AMERICAN JOURNAL OF PHARMTECH RESEARCH

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Development and Validation of RP- HPLC Method for the Simultaneous Estimation of Vildagliptin and Metformin In Tablet Dosage Form

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ABSTRACT

A simple, rapid, sensitive, reversed phase-HPLC method was developed and validated to measure simultaneously the amount of Metformin and Vildagliptin at single wavelength (210 nm) in order to assess quantification in its tablet formulation and its subsequent stability studies. An isocratic elution of filtered sample was performed on Hypercil BDS C18 column with buffered mobile phase (0.1 M potassium dihydrogen ortho Phosphate buffer (Ph 4.8) and acetonitrile in the ratio of 60:40 v/v) with Hypercil BDS detection at 210 nm. The linearity for concentrations between 12.5µg/ml–75µg/ml for Metformin and 1.25µg/ml – 7.5µg/ml for Vildagliptin were established. The limits of detection (LOD) and quantification were 1.75 and 5.29 µg/ml for metformin and 0.46 and 1.39 µg/ml for vildagliptin. The determination of the two active ingredients was not interfered by the excipients of the products. This method was satisfactorily applied to the analysis of the tablet formulations and proved to be specific and accurate for the quality control of the cited drugs in tablet dosage form.

Keywords: RP-HPLC, Metformin, Vildagliptin, 0.1 M potassium dihydrogen ortho Phosphate buffer.

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Received 30 July 2014, Accepted 13 August 2014

Please cite this article as: Sai Ch *et al.*, Development and Validation of RP- HPLC Method for the Simultaneous Estimation of Vildagliptin and Metformin In Tablet Dosage Form. American Journal of PharmTech Research 2014.

INTRODUCTION

Vildagliptin chemically (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile], is a potent dipeptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. DPP IV inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas.

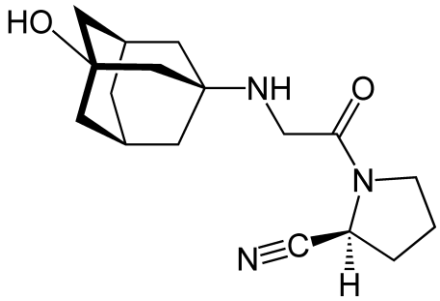

Figure: 1 Chemical structure of Vildagliptin

Metformin hydrochloride (MET) chemically *N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride) is an orally administered biguanide widely used in the treatment of type 2 (non-insulindependent) diabetes mellitus.[5,6] It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis commonly found with its analogue, phenformin. MET is a hydrophilic drug with an oral bioavailability of 50–60% and a relatively short half-life of 1.5–4.5 h

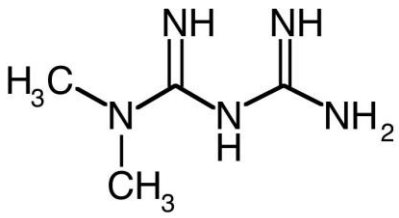

Figure: 2 Chemical structure of Metformin

MATERIAL AND METHODS:

Drugs and Instruments:

Waters e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. A gift sample given by Spectrum Labs, Hyderabad, Andhra Pradesh. Formulation tablets were purchased from Novartis. Potassium hydrogen ortho phosphate A.R grade purchased form

Fisher scientific chemicals, Mumbai. Acetonitrile and methanol were purchased form Ranbaxy Pvt. limited, Delhi, India. Water for HPLC was purchased from Fisher scientific, Mumbai.

Standard Preparation:

Accurately Weighed and transferred 50mg of Metformin and 5mg of Vildagliptin working Standards into a 10 ml clean dry volumetric flask, add 30ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample preparation:

5 tablets were weighed and calculate the average weight of each tablet. then the weight equivalent to 5 tablets was transferred into a 500 ml volumetric flask 300mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

Chromatographic Conditions:

Mobile phase ratio 60:40, column C18 hypercil BDS, flow rate 0.9 ml/min, sample temperature 25^{o}c, column temperature 30^{o}c and wave length 210 nm.

