
2886303	301.86	300.11	10	
2863248	301.86	297.71	99	

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Vildagliptin and Metformin were added to pre-analyzed samples and were subjected to the proposed HPLC method. The measured value was obtained by recovery test. Spiked amount of both the drugs were compared against the recovery amount. % Recovery was 99.66% for Vildagliptin and 101.66 for Metformin.

Precision

Table 5: Intraday and inter day precision result of vildagliptin and metformin

Drug	**%RSD (intra-day)**	**%RSD (inter-day)**
Metformin	0.59	0.90
Vildagliptin	0.63	0.80

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Robustness of Method

Table 6: Results for robustness test of vildagliptin and metformin

Parameters count	**Changes**	**RT**	**USP Tailing**	**USP Plate**
Metformin				
Flow rate(ml/min)	0.8	2.569	1.238	3082
	1.2	2.569	1.238	3082
Temperature	40	2.567	1.200	2743
	50	2.570	1.261	2724
	Vildagliptin			
Flow rate(ml/min)	0.8	11.395	1.101	8260
	1.2	11.445	1.108	9204
Temperature	40	11.537	1.193	10160
	50	11.681	1.101	9781

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature [5-8].

RESULTS AND DISCUSSION

The developed method was validated as per the parameters like System suitability parameters, selectivity, linearity, precession, accuracy, robustness, ruggedness and the values all above parameters are within the limit so the developed method was validated according to ICH guidelines. It will be use full for further analysis in quality control and other departments

CONCLUSION

The new Reverse phase HPLC method developed and validated for simultaneous determination of Vildagliptin and Metformin pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid .this method was doing in simple manor but founded rapidly accurate values so method will be use full for quality control department ,formulation and also stability.

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