STABILITY INDICATING RP-HPLC METHOD FOR QUANTIFICATION OF VILDAGLIPTIN AND METFORMIN

Lalit K Sahu[1]*, Sudam Si[2], Saroja Kumar Patro[3]

Abstract

A novel, simple, robust and stability-indicating RP-HPLC method has been developed and validated for the simultaneous determination of Vildagliptin and Metformin hydrochloride in the tablet dosage form. The method shows best separation of Vildagliptin and Metformin from their degradation products. Separation was effected on a Sunniest ECO C_{18}, 250 mm x 4.6 mm, 5 mm analytical column at wavelength of 210 nm, using a mobile phase buffer (pH-6.5): acetonitrile (77:23) in an isocratic elution mode at a flow rate of 2.0 ml/min, Injection volume: 10 µl and run time 6mins.All the validation parameters of Analytical Performance like linearity, recovery, and intraday and inter day precision, Limit of Detection (LOD), Limit of Quantitation (LOQ) Robustness and specificity were found to be within acceptance criteria as per ICH guidelines. Retention times under the optimized condition were 2.458 and 4.299 min for Metformin and Vildagliptin, respectively. The results of recovery studies were found to be between 99.0% and 100.38% which indicates the accuracy of the method. The % RSD for inter day and intraday precision studies was found to be less than 1.5%. Robustness and ruggedness were expressed in terms of %RSD which was also within the acceptable limits. The newly developed method was precise, robust and stability indicating as no interfering peaks of degrades and excipients were observed. The proposed method is fast, sensitive and suitable for routine adoption in quality-control laboratories for quantification of both the drugs individually and in combined dosage form and with tremendous precision and accuracy.

Keywords: RP-HPLC, Stability indicating, Vildagliptin, Metformin, Validation, ICH guidelines.

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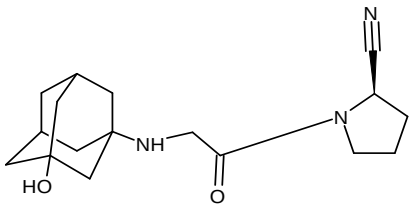
DOI: - 10.31838/ecb/2023.12.si6.00xyz

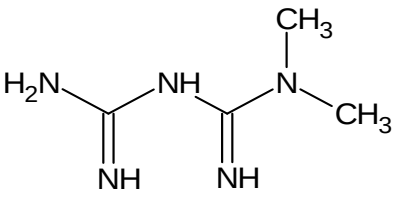
INTRODUCTION

About 463 million adults are living with diabetes globally. The proportion of people with type 2 diabetes is increasing in most countries [1]. Diabetes mellitus type 1 is a disease caused by the lack of insulin secretion, and type 2 diabetes mellitus (T2DM) is a disease caused by insulin resistance by cells. Anti-diabetic drugs are used to treat diabetes mellitus by reducing the glucose level in the blood. Type 2 diabetes mellitus, which is characterized by polyphagia, polyuria, and polydipsia and needs a lifetime treatment with anti diabetic drugs [2]. Metformin hydrochloride (MTF), [3-(diaminomethylidene)-1,1-dimethylguanidine] is used for the management of T2DM as it improves glucose tolerance, and decreases the postprandial and basal plasma glucose. It improves insulin sensitivity by decreasing glucose intestinal absorption and hepatic production, and it is the most important therapy which is used in combination with other orally administered hypoglycemic[3]. Vildagliptin (VLD) [(2*S*)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile] is also used for the treatment of T2DM. It is an orally administered anti-hyperglycemic agent of DDP-4 class of drugs [4-5].

In many cases, therapy with a single glucose-lowering agent does not provide adequate glycemic control and consequently, most patients with T2DM require therapy with multiple oral anti diabetics. Fixed Dose Combinations (FDCs) have certain advantageous over multiple pills, including convenience, ease of administration, and a reduction in the pill burden. Thus, FDCs potentially improve patients' treatment adherence and optimize the achievement and maintenance of glycemic targets [6]. The FDC of Metformin and Vildagliptin produce synergic effect and offers advantages over other oral anti-hyperglycemic combinations, with almost no risk of hypoglycemia. So the combination of these drugs in a single tablet is mostly used to achieve best glucose control in blood and for better compliance to therapy[7]. The proposed study aims to development and validation of RP-HPLC method for quantification of Vildagliptin and Metformin in combination tablets.



(a)



(b)

Fig 1. (a) Vildagliptin (b) Metformin

Various analytical techniques are described in the literature to analyze Metformin and Vildagliptin either individually or in combination with other drugs by spectrophotometry[8-12], High-Performance Liquid Chromatography (HPLC) [12-18] and GC-MS [19]. However, the proposed stability-indicating chromatographic method for determining Vildagliptin and Metformin HCl in the dosage form is less retention times and higher resolution in comparison to reported methods. The major goal of the present study is to develop a faster validated stability-indicating HPLC method for the estimation of Vildagliptin and Metformin simultaneously in bulk and dosage formulation

MATERIALS AND METHOD

Chemicals and Reagents

Reference sample of Vildagliptin and Metformin HCl, Acetonitrile, Water (HPLC grade) Potassium dihydrogen orthophosphate (AR grade), 1-Octance sulphonic acid sodium salt anhydrous (AR Grade), Potassium Hydroxide (AR Grade), Water (HPLC Grade) were used in the analysis.

Instruments and Software

A Schmidazu LC 2010 CHT with quaternary constant flow system and equipped with auto sample injector was utilized in the study. Shimadzu LC Solution software was employed to monitor and integrate the output signals. Other instrument and apparatus such Contech Analytical balance, Eutech pH meter, Leela Sonic Ultra-sonicator were used in this study.

Optimized Chromatographic conditions

The separation of Metformin and Vildagliptin was achieved by using Column: Sunniest ECO C_{18}, 250 mm X 4.6 mm, 5µm at a Column temperature of 35°C with Phosphate Buffer: Aetonitrile (77:23) as mobile Phase, at a Flow rate of 2.0 ml/minute. Elution effected in isocratic mode with Injection volume of the analyte sample of 10 µl and detection at 210 nm with Run time 6.0 minutes

Preparation of Mobile Phase and Diluent

The buffer is prepared by dissolving 2.72gm of Potassium dihydrogen orthophosphate and 1.62gm of 1-Octance sulphonic acid sodium salt anhydrous in 1000ml of HPLC grade water and its pH was adjusted to 6.5 with Potassium hydroxide solution. The mobile phase is prepared with the ratio of buffer and Acetonitrle 77:23(v/v). The diluents solution was prepared by mixing of acetonitrile and water in ratio 40:60 (v/v).

Standard Stock Solutions

Standard Stock solutions of Vildagliptin and Metformin HCl were prepared separately weighing 50mg of Vildagliptin and 500mgof Metformin HCl into two separate 100mlvolumetric flask and was added 40ml of diluent. Then the flasks were sonicated with the help of ultra sonicator to dissolve the drug. Then the flask was diluted up to the mark with the diluent to produce 500µg/ml of Vildagliptin and 5mg/ml (5000µg/ml) of Metformin.

Preparation of Working Standard Solution

2ml, 3ml, 4ml, 5ml, 6ml, 7ml of Standard stock solutions of Vildagliptin and Metformin HCl were transferred into six different 50ml volumetric flasks and then the volume was made up to the mark with diluents to produce the concentration of the drug in the given Table 1.

Preparation of Sample Solution from Marketed Tablets

Commercial tablets available in local market procured and an average weight of 20 tablets was determined and powdered finely in a mortar. Powdered tablet equivalent to 50 mg of Vildagliptin and 500mg of Metformin HCl was accurately weighed and transferred into a 100 ml clean dry volumetric flask which was dissolved with diluent and sonicated and made volume up to volume with the diluents and filtered. Further transferred 3ml of filtrate into another 50ml volumetric flask and made up to the mark with the diluent.

Method Validation [20]

The HPLC method was validated in terms of System linearity, specificity, sensitivity, precision and accuracy, robustness in accordance with ICH Q2 (R1) guideline and system suitability test as per USP.

Forced degradation study

Force degradation studies have done to develop a stability-indicating assay by involving the proposed optimized RP-HPLC conditions by utilizing acidic, basic and oxidative, conditions at final concentration of 50µg/mL of Vildagliptin and 500 µg/mL of Metformin from tablet under investigation.

***Acid Degradation*:** Taken 1.0mL of the stock solution into the 10 ml graduated flask and added 1.0mL of 0.1 N HCl solutions and mixed well with this solution it was kept for 2hrs at room temperature. Then the volume was adjusted with the diluent. After making final solution run into HPLC, the peak area and shape were observed under optimized chromatographic conditions.

***Base Degradation*:** Taken 1.0 mL of stock solution, which transferred into the 10 ml volumetric flask, then added 1.0 ml of 0.1 N NaOH solution it mixed well, and it was kept for 2hrs at room temperature. Then the volume was adjusted with the diluents. After making the final solution run into HPLC and the peak area and shape observed under optimized chromatographic conditions.

***Oxidative Degradation*:** 1 mL of stock solution was taken and transferred into the 10 mL volumetric flask, and then added 1 mL of 3.0% H_2O_2 solution was mixed well and kept for 2hrs at room temperature. Then the volume was adjusted with the diluent. After making the final solution run into HPLC, the peak area and shape were observed under optimized chromatographic conditions.

RESULTS

Method Development and Optimization

The main objective of the proposed method was obtaining an optimized chromatographic condition that result effective resolution of both analyte in acceptable retentions and better chromatographic peaks in terms of sharpness and symmetry within a short run time. After several systematic trials the optimized condition was obtained on Sunniest ECO C_{18} (250 mm X 4.6 mm, 5µm) column, with a mobile phase composed of Phosphate Buffer: Aetonitrile (77:23 v/v) in an isocratic flow at a rate of 2.0 ml/min. The volume of auto sampler injection was adjusted to 10 µl, column temperature was set at 35 ℃, and detection wavelength was chosen at 210 nm. Metformin and Vildagliptin were successively eluted at the retention time (Rt) of 2.458 min and 4.299 min respectively, using the optimized condition. The optimized chromatogram is presented in Fig 2.

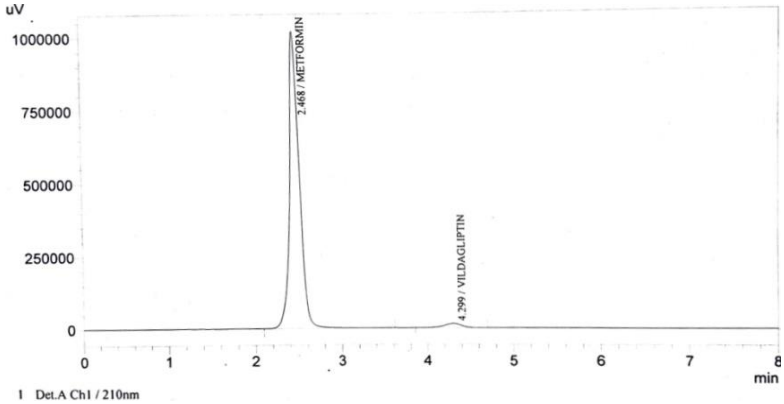


Fig 2. Representative chromatogram of Vildagliptin and Metformin (50µg/ml and 500µg/ml)

Linearity: The working standard Solutions prepared by diluting 2ml, 3ml, 4ml, 5ml, 6ml, 7ml of each of Vildagliptin and Metformin Standard stock solutions into six different 50ml volumetric flasks and then the volume was made up to the mark with diluents to produce the concentration of the drug in the given Table 1.

Specificity

The specificity chromatograms of the blank depicted in Fig 3. indicate that no interfering peaks were observed at the retention times of Metformin and Vildagliptin

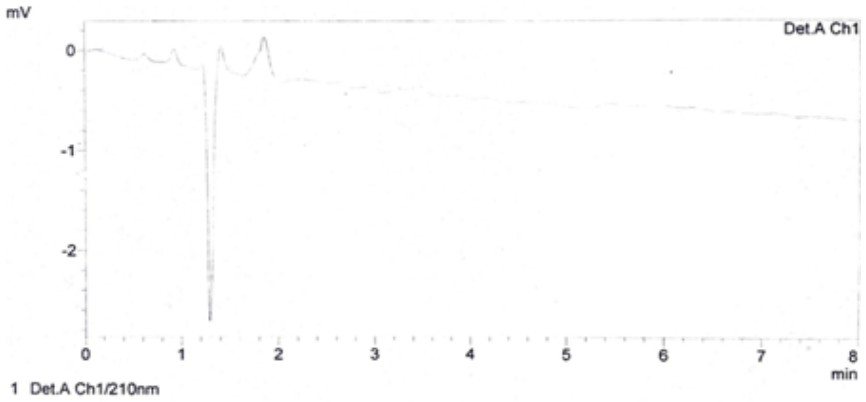


Fig 3. Representative chromatogram of Blank

The peak purity of Vildagliptin and Metformin were assessed by comparing the retention time (R_t) of samples of standard mixture and tablet samples of Vildagliptin and Metformin. Overlay chromatogram in Fig 4., depicts good correlations between the retention time of standard and dosage form drugs.

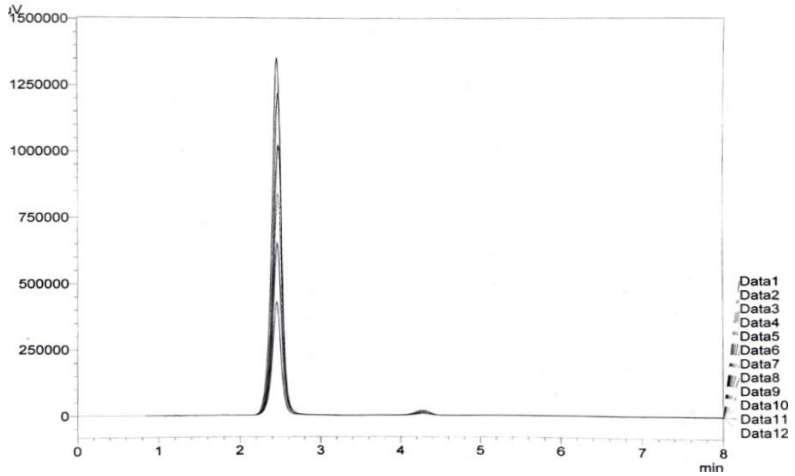


Fig 4. Overlay Chromatogram of Standard and Tablet Test Samples

Linearity

The proposed method was linear for both drugs in the investigated concentration ranges of 200-700 µg/ml for Metformin HCl, and 20-70 µg/ml for Vildagliptin. The linear regression equation was found to be for Metformin Y = 16,084.2825 X + 156,808.8770 and r^2 = 0.9985. The linear regression equation was found to be for Vildagliptin Y = 4191.0352 X – 1137.0738 and r^2 = 0.9998. Here Y denotes peak area and X represents the corresponding concentration. The linearity curves of Vildagliptin and Metformin are shown in the Fig 5 and 6 respectively.

Table 1. Linearity data of Metformin and Vildagliptin

Sl. No.	Metformin Conc.(µg/ml)	Area	Vildagliptin Conc. (µg/ml)	Area
1	0	0	0	0
2	200	3362945	20	82188
3	300	5157493	30	123665
4	400	6673903	40	165483
5	500	8249662	50	207693
6	600	9905478	60	253067
7	700	11175744	70	291524

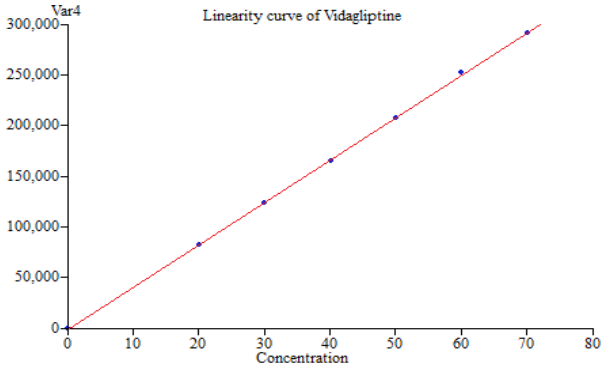


Fig 5. Linearity curve of Vildagliptin

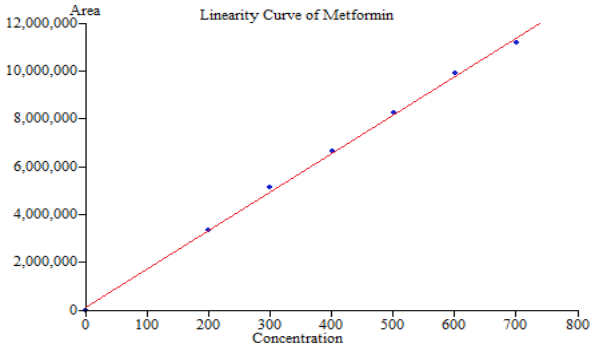


Fig 6. Linearity curve of Metformin

Analysis of Commercial Tablets

The test samples solutions prepared from commercial tablets were injected into instrument for tablet analysis and the results are given in the Table 2. The representative tablet analysis chromatogram of Vildagliptin and Metforminis shown in the Fig 7.

Table 2. The results of tablet analysis

Analyte	Label claim (mg/Tablet)	**Amount Found (mg/Tablet)***	C.I.	%RSD	SE	t
Vildagliptin	50	51.2	100.70±1.47	1.179	0.532	1.13
Metformin	500	500.342	100.068±0.26	0.207	0.093	0.72

* Average of five determination

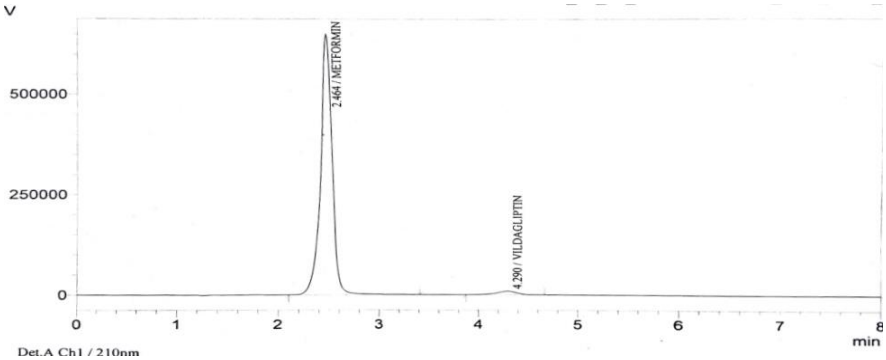


Fig 7. Representative chromatogram of tablet sample of Vildagliptin (30 µg/ml) and Metformin(300 µg/ml)

Method Validation[20]

The HPLC method was validated in terms of linearity, sensitivity, precision, and accuracy, robustness in accordance with ICH Q2 (R1) guideline and system suitability test as per USP.

System Suitability

The system suitability test was carried out on 30µg/ml and 300µg/ml of Vildagliptin and Metformin respectively by using five replicate injections. The system suitability is conformity of chromatographic parameters that ensures the performance of the analytical system. All the parameters of system suitability met the acceptance criteria. The system suitability parameters that were monitored for Vildagliptin and Metformin are given in the below Table 3.The tailing factors were less than 1.6, the number of theoretical plates was greater than 3000, and the resolution was more than 8.

Table 3. Results of the system suitability parameters

Sl. No.	Parameter	Specification	Vildagliptin	Metformin
1	Retention Time	----	4.299 ± 0.003	2.458 ± 0.003
2	Tailing factor	< 2.0	1.181	1.511
3	Theoretical Plates	> 2000	7494	3421
4	Area (% RSD)	< 2	0.21	0.38
5	Resolution (Rs)	> 2.0	8.02	----
6	Capacity factor (k′)	> 1.0	4.56	2.44

Accuracy

Recovery studies using the conventional addition method was performed to test the accuracy of the proposed method. It was performed at 50 % and 100 % level. The results are shown in the below Table 4

Table 4. Recovery study of the drugs

% Level of recovery	Analyte	Conc. of Drug in Sample of Formulation	Amount of drug added (µg/ml)	Conc. of drug found (µg/ml)	% RSD	SE	CI	t
50	VLD	30	15	45.122	1.333	0.599	100.271 ±1.66	0.453
	MTF	300	150	450.288	0.366	0.164	100.064±0.454	0.39
100	VLD	30	30	60.65	1.837	0.817	101.083±2.265	1.327
	MTF	300	300	600.812	0.21171	0.09507	100.135±0.264	1.423

SD: Standard deviation, SE: standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis of Recovery study (n = 5). Theoretical 't' values at 95% confidence level for n - 1 degrees of freedom t (0.05, 4) = 2.776

Precision

The intraday precision/repeatability can be determined by injecting three working standard solutions and test sample injections. The areas of all the injections were taken and % Relative standard deviations were calculated. The % RSD was found to be less than 1.5 standard and test sample solutions.

The inter day precision can be determined by utilizing three different concentration working standard solutions and three different test sample

solutions were injected on three different days. The areas of all the injections were taken and % Relative standard deviation was calculated. The results obtained were found to be less than 1.5% in standard and test sample solutions.

Robustness

The robustness of the proposed assay method was determined by introducing small changes in the chromatographic condition which included wavelength (208 nm212 nm), flow rate (1.8 and 2.2 mL/min) and organic phase (+5% to -5%). The % RSD shows with in the 1.5. So it indicates that the method is robust.

Sensitivity

The sensitivity of the method was estimated by calculating LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve. The Lower limit of detection and limit of quantization were found to be 0.276, and 0.796µg/ml respectively.

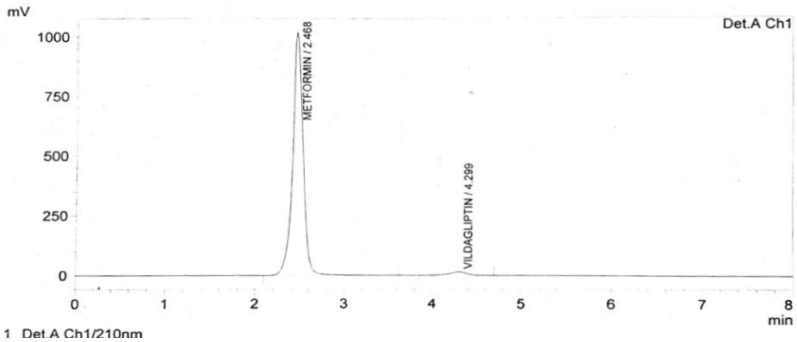


Fig 8. Representative chromatogram of Vildagliptin and Metformin (50µg/ml and 500µg/ml)

Forced degradation study

Force degradation study results showed degradant peaks observed when the drug sample were stressed with alkali and hydrogen peroxide, while no apparent degrading peaks were seen in acid degradation. The percent degradation of Metformin ranged from 24.4 - 1.2, the maximum and minimum recorded in alkali and acidic conditions respectively. For Vildagliptin percent degradation ranged from 39.2 -3.8, the maximum and minimum recorded in oxidative and acidic conditions respectively. All peaks of degradation studies are well resolved. Results of forced degradation data are presented in table and chromatograms are presented in Fig 9-11.

Table 5. The results of forced degradation study of the Vildagliptin and Metformin

Stress conditions	Degradation (Time) (hrs)	Metformin		Vildagliptin	
		% Assay	% Degradation	% Assay	% Degradation
Acid (0.1 M HCl)	2	98.77	1.22	96.18	3.82
Base (0.1 NaOH)	2	75.62	24.38	88.038	11.962
Hydrogen Peroxide (3%)	2	83.130	16.86	60.78	39.22

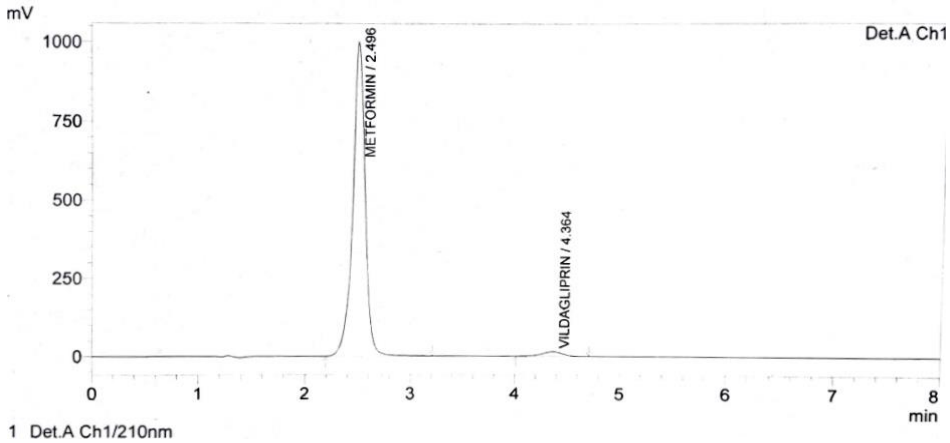


Fig 9. Chromatogram of Acid degradation Study

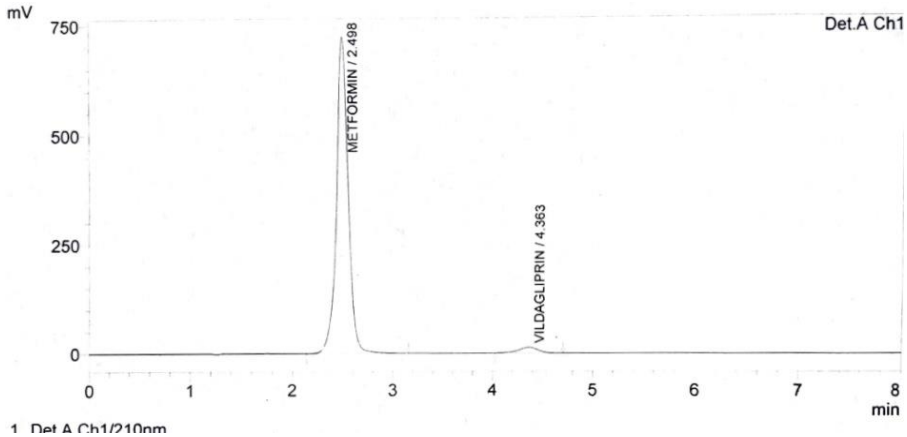


Fig 10. Chromatogram of Alkali degradation Study

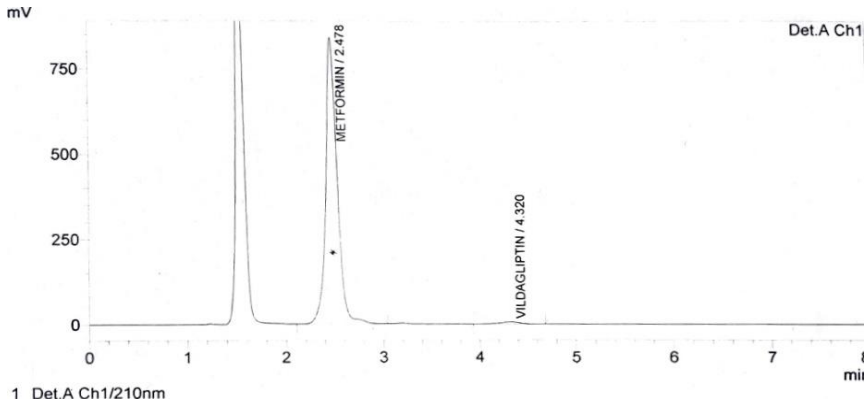


Fig 11. Chromatogram of Peroxide degradation Study

DISCUSSION

Chromatographic parameters were preliminary optimized to develop a stability-indicating method for Metformin HCl and Vildagliptin. The method development process was carried out by examining different conditions like mobile phase compositions like Water: Methanol, Water: Acetonitrile, buffer: methanol, Acetonitrile: buffer with different ratios. The method was tried by utilizing different types of mobile phase compositions and different ratios of buffer and acetonitrile i.e. 70+30, 30+40, 50+50, 40+60 and 77 + 23. The buffer and acetonitrile ratio was having 77% and 23% given the best chromatographic peak having Retention time of 2.458 and 4.299mins for Metformin and Vildagliptin respectively. In comparison to previously reported HPLC methods [12-18], the proposed method is fast and requires less solvent consumption as the total run time was less than 6 min, The modalities adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries was obtained in the range of 98.65 to 101.13. The results of analysis of average recoveries obtained in each instance were compared with the theoretical value of 100 percent by means of Student's 't' test at 95 percent confidence level. As the calculated 't' values are less than theoretical 't' values (Table 4.), it is concluded that the results of recoveries obtained are in agreement with 100 percent for each analyte. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets.

As per the validation results, all the parameters were within the acceptable limits of ICH guidelines [20]. The system suitability test results evidenced that more efficient separation (N>2000), with well-resolved (R>2) and symmetric peaks (T<2) were obtained consistently. The method was found to be selective because formulation excipients didn't interfere with blank determinations. The r^2 obtained from the least square regression analysis for both drugs was closer to 1 which indicates the better linearity of the developed method. The lower limit of detection and limit of quantization were found to be 0.276 and 0.796 µg/ml respectively. Percentage relative standard deviation (% RSD) was found to be less than 1.5% for all the parameters in the robustness study, which proves that method is robustness.

Chemical stability of pharmaceutical compounds is a major concern since it impacts the drug's safety and efficacy. