AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

RP-HPLC Method Development and Validation of Metformin and Vildagliptin in Bulk and Its Pharmaceutical Dosage form and their Bio-Analytical Studies

G.Alekya *[1], Naira Nayeem[1], T.Mahati [1]

1. Krupanidhi College of Pharmacy.Bangalore

ABSTRACT

The present work is simple and sensitive RP-HPLC Method Development and Validation for the simultaneous estimation of Metformin and Vildagliptin in bulk and its pharmaceutical dosage form and their Bio-Analytical studies. Chromatography was carried out on Kromosil C18 (4.6 x 250mm, 5µm) column using Phosphate buffer pH 5.8 and Acetonitrile in the ratio of 80:20 as the mobile phase at a flow rate of 1 ml/min with UV detection at 215 nm. The Retention time of Metformin and Vildagliptin is 2.589 mins and 4.296 mins respectively. The detector response is linear. The Limit of Detection for Metformin and Vildagliptin is 0.06 µg/ml and 0.1 µg/ml and Limit of Quantification for Metformin and Vildagliptin is 0.2 µg/ml and 0.4 µg/ml respectively. The Percentage assay for Metformin and Vildagliptin is 99.6% and 99.2% respectively and Percentage Recovery for average of three different concentrations for Metformin and Vildagliptin is 99.9% and 100.1% respectively. The method was validated by determining its selectivity, robustness, linearity, accuracy and precision. The developed method is simple, fast, sensitive, linear, accurate, rugged and precise and hence can be applied for routine quality control of Metformin and Vildagliptin in bulk and its pharmaceutical dosage form.

Keywords: Reverse Phase-High Performance Liquid Chromatography, Metformin, Vildagliptin, Bio-Analysis studies.

*Corresponding Author Email: naira_64@yahoo.co.in

Received 24 May 2013, Accepted 06 June 2013

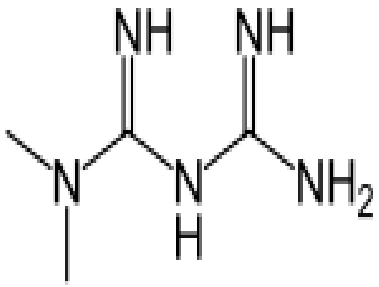
Please cite this article in press as: Alekya G. *et al.*, RP-HPLC Method Development and Validation of Metformin and Vildagliptin in Bulk and Its Pharmaceutical Dosage form and their Bio-Analytical Studies. American Journal of PharmTech Research 2013.

INTRODUCTION

Pharmaceutical products formulated with more than one drug, i.e. combination products, are intended to satisfy previously unmet needs of the patients by combining the therapeutic effects of two or more drugs in a single product. These combination products can be challenging to the analytical chemist who are involved in the development and validation of analytical methods. Simultaneous estimation of drug combination is done by separation using chromatographic methods like HPLC,HPTLC,GC etc as these methods are accurate and precise having good reproducibility. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation.

Metformin:-

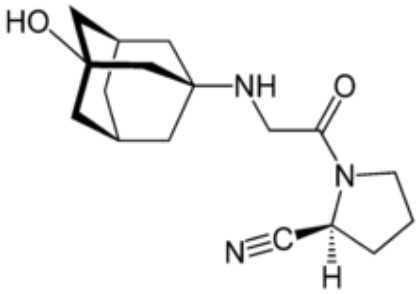
It is an oral anti-diabetic drug in the biguanide class. Metformin is chemically known as N,N-Dimethylimidodicarbonimidicdiamide. It is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function. It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor. Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance and metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells[1-2].