System Suitability:

System suitability is performed by six replicate standards inject into HPLC. It can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The USP defines parameters that can be used to determine the system suitability prior to analysis. These parameters are retention time, plate count, resolution, tailing and %RSD.

Selectivity:

Selectivity of the method was carried out by standards of Vildagliptin and metformin were inject into HPLC after that commercial product and placebo, excipients are one after one. It determines interference excipients peaks with analyte peaks.

Linearity:

Method linearity was deter mind by prepare five replicate standard solutions of those drugs in different (25%,50%,75%,100%, 125%,150%) concentration levels were inject in to the HPLC . plot the graph standard area verses concentration levels.

Accuracy (Recovery Studies):

Recovery studies were carried out by prepare triplicate standard solutions in 50%, 100%, 150% concentration levels and pre analyse the known amount of samples.

Precision :

Method precision was performed by prepare six replicate samples from single formulation and inject into HPLC at the same manner after 24 hours or day to day variation prepare six replicate samples from same formulation and inject into HPLC observe uniformity of test result and calculate the %RSD.

Robustness:

Method robustness was determined by the small changes in chromatographic conditions like as 0.2ml flow rate and ±5ºc temperature and inject the sample observe the result there were no marked changes compare to other analysis.

RESULTS AND DISCUSSION:

Results of system suitability are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, linearity range, count, tailing factor, usp plate count, LOD, LOQ and resolution for both the drugs which indicate a good system for analysis.

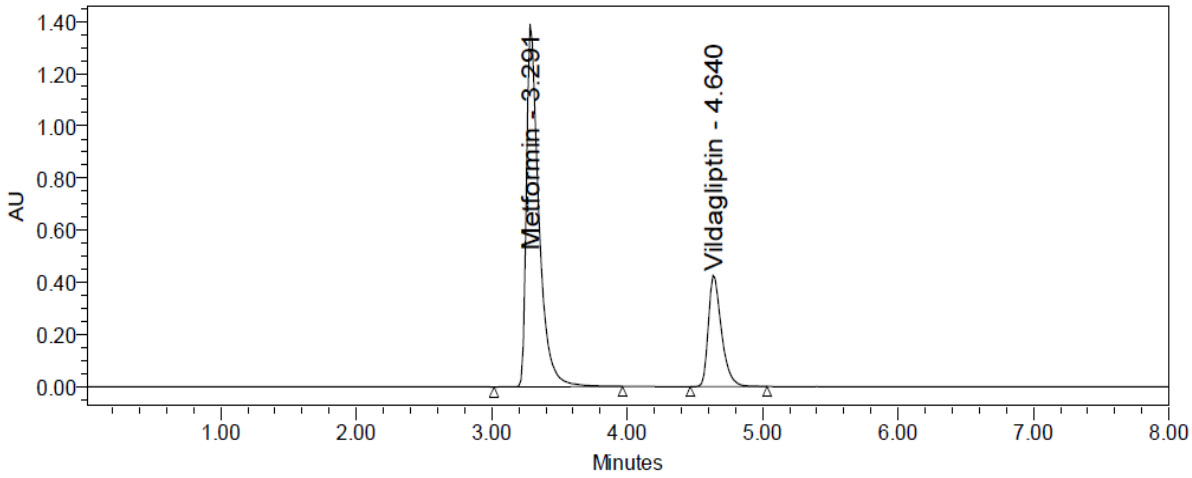

Figure: 1 Standard Chromatogram of Metformin and Vidagliptin

Table: 01 Results from Analysis of Metformin

	PEAK NAME	RT	AREA	USP PLATE COUNT	TAILING
1	Metformin	3.289	8849320	6671	1.70
2	Metformin	3.291	8823046	6586	1.62
MEAN			8836183		
STD.DEV			18578.6		
% RSD			0.2		

Table: 2 Results from Analysis of Vildagliptin

	PEAK NAME	RT	AREA	USP PLATE COUNT	TAILING
1	Vildagliptin	4.640	2864556	11072	1.39
2	Vildagliptin	4.656	2868286	11049	1.38
MEAN			2866421		
STD.DEV			2637.5		
% RSD			0.1		

Table: 3 Results from validation of Metformin and Vildagliptin

Parameters	Metformin HCl	Vildagliptin
Linearity range	12.5-75 µg/ml	1.25-7.5 µg/ml
Retention time	3.273	4.697
Usp plate count	7674	12517
Tailing factor	1.69	1.36
Limit of detection(LOD)	1.7456	0.4574
Limitof quantification(LOQ)	5.2898	1.3863

Results of system suitability test of metformin and vildagliptin:

Chromatograms shown in figure 1 explain that retention time for standard sample and commercial product of Metformin HCl and Vildagliptin 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. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Metformin HCl and Vildagliptin in the range of 50% to 150% of nominal concentration.

