STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF VILDAGLIPTIN AND METFORMIN HCL IN PHARMACEUTICAL FORMULATION IN THE PRESENCE DEGRADATION PRODUCTS.

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ABSTRACT: Main purpose of the work is to validate drug efficiency of anti-diabetic drugs vildagliptin and metformin which is used in diabetic patients. Thus we illustrate a new and simple chromatographic method to analyze both drugs simultaneously in their marketable pharmaceutical dosage forms. A reverse Phase-high performance liquid chromatography (RP-HPLC) separation method was applied using an Thermo scientific C_{18} column of dimensions 250 mmL×4.6 mm I.D × 5µ. Separation was carried out using acetonitrile: phosphate buffer (pH 6.8) in ratio of 70:40 v/v as a mobile phase at a flow rate of 1.0 ml/min. Quantification of these drugs by this technique was achieved using an ultra violet detector at λ = 237 nm. The limit of detection (LOD) for vildagliptin was 0.040 µg/ml and 0.25 µg/ml for metformin using this RP-HPLC method. A linear calibration curves were reached at a concentration range of 5-30 µg/ml and 10-60 µg/ml for vildagliptin and metformin, respectively. Stability testing of both drugs were carried out which shown below 12 % degradation in acidic, alkali, oxidative and photolytic conditions. The developed technique was validated for concentration linearity, robustness, accuracy and precision, and results were statistically examined according to the International Conference on Harmonization (ICH) guidelines. The results presented in this report revealed the development of simple, rapid, precise and accurate RP-HPLC method for immediate determination and validation of vildagliptin and metformin in their pharmaceutical dosage forms.

Keywords: Stability, Validation, Vildagliptin, Metformin, RP-HPLC

INTRODUCTION: Diabetes mellitus type two (T2DM) is a chronic disease that wants a mixture of anti-diabetic drugs to have different mechanisms of action to succeed glycaemic goals. The widely used of metformin and a sulphonylurea (SU) as dual therapy fails to improve glycaemic control and the adding of a third anti-hyperglycaemic drug is necessary.

Metformin (MTF) is chemically known as [1- carbamimidamido-N, N-dimethylmethanimidamide in Fig. 1 is an oral anti-diabetic drug in the class of biguanides. It is used as the first-line drug for noninsulin-dependent diabetes mellitus treatment. It works as improving glycemic control factor through decreasing hepatic glucose production, decreasing glucose absorption, and increasing the insulin-mediated uptake of glucose. Vildagliptin (VGT) [(S)-1-[N-(3-hydroxy-1-ada-mantyl) glycyl] pyrrolidine-2-carbonitrile], Fig. 2, is a new oral anti-diabetic drug belonging to the class of dipeptidyl peptidase-4 inhibitor (reduces glucose-induced glucagon-like peptide 1 and gastric inhibitory polypeptide secretion) 3 and is used as mono therapy in adults with type 2 diabetes mellitus treatment especially in patients inadequately controlled by diet and exercise alone. Vildagliptin can be used as dual oral therapy in combination with; metformin, in patients with insufficient glycemic control despite maximal tolerated dose of monotherapy with metformin. It has similar efficacy as it is used with metformin when compared to sulphonylurea. In addition, vildagliptin used with a sulphonylurea, in patients with insufficient glycaemic control despite maximal tolerated dose of a sulphonylurea and for whom metformin is inappropriate due to contraindications or intolerance. Also, used with a thiazolidinedione, in patients with insufficient glycaemic control and for whom the use of a thiazolidinedione is appropriate. Furthermore, it was used with sulphonylurea and metforminas triple oral therapy when diet and exercise plus dual therapy with these medicinal products do not provide adequate glycaemic control [1-6].

Several methods were developed for the analysis of both vildagliptin and metformin in combination such as UV-Vis spectroscopies, HPLC and LCMS/ MS methods. Instantaneous estimation of these compounds by RP-HPLC methods were showing more time of analysis and complicated procedures; hence the present study was focused on chromatographic analysis of vildagliptin and metformin in a less time consuming simultaneous analysis of these compounds inactive ingredient (API) and Pharmaceutical dosage form which found in the pharmaceutical market[7-17].

