Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION of VILDAGLIPTIN AND METFORMIN IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of vildagliptin and metformin in combination. The separation was carried out using a mobile phase consisting of 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with ortho phosphoric acid in the ratio of 70: 30%v/v. The column used was Phenomenex C18, (250 mm x 4.6 mm i.d, 5µm) with flow rate of 1 ml / min using PDA detection at 293 nm. The described method was linear over a concentration range of 5-50 µg/ml and 12.5-125 µg/ml for the assay of Vildagliptin and Metformin respectively. Gatifloxacin (50 µg/ml) was used as internal standard. The retention times of Vildagliptin, Metformin and Gatifloxacin were found to be 2.1, 2.5 and 5.5min respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Vildagliptin and Metformin were found to be 5 and10 µg/ml 10 and 25 µg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Vildagliptin and Metformin bulk drug and in its pharmaceutical dosage form.

Keywords: Vildagliptin, Metformin, RP-HPLC, Pharmaceutical dosage form.

INTRODUCTION

Vildagliptin (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile Figure 1 is an oral anti-hyperglycemic agent (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Vildagliptin inhibits the inactivation of GLP-1[1,2] and GIP by DPP-4, 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. Vildagliptin has been shown to reduce hyperglycemia in type 2 diabetes mellitus. Novartis has since withdrawn its intent to submit vildagliptin to the FDA, as of July 2008 [3]. The Food and Drug Administration had demanded additional clinical data before it could approve vildagliptin including extra evidence that skin lesions and kidney impairment seen during an early study on animals have not occurred in human trials. While the drug is still not approved for use in the US, it was approved in Feb 2008 by European Medicines Agency for use within the EU [4] and is listed on the Australian PBS with certain restrictions [5]. The EMEA has also approved a new oral treatment released by Novartis, called Eucreas, a combination of vildagliptin and metformin [6].

The DPP-4 enzyme is known to be involved in the suppression of certain malignancies, particularly in limiting the tissue invasion of these tumours. Inhibiting the DPP-4 enzymes may allow some cancers to progress [7, 8]. A study of DPP-4 inhibition in human non-small cell lung cancer (NSCLC) concluded that "DPPIV functions as a tumor suppressor, and its down regulation may contribute to the loss of growth control in NSCLC cells [9]. The risk of cancer suppression with DPP-4 down-regulation applies to all the DPP-4 inhibitors on the market in addition to vildagliptin (sitagliptin and saxagliptin)

Metformin (N, N-dimethylimidodicarbonimidic diamide) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function [10-13]. Its use in gestational diabetes has been limited by safety concerns. 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 works by suppressing glucose production by the liver.

Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It helps reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain. As of 2010, metformin is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide) [4]. When prescribed appropriately, metformin causes few adverse effects (the most common is gastrointestinal upset) and is associated with a low risk of hypoglycemia. Lactic acidosis (a buildup of lactate in the blood) can be a serious concern in overdose and when it is prescribed to people with contraindications, but otherwise, there is no significant risk.

First synthesized and found to reduce blood sugar in the 1920s, metformin was forgotten for the next two decades as research shifted to insulin and other antidiabetic drugs. Interest in metformin was rekindled in the late 1940s after several reports that it could reduce blood sugar levels in people, and in 1957, French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes. It was introduced to the United Kingdom in 1958, Canada in 1972, and the United States in 1995. Metformin is now believed to be the most widely prescribed antidiabetic drug in the world; in the United States alone, more than 48 million prescriptions were filled in 2010 for its generic formulations [5, 6].
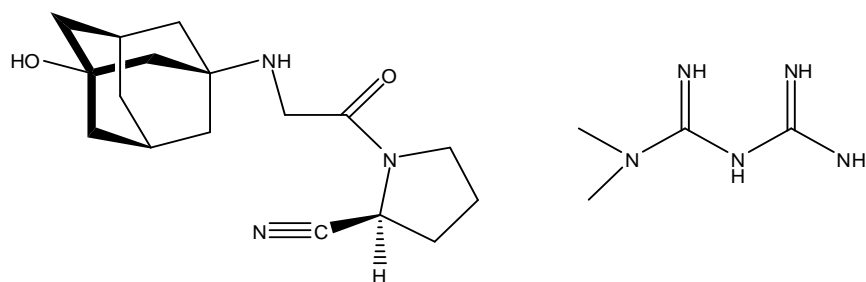

Fig. 1: Chemical structures of vildagliptin and metformin

MATERIALS AND METHODS

Chemicals and Reagents

Samples of Vildagliptin, Metformin and Gatifloxacin confirmed by spectral characterization and SOR (specific optical rotation) were obtained from Process Research Department of Emmanthi Laboratories Ltd, Hyderabad, India. HPLC-grade Acetonitrile was procured from Sigma Aldrich, India.