Linearity of metformin HCl:

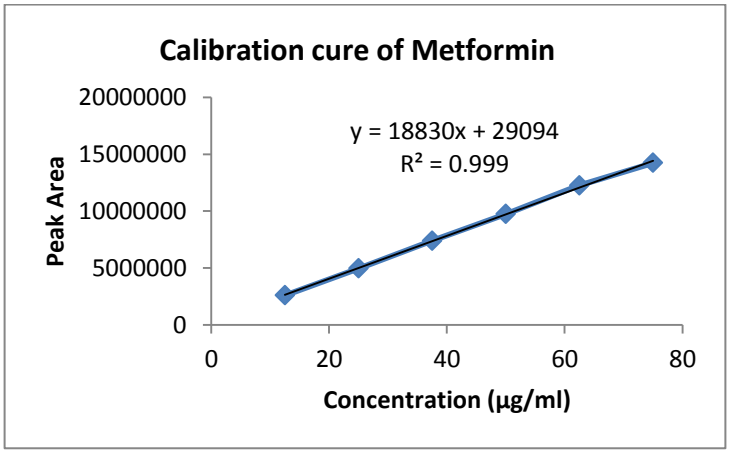

Figure 3 Calibration Curve of Metformin

Linearity of Vildagliptin:

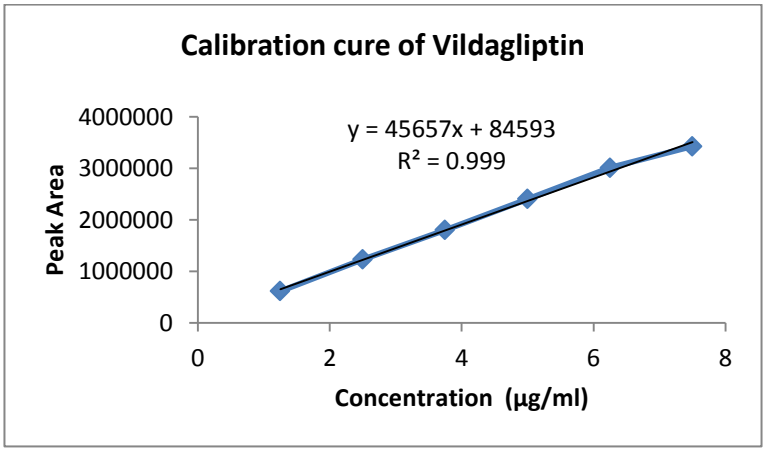

Figure 4 Calibration Curve of Vildagliptin

Table: 4 Result of %Linearity Level

Linearity level	Metformin		Vildagliptin	
Level	**Concentration (µg/ml)**	**Area**	**Concentration (µg/ml)**	**Area**
1	12.5	2601134	1.25	619044
2	25	4959437	2.5	1231006
3	37.5	7393994	3.75	1802551
4	50	9732251	5.0	2403308
5	62.5	12255479	6.25	3010305
6	75	14232090	7.5	342629
Correlation co-efficient	0.999		0.999	
Slope	19337x		48326x	
Intercept	10229		6687	

CONCLUSION:

We had run various trail runs at different chromatographic conditions finally we founded the above conditions are suitable for development and validation for simultaneous estimation of Vildagliptin and metformin in formulation dosage forms. According to previous method our method is cost effective, and less time consuming. This HPLC new method was very simple and accurate and also we observed validation parameters all are within the limit and % RSD is very low so it will be use full for routine analysis of quality control stability and further studies.

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