Vildagliptin:-

It is previously identified as LAF237, belongs to a new oral class of Anti Hyperglycemic agent of the new Dipeptidyl peptidase-4 (DPP-4) inhibitor class of the drug. Vildagliptin is chemically known as (S)-1-[2-(3-Hydroxyadamantan-1-ylamino)acetyl]pyrrolidine-2-carbonitrile. It has become a new once-daily oral treatment for type 2 diabetes with impressive efficacy, advantage of improving glycaemic control with a low risk of hypoglycaemia, as well as weight loss benefits in obese patients. It works to competitively inhibit the enzyme DPP-4. This enzyme breaks down the incretins GLP-1, GIP and gastrointestinal hormones released in response to a meal. By

preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the pancreas. This drives blood glucose levels towards normal[3-5].



Literature survey shows that there are many methods for the estimation of Metformin and Vildagliptin separately and in combination with other drugs[6-8]. To our knowledge, simple and sensitive simultaneous estimation of metformin and vildagliptin by RP-HPLC and their Bio-Analytical studies has not been developed and reported so far. So, an attempt was made to develop and validate an economic and rapid RP-HPLC method for the quality control of metformin and vildagliptin in bulk and pharmaceutical dosage form. The method was validated as per ICH guidelines [9-10].

MATERIALS AND METHODS:

Apparatus and Reagents:-

Waters e2695 Alliance HPLC system connected with a UV Detector 2487 and Empower 2 Software was used for the study. The drug analysis data were acquired and processed by using Empower 2 software. Pharmaceutical grade Metformin and Vildagliptin bulk samples were supplied by Pharma Train labs, Kukatpally, Andhra Pradesh, India. Acetonitrile used was of HPLC grade and Phosphate buffer was of analytical grade. Water HPLC grade was obtained from a Milli-QRO purification system.

Commercial Formulation:-

Metformin and Vildagliptin combination tablets are available in the market by the brand name as Galvus in composition of Metformin (500 mg) and Vildagliptin (50 mg). The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and are stored properly.

Chromatographic Conditions:-

The mobile phase consists Phosphate buffer and Acetonitrile (80:20) pumped at a flow rate of 1 ml/min through the Kromosil C18 column (4.6 x 250mm, 5µm) at ambient temperature. The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane

filter. Both the drugs showed good detection wavelength absorbance at 215 nm, which was selected as wavelength for further analysis.

Preparation and Selection of Mobile Phase:-

The preliminary isocratic studies on a reverse phase column with different mobile phase combination of potassium dihydrogen phosphate buffer and acetonitrile were studied for simultaneous estimation drugs. Weigh 7.0 grams of phosphate buffer into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. Adjust the pH to 5.8 with sodium hydroxide. Mixed a mixture of above phosphate buffer 800 ml (80%) with 200 ml of acetonitrile HPLC (20%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter under vacuum filtration.

Preparation of Standard solution:-

Accurately weighed and transferred 10 mg of metformin and 10 mg of vildagliptin working standard into a 10 ml and 100 ml clean dry volumetric flask and added about 7 ml and 70 ml of diluent and sonicated to dissolve it completely and made the volume up to the mark with the same solvent (Stock solution). Further pipette 2 ml of metformin and vildagliptin from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

Preparation of Sample solution:-

Accurately weighed and transferred 936.9 mg of metformin and vildagliptin tablet powder into a 100 ml clean dry volumetric flask and added about 70 ml of diluent and sonicated to dissolve it completely and made the volume up to the mark with the same solvent (Stock solution). Further pipette 0.4 ml of metformin and vildagliptin from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent.

RP-HPLC METHOD DEVELOPMENT AND VALIDATION:

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for RP-HPLC determination of Metformin and Vildagliptin in bulk and dosage form and their Bio-Analytical studies. The experiment was carried out according to the official specifications of ICH guidelines. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, LOD, LOQ and robustness.

System Suitability:-

The purpose of the system suitability test is to ensure that the complete testing system is suitable for the intended application. System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of metformin and vildagliptin. Various chromatographic parameters such as retention time, peak area, tailing

factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Specificity :-

Specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products and excipients. To determine the specificity of the method, standard sample of metformin and vildagliptin were injected first and then the commercial product, blank and excipients solution were run in the instrument one after another.

Linearity:-

The linearity of an analytical procedure is its ability (within a given range) to elicit test results which are directly proportional to the concentration of analyte in the sample. Standards are prepared at the concentration ranging from 100 ppm to 300 ppm for metformin and 10 ppm to 30 ppm for vildagliptin using at least five concentrations over the whole working range and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curve and correlation coefficient.

Accuracy :-

Accuracy is the percentage of analyte recovered by assay from a known added amount. It is assessed using a minimum of nine determinations over a minimum of three concentration levels in the specified range. It is determined by calculating Percentage Recovery and Percentage Relative Standard deviation (%RSD) of individual measurements by analyzing samples at least in triplicate and at each level 50%, 100% and 150% is recommended.

Precision:-

It is a measure of the degree of reproducibility or repeatability of the analytical method. 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.

Limit of Detection (LOD) and Limit of Quantification (LOQ) :-

LOD and LOQ were calculated for the sensitivity of the method. They were quantified based on the signal to noise ratio and are expressed as concentration of analyte (% parts per million). LOD is lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines.

$$LOD = 3.3 \times SD/SLOPE$$

$$LOQ = 10 \times SD/SLOPE$$

Robustness :-

Robustness is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters like pH, mobile phase composition, flow rate, column, buffer temperature, injection volume and instrument settings which provides an indication of its reliability during normal usage.

Bio-analytical studies:

The focus of bioanalysis in the pharmaceutical industry is to provide a quantitative measure of the active drug or its metabolites for the purpose of pharmacokinetics, toxicokinetics and forensic investigations[11-12]. Measurement of drug concentrations in biological matrices (such as serum, plasma, blood, urine and saliva) is an important aspect of medicinal product development. The samples are validated by measuring a known amount of plasma sample, acetonitrile and stock solution of metformin and vildagliptin and then allow it to cyclomix for 5 mins centrifuge for 20 mins. Collect the upper organic layer for HPLC analysis.

RESULT AND DISCUSSIONS:

Results are summarized below in the following figures and tables and the chromatograms of both the drugs are shown both in marketed formulation and bulk form along with blank chromatogram.