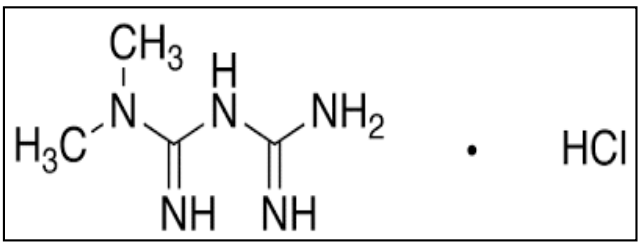

FIG. 1: STRUCTURE OF METFORMIN HCl

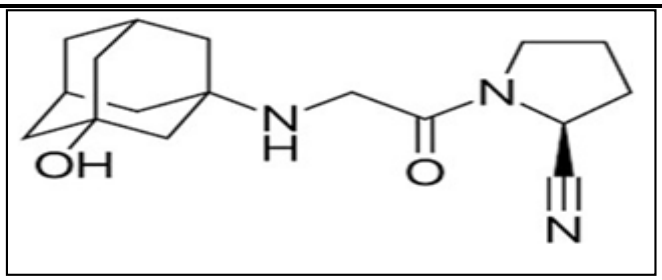

FIG. 2: STRUCTURE OF VILDAGLIPTIN

MATERIALS AND METHODS:

Chemicals Reagents and Instrumentation: Chemicals and reagents used in this experiment, Active Pharmaceutical Ingredients (API) of vildagliptin and metformin HCl as gift sample from Ipca laboratory, Mumbai. HPLC grade acetonitrile (Merck), potassium dehydrogenate orthophosphate (sigma chemicals) Ortho phosphoric acid (sigma chemicals), HPLC grad water (Hikma - Pharma) and marketed formulation obtained from local Market

HPLC (Jasco EQ, Aliance 2796 Model, and Detector 2678 with Em.power 4.5 software), UV spectrophotometer (Make: Jasco Model: UV- 3000 with chrom nav win 5 software

Chromatographic Conditions: Jasco HPLC with Chrom Nav power 4.5 software. The column used was symmetry C_{18} of dimensions 4.6 x 250mm, 5 µm, Make: X. Terra 1. The mobile phase consisting of phosphate buffer pH 6.8 and acetonitrile in ratio of 30:70 v/v with flow rate 1.0 ml/min and the run time was 10 min, columns temperature was maintained at room temperature in normal laboratory condition, injection volume 20 µl loop and detection wavelength 237 nm.

Standard Stock Solution Preparation: Stock solution of concentration 500µg/ml and 1000 µg/ml of Vildagliptin and metformin, respectively were prepared by taking exactly weighing 5 mg of Vildagliptin (VLG) and 10 mg of Metformin (MET) working standard in to a clean 10ml volumetric flask individually. 2, 5 -3.0 ml of mobile phase was used for dissolving completely and then the volume was made up to the mark with mobile phase. More dilutions were prepared with mobile phase. 0.3 ml of both the standard stock was diluted with mobile phase up to 10ml to get mixed standards of 15µg/ml Vildagliptin and 30µg/ml metformin. Analysis of marketed formulation weigh an equivalent to 10 mg of marketed formulation was transferred to a clean 10ml volumetric flask and added with 3 ml of mobile phase for dissolving the content and the volume was made up to 10 ml with mobile phase. The resulting solution was sonicated and filtered through nylon filter 0.45µ membrane filter.

FORCED DEGRADATION STUDIES:

Above mixed sample solution were taken to study forced degradation parameters such as Acid,Alkali , oxidative and photolytic parametrs.For acid hydrolysis take 2 ml of each 1 N HCl solution and adjust volume 10ml with mixed sample solution.This sample was kept it at 80^0c about 1 hours in water bath and then chromatogram was recorded. For alkaline hydrolysis Take 2 ml of each 1 N sodium hydroxide solution and mixed sample solution. This sample was kept it at 80^0c about 1 hours in water bath and then chromatogram was recorded. For oxidative degradation take 2 ml of each 5 % hydrogen peroxide and mixed sample solution. This sample was kept it at 80^0c about 1 hours in water bath and then chromatogram was recorded. For Photolytic degradation matrix solution was kept in sunlight for 24 hrs.

Validation Method: The validation method was carried out as per the ICH guidelines and accordingly the parameters evaluated were specificity, pre-cision, accuracy, linearity, ruggedness, and robustness and system suitability studies. %RSD was calculated for all the parameters[18-21].

Linearity: Linearity of an analytical procedure is its ability to extract test results that are directly proportional to the concentration of analyte in samples contained by given range.

Linearity of the method was studied by analyzing six analyte concentrations of drug ranging from 5- 30 ppm for VGT and 10-60 ppm MTF are tabulated in Table 1 and linearity plot is given in the Fig. and .

Limit of Detection (LOD) and Limit of Quanti- fication (LOQ): The detection and quantification limits for the VGT and MTF were performed and calculated using S/N ratio method.