Instrumentation

Chromatographic system consisted of a Waters Model Alliance 2695 separation module equipped with auto sampler Photodiode array ultraviolet (UV) detector. The data recorded using empower software. The separation was carried out using a mobile phase consisting of 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with ortho phosphoric acid in the ratio of 70: 30%v/v. The column used was Phenomenex C18, (250 mm x 4.6 mm i.d, 5μm) with flow rate of 1 ml / min using PDA detection at 293 nm.

Preparation of stock, working standard solutions, and sample solution

A stock solution of vildagliptin and metformin (100 μg/mL) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-mL volumetric flasks. They were dissolved in 25 mL of mobile phase and then the volume was made up to the mark to get 100 μg/mL. The internal standard solution was prepared by taking 10 mg of gatifloxacin in a 100 ml standard flask. It is dissolved by adding 25 ml of mobile phase, shaken for few minutes to get a clear solution and the final volume was made up to 100 ml. For each drug, appropriate aliquots were pipetted out from the standard stock solution into a series of 10 mL volumetric flasks to get a concentration of 5, 10,20,30,40 and 50 μg/ml of vildagliptin, 12.5, 25, 50, 75,100 and 125 μg/ml of metformin and 50 μg/ml of gatifloxacin (Internal Standard).

Development and validation of HPLC Method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of vildagliptin and metformin in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996, and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness.

System suitability

The system suitability was assessed by six replicate analysis of vildagliptin and metformin at a 100% level to verify the resolution and reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for both vildagliptin and metformin tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks of vildagliptin and metformin.

Selectivity

Selectivity was determined in the presence of common excipients used in the tablet formulation. Sample containing 100% vildagliptin and metformin was injected first. Then the samples mixed with three different placebo formulations were injected to find out the selectivity of the method

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of vildagliptin and metformin at different concentrations level (80%, 90%, 100%, 110%, and 120%) were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of vildagliptin and metformin to obtain the calibration curves. The five concentrations of the standard were

subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments, by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) study. Repeatability was determined by performing four repeated analysis of the three standard solutions (90%, 100% and 110% of target concentration) of vildagliptin and metformin on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis of previous standard solutions on three different days (inter-day) in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 ml/min, amount of acetonitrile (10% to 15%,) the temperature of the column (28 °C to 32 °C) and PH of the mobile phase.

RESULTS AND DISCUSSION

The developed method has been validated as per ICH guidelines10. Every 20 μL of the working standard solution of vildagliptin in the mass concentration range of 5 to 50 μg/mL, and that for metformin in the mass concentration range of 12.5 to 125 μg/mL were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of vildagliptin and metformin were obtained by plotting the peak area ratio versus the applied concentrations of vildagliptin and metformin. The linear regression coefficients were found to be 0.9998 and 0.9991for vildagliptin and metformin respectively.

Repeatability of the method was checked by injecting replicate injections of the solution 10 μg/mL and 25 μg/mL of vildagliptin and metformin respectively and the RSD was found to be 0.326% and 0.435%. Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter- day precision). The results obtained for intra-day precision (RSDs) were 0.879 %& 0.945 % respectively, at *n* = 6, for both vildagliptin and metformin. The inter-day precisions (RSDs) were 0.254 % and 0.364 %, respectively, at *n* = 6, for both vildagliptin and metformin.

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, results are shown in Table 1.

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 μg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both vildagliptin and metformin from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The results of validation parameters and System suitability parameters were shown in Table 2.

Table 1: Recovery studies of vildagliptin and metformin.

Drug	Conc. Of Std. (µg/mL)	Conc. Of solution (µg/mL)	Amount found (µg/mL)	% Recovery	%RSD
Vildagliptin	10	10	19.90	99.50	0.324
	10	20	30.14	100.46	0.687
	10	30	40.87	102.17	0.652
Metformin	25	25	49.69	100.53	0.517
	25	50	74.82	99.38	0.674
	25	75	99.83	99.83	0.285

Table 2: System suitability of parameters

Validation parameters	Vildagliptin	Metformin
Linearity range (µg/mL)	5-50	12.5-125
LOD (ng/mL)	5	10
LOQ (ng/mL)	10	25
Intraday (%RSD)	0.879	0.945
Inter day (%RSD)	0.254	0.364
Repeatability (%RSD)	0.3251	0.4250
Accuracy	99.50-102.17	99.38-100.53
Peak purity index	1.0000	1.0000
Resolution factor (Rs)	-	19.618
r	0.9998	0.9991
Asymmetry factor (As)	**0.95**	
No. of theoretical plates (N)	3059	12500
Capacity factor (K)	-	0.330
High equivalent to theoretical Plates (HETP)	49.029	11.999
Tailing factor	1.149	1.140
Separation factor	-	7.902

Analysis of Formulation

Twenty tablets of vildagliptin and metformin in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 20 mg of vildagliptin and 50 mg of metformin was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase, and then the volume was made up to the mark with the mobile phase. The content was ultrasonicated for 30 min for complete dissolution. The solution was then what Mann's filter paper No-41. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 10 µg/mL of vildagliptin and 25 µg/mL of metformin, which was falling in the Beer's-Lamberts range with 50 µg/mL internal standards, showed good results and was selected for the entire analysis. The results of tablet analysis (*n* = 6) were found to be 99.81 and 99.44 for vildagliptin and metformin respectively.

From the typical chromatogram of vildagliptin, metformin and Gatifloxacin (Internal standard) **(Fig. 2)**, it was found that the retention time of vildagliptin was 2.1 min, Gatifloxacin was 2.5 min and metformin was 5.5 min, which were well-resolved peaks with a resolution factor of 3.3 for Gatifloxacin and 19.618 for metformin. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and the respective peak purity curve and overlay UV Spectrum were shown in **Fig 3, 4, & 5.** The results analysis was shown in **Table – 3.**
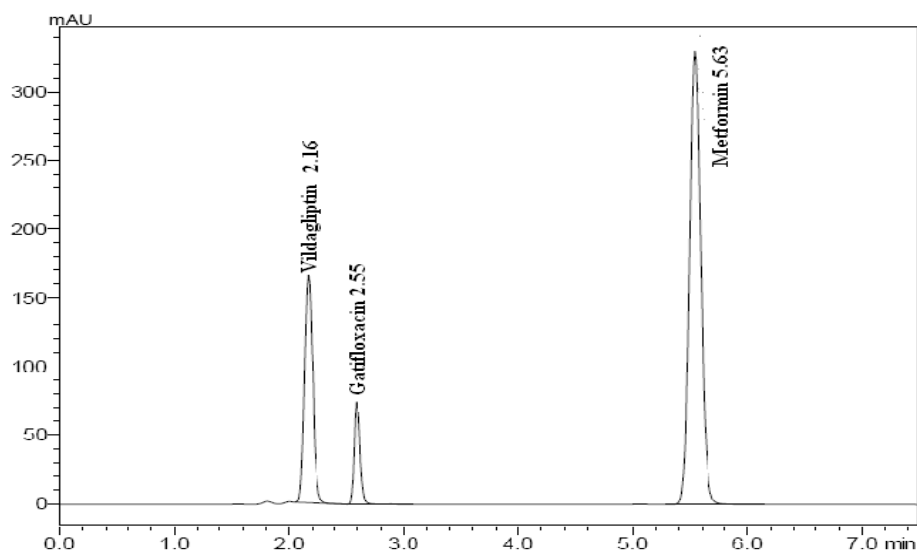

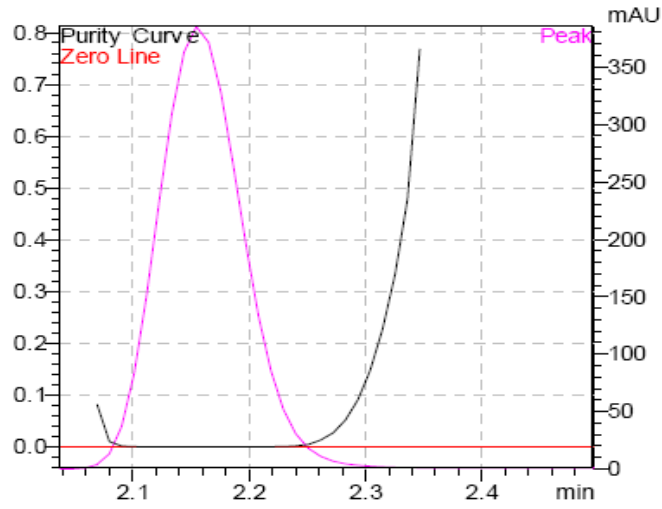**Fig. 2: Typical chromatogram of vildagliptin, Gatifloxacin and metformin.**

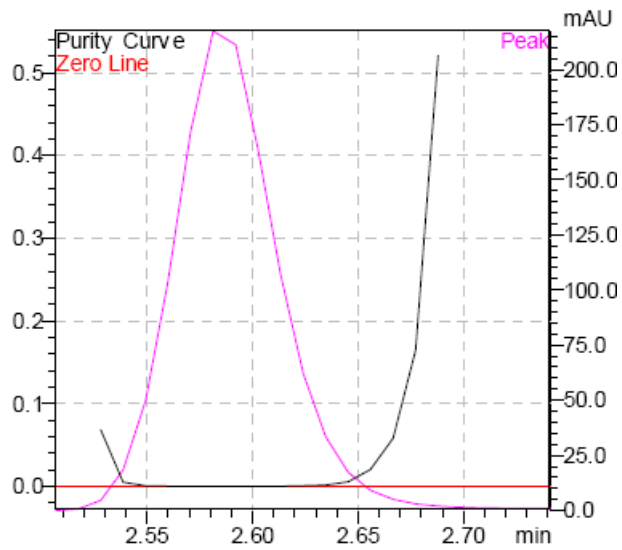**Fig. 3: Peak purity curve of vildagliptin**
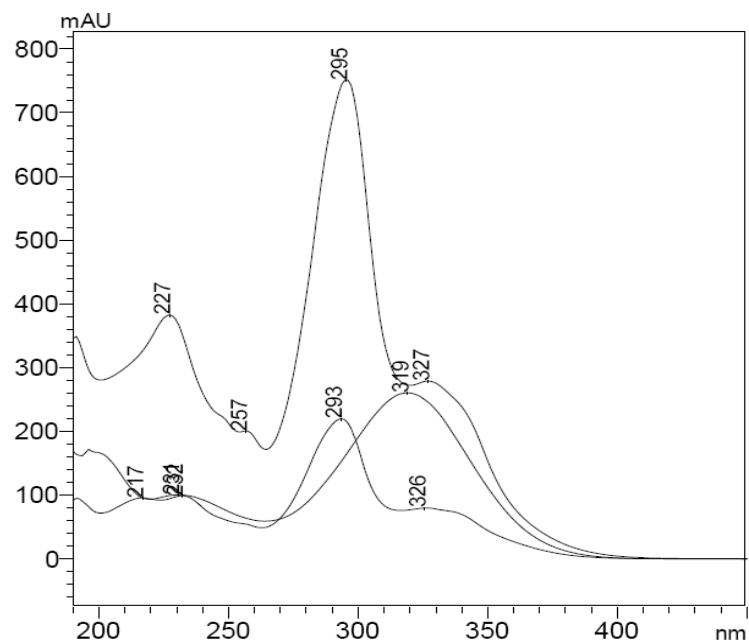

Fig. 4: Peak purity curve of metformin

Fig. 5: Overlaid UV spectrum of Vildagliptin, Gatifloxacin and metformin

Table 3: Analysis of formulation

Drugs	Labeled amount % lable claim	Amount taken(mg)	Amount found for assay (µg/mL)	(mg)
Vildagliptin	20	10	9.981±0.153	99.81
Metformin	50	25	24.86±1.025	99.44

CONCLUSION

The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for vildagliptin and metformin in the concentration ranges of 5 –50 µg/mL and 12.5– 125 µg/mL respectively. The correlation coefficient for vildagliptin was found to be 0.9998 and that for metformin was 0.9991. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of vildagliptin and metformin estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of vildagliptin and metformin simultaneously, from its pharmaceutical formulations and bulk drug.

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