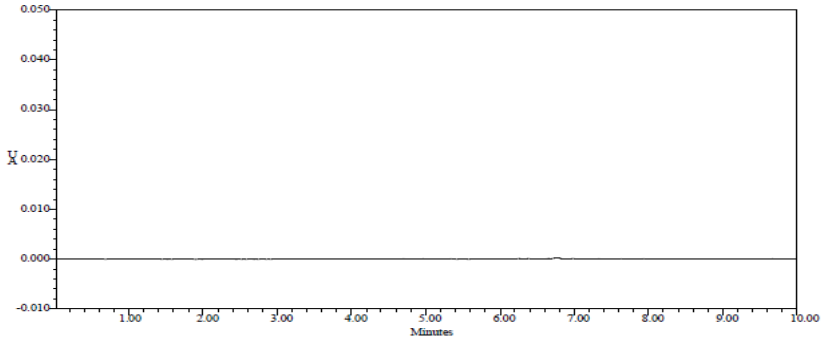


Figure 1: Blank chromatogram

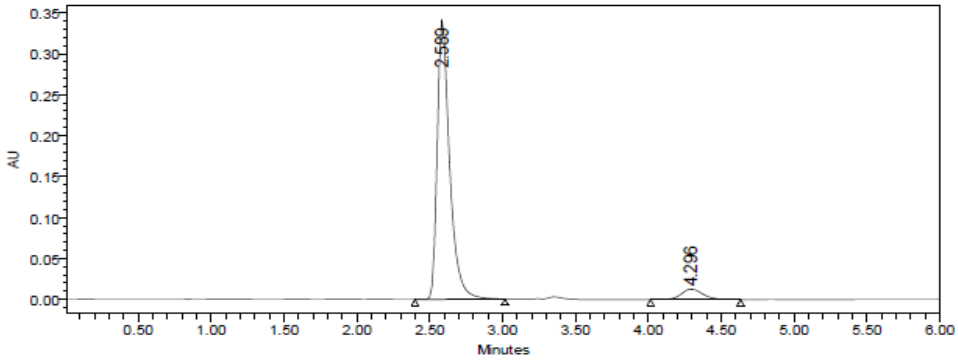


Figure 2: Chromatogram of Metformin and Vildagliptin in marketed formulation

Table 1: Data showing Metformin and Vildagliptin in marketed formulation

S.NO	Name	RT	Area	Height
1.	Metformin	2.589	2026717	433087
2.	Vildagliptin	4.296	129476	12963

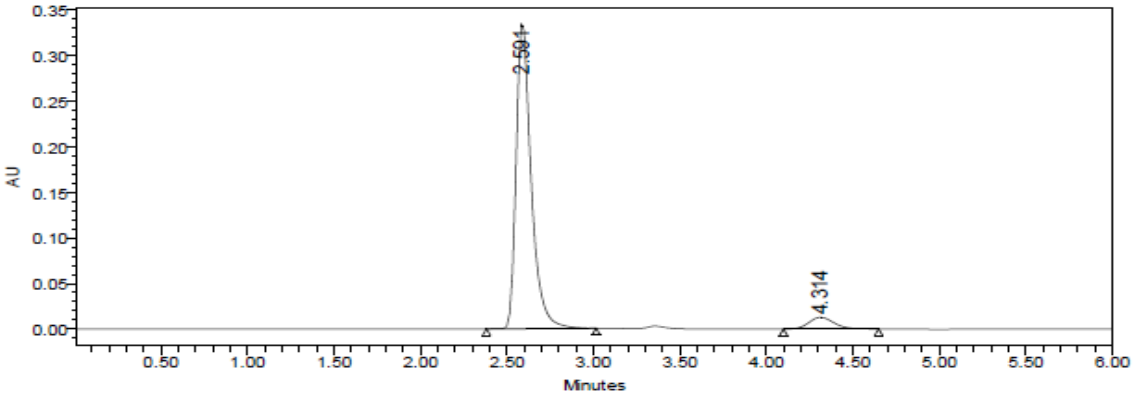


Figure 3: Chromatogram of Metformin and Vildagliptin in bulk form

Table 2: Data showing Metformin and Vildagliptin in bulk formulation

S.NO	Name	RT	Area	Height
1.	Metformin	2.591	2024664	336338
2.	Vildagliptin	4.314	131114	12990

Chromatogram shown in figure 1 and 2 explain the retention time for marketed formulation and bulk sample of metformin and vildagliptin are same and 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.

System Suitability parameters are explained below in the following figure and the consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both drugs and thus indicate a good system for analysis

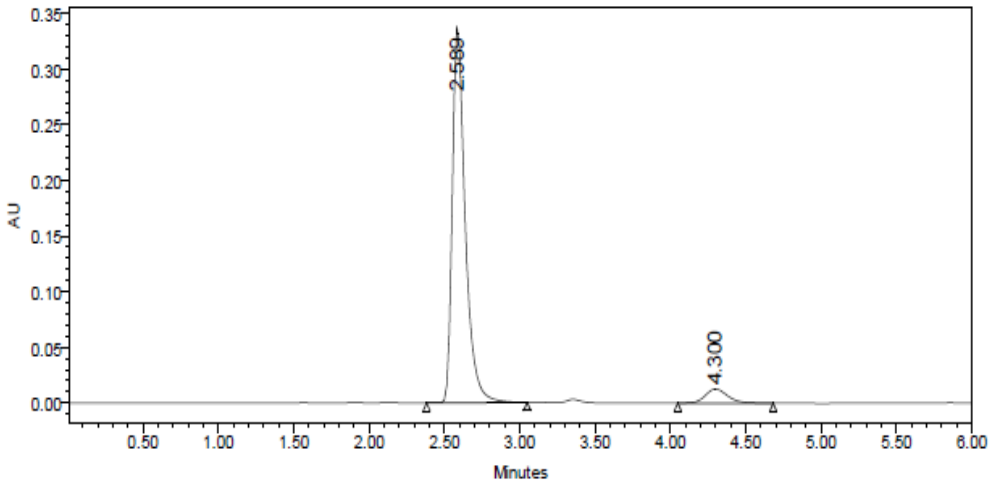


Figure 4: Chromatogram showing System Suitability parameters

Table 3: Data showing System Suitability parameters for Metformin and Vildagliptin