Accuracy: Accuracy is the closeness of a measured value to a standard value. Accuracy was studied by means of recovery experiments for 80%, 100% and 120 %.Each level was injected three times. The accuracy was calculated in the form of percentage the test of the analyte recovered by the assay and the dated are given in **Table 3**

Precision: The precision expresses the closeness of agreement between a series of measurement obtained from multiple sampling of same homogenous sample under prescribed conditions. This experiment was conducted to prove the repeatability of the assay results obtained by quantification methodology. System precision, method precision and intermediate precision was performed for the homogeneous sample, according tom ICH guideline.

Sample concentration containing 15 ug/ml (MET) and 30 ug/ml (VLD) was injected for six times and calculated the peak area for all six injections in HPLC. The % RSD for the area of six replicate injections was calculated for system precision and shown in **Table 4**.

Robustness: Robustness measures the lack of internal controls on the test results. As part of the robustness, deliberate change in the wavelength, flow rate and mobile phase composition was made to evaluate the impact on the method.

Change in flow rate was varied at ±0.1 ml/min, wavength at ± 1nm and mobile phase ratio at ±2 ml. Standard solution 15 µg/ml Of VGT and 30µg/ml of MTF were prepared and analyzed using the different flow rates along with method flow rate. Results were given in **Table 5** and **6**.

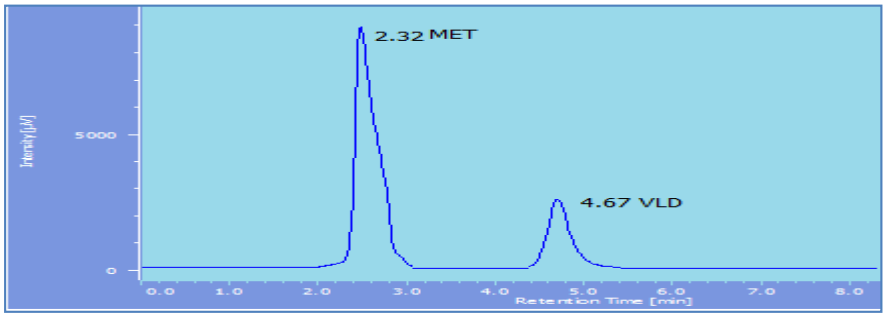

FIG 3:STANDARD CHROMATOGRAM OF METFORMIN AND VILDAGLIPTIN

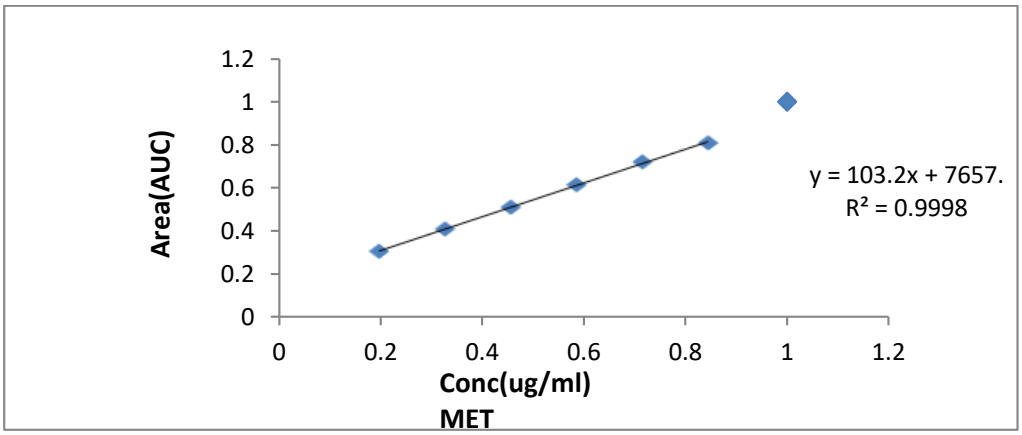

FIG 4: CALIBRATION GRAPH OF METFORMIN

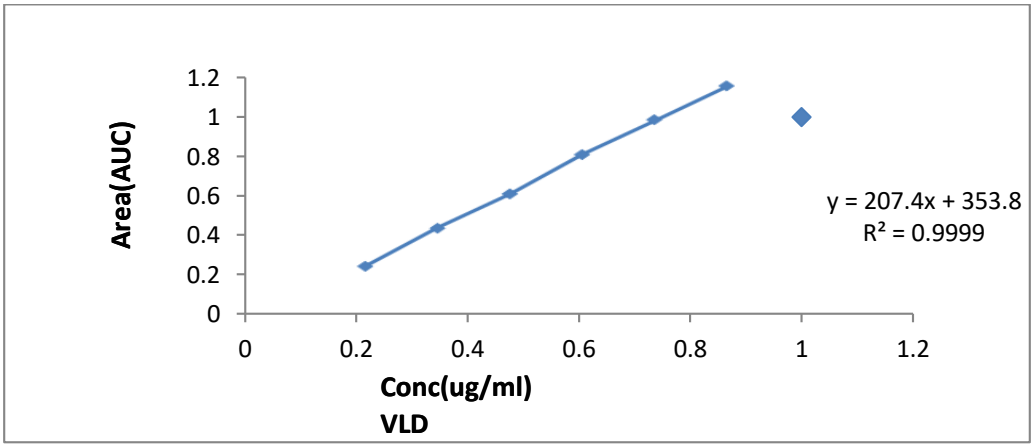

FIG 5: CALIBRATION GRAPH OF VILDAGLIPTIN

TABLE 1: LINEARITY RESULTS OF METFORMIN AND VILDAGLIPTIN

Sr.no	Concentration (ppm)	Area (AUC)	Concentration(ppm)	Area (AUC)
1	10	8669	5	1361
2	20	9721	10	2453
3	30	10752	15	3437
4	40	11810	20	4563
5	50	12886	25	5562
6	60	13784	30	6532

*(n=3)

TABLE 2: RECOVERY STUDIES OF METFORMIN

Accuracy %	Standard addition	Formulation	Percent recovery %	Mean recovery %
80%	15	30	99.50%	
Low	15	30		
	15	30		
100%	30	30	99.60%	
Mid	30	30		99.60%
	30	30		
120%	45	30	99.70%	
High	45	30		
	45	30		

TABLE 3: RECOVERY STUDIES OF VILDAGLIPTIN

Accuracy %	Standard addition	Formulation	Percent recovery %	Mean recovery %
80%	7.5	15	99.50%	
Low	7.5	15		
	7.5	15		
100%	15	15	99.60%	
Mid	15	15		99.60%
	15	15		
120%	22.5	15	99.70%	
High	22.5	15		
	22.5	15		

TABLE 4:FORCED DEGRADATION STUIDES

Sr no	Degradation conditions	% Percent Degradation	
		MET	VLD
1	Acidic Degradation 1 N HCl	11.9 %	12.08%
2	Base degradation 1N NaOH	10.54 %	11.09%
3	Oxidtive Degradation 5% H_2O_2	7.6 %	8.30%
4	Photolytic Degradation Sun light 24Hrs	4 %	3.50%

RESULTS AND DISCUSSION:

Vildagliptin and metformin can be effectively analyzed by the RP- HPLC method with phosphate Buffer, pH around 6.8: acetonitrile (70:30 v /v) at a flow rate of 1.0 ml/minute and detection wavelength of 237 nm.

The retention time of the drugs was 2.32 and 4.29 minute for vildagliptin and metformin respectively. The assay limits for vildagliptin and metformin was 92-109% and the results were within the limits.

The forced degradation studies were performed to analyze environmental factors affecting drug efficacy and potency. Vildagliptin and metformin drugs were shown degradation in acidic, alkaline, oxidative and photolytic conditions. Both drugs were more degradable in a 1 N HCl acidic condition. Vildagliptin was shown more alkaline and oxidative degradation than Metformin. Very less degradation was shown in photolytic stress testing.

Linearity: The linearity range was found to be 5- 30 µg/ml for Vildagliptin and 10-60 µg/ml for metformin. Calibration curves were plotted between the peak area and the concentrations and the linear regression coefficients for both drugs VGT and MTF were found to be 0.9999 and 0.9998 respectively (**Table 2**, **Fig. 4** and **5**). Hence the results obtained within the limits.

Limit of Detection and Limit of Quantification: The limit of detections (LOD) was calculated on SD and slope ratio which was 0.0040 µg/ml for vildagliptin and 0.025 µg/ml for metformin. Limit of quantification was 0.00134 ug/ml for VLD and 0.075 ug/ml for MET.

Accuracy: The accuracy studies were shown as % recovery for Vildagliptin (VGT) and Metformin (MTF) at three levels; 80 %, 100 % and 120 % (**Table 3**). The mean % recovery of the vildagliptin was 99.6% and of the metformin was 99.8 %.

Precision

In the intraday method precision studies % RSD was found to be less than 2 % that is VGT 1.19 % and MTF 0.171 % which specifies that the method has good repeatability. In the Intermediate System precision studies % RSD was found to be less than 2 % that is VGT 0.67 % and MTF 0.92 % which has shown that the system has good reproducibility.

CONCLUSION:

The developed and validated stability indicating HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous determination of vildagliptin and metformin in pharmaceutical dosage form.

Moreover, this method brings in a simple origin procedure with a little chromatographic run time, that make this method suitable for the analysis of large number of samples for the pharmacokinetic, bioavailability or

bioequivalent studies of vildagliptin and metformin hydrochloride. This developed method was validated as per ICH guidelines.

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CONFLICT OF INTEREST: The authors declare that no conflict of interest for this research.

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