S.No	Name	RT	Area	Height (µV)	USP Plate count	USP Resolution	USP Tailing
1.	Metformin	2.589	2007494	337368	4794.9	-	1.5
2.	Vildagliptin	4.300	130459	12786	4218.1	7.9	1.2

A linear relationship between peak areas (average peak areas of five replicates) versus concentrations was observed in its nominal concentration range. Correlation coefficient was 0.999 for metformin and vildagliptin which prove that the method is linear and calibration curve is shown below:

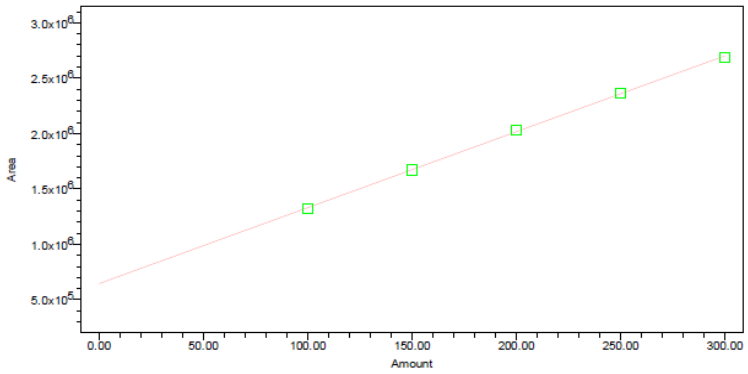


Figure 5: Linearity graph of Metformin

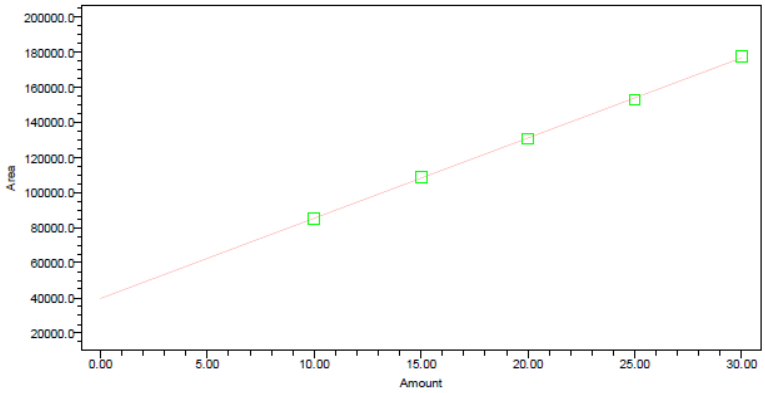


Figure 6: Linearity graph of Vildagliptin

Precision values for metformin and vildagliptin were summarized below in table 4 and 5. Five replicate injections were given and the %RSD was calculated by its values.

Table 4 : Data showing Precision for Metformin

Injection	Area
Injection-1	1988914
Injection-2	2025739
Injection-3	2019189
Injection-4	2018510
Injection-5	2033936
Average	2017258
Standard Deviation	17020.5
%RSD	0.84

Table 5 : Data showing Precision for Vildagliptin

Injection	Area
Injection-1	128478
Injection-2	130962
Injection-3	130097
Injection-4	130484
Injection-5	130460
Average	130096
Standard Deviation	955.3
%RSD	0.73

Table 6 : Data showing Accuracy values for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1009442	5.0	4.94	98.8%	99.9%
100%	2047373	10.0	10.0	100.2%	
150%	3085210	15.0	15.1	100.7%	

Table 7: Date showing Accuracy values for Vildagliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65699.3	5.0	4.95	99.1%	100.1%
100%	133312	10.0	10.0	100.5%	
150%	200131	15.0	15.0	100.6%	

Table 8: LOD and LOQ values for Metformin and Vildagliptin

Parameter	Metformin	Vildagliptin
Limit of Detection	0.06 µg/ml	0.1 µg/ml
Limit if Quantification	0.2 µg/ml	0.4 µg/ml

Table 9: Robustness for Metformin and Vildagliptin

Parameters	Changes	USP plate count	USP tailing
METFORMIN			
Flow rate (ml/min)	0.8	3421.6	1.4
	1.0	4817.5	1.5
	1.2	2398.9	1.4
Change in Mobile phase	10% less	3815.9	1.4
	Actual	4817.5	1.5
	10% more	2891.5	1.4
VILDAGLIPTIN			
Flow rate (ml/min)	0.8	3023.0	1.2
	1.0	4267.5	1.2
	1.2	2264.6	1.3
Change in Mobile phase	10% less	3128.9	1.2
	Actual	4267.5	1.2
	10% more	2759.6	1.3

Accuracy (% recovery) for Metformin and Vildagliptin were summarized below in table 6 and 7. Standard solutions of 50%, 100% and 150% concentrations were prepared and injected and % recovery and Mean recovery values are calculated.

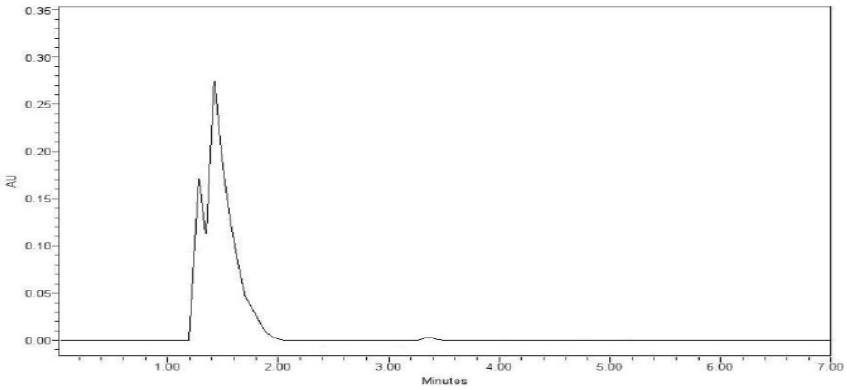


Figure 7: Chromatogram showing blank peak for plasma sample

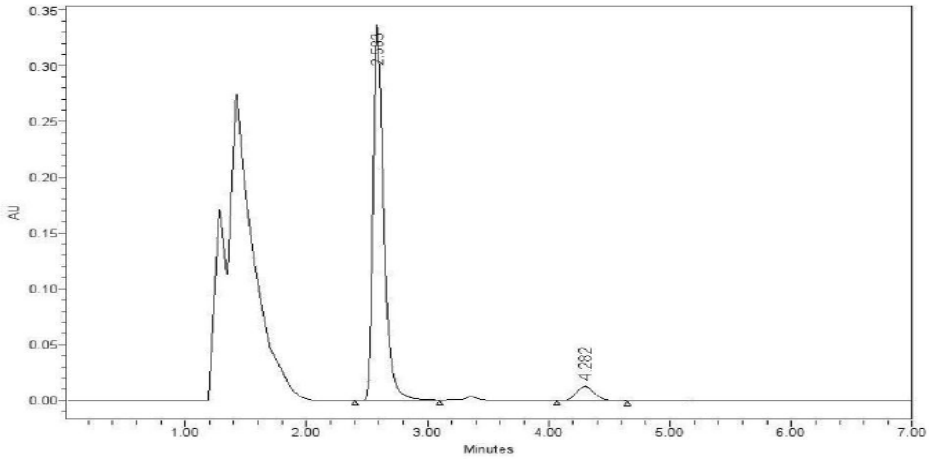


Figure 8: Chromatogram of Metformin, Vildagliptin and Plasma peak

Table 10: Data showing plasma peak

S.NO	Name	RT	Area	Height
1	Metformin	2.583	1818870	336354
2	Vildagliptin	4.282	104159	12549

Results of Precision were summarized in table 4 and 5. Five replicate injections were calculated and the method is highly precise because % RSD peak area is less than 2% and results obtained are within the limits. Results of Accuracy study are presented in table 6 and 7. The measured value was obtained by recovery test. Spiked amount of both drugs were compared against the recovery amount. % Recovery was 99.9% for metformin and 100.1% for vildagliptin. All the results indicate that the method is highly accurate.

The results LOD and LOQ were summarized in table 8. The results of robustness in table 9 showed that small changes were made in the flow rate and mobile phase did not produce

significant changes in analytical results which are not significant. Hence, we can say that the method is robust.

Bio-Analysis sample were also validated by spiking the plasma sample with metformin and vildagliptin. A known amount of plasma sample was mixed with equal concentration of acetonitrile and stock solution of metformin and vildagliptin. The mixture is centrifuged and the upper organic layer is collected and validated for HPLC analysis. The results show no change in retention time and resolution of the peak of the drugs in the presence of plasma.

CONCLUSION:

The new method developed was found to be simple, accurate, economical and rapid. It was shown that the above proposed method was linear, accurate, reproducible, repeatable and precise which proves the reliability of the method. All the validation parameters are within the limits and % RSD is very low. Hence, it is concluded that the proposed analytical method validation satisfies all the validation criteria mentioned under acceptance limits and hence it is suitable for the routine determination Quality control of Metformin and Vildagliptin in bulk and dosage form.

REFRENCES:

1. Ramachandran D, Pushpalatha K. Method Development and Validation for the simultaneous estimation of vildagliptin and metformin in tablet dosage form by RP-HPLC. Int J Pharm Pharm Sci. 2013; 5(1): 459-463.
2. Kar M, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Ind J Pharm Sci. 2009; 71(3): 318-320.
3. Ramzia.EB, Ehab E, Bassam.A. Spectrophotometric methods for the determination of sitagliptin and vildgliptin in bulk and dosage forms. Int J Biomed Sci. 2011; 7(1): 55-61.
4. Aparjita M, Bishwajit Bokshi, DilrubaNasrin. Development and validation of RP-HPLC method for estimation of vildagliptin from tablet dosage form. Int J Pharm Life Sci. 2012; 1(1): 1-6.
5. Amanda TB, Barbara S,Elfrides ES, Martin Steppe. Stability-Indicating RP-LC method for the determination of vildagliptin and Mass spectrometry detection for a main degradation product. J Chromatogr Sci. 2012; 50(5): 426-432.
6. Sumithra M, Shanmugasudaram MRP, Sankar ASK, Niharika MRS. Development of RP-HPLC method and it's validation for simultaneous estimation of sitagliptin and metformin. Int J Pharm Chem Sci. 2012; 1(1): 360-364.

7. Santhakumari B, Pharne AB, Ghemud AS, Jain HK, Kulkarni MJ. Bioanalytical method development and validation of vildagliptin a novel dipeptidyl peptidase IV inhibitor by RP-HPLC method. Int J Pharm Pharm Sci. 2012; 4(3): 119-123.
8. Xiaoyan C, Qi-Gu, Freng Q, Dafang Z. Rapid determination of metformin in human plasma by liquid chromatography-tandem mass spectroscopy method. J Chromatogr B. 2004; 802: 377-381.
9. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
10. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
11. En.wikipedia.org/wiki/Bioanalysis [Retrieved on 12-03-2013].
12. http://www.fda.gov/downloads/Drugs/.../Guidance/ucm070107.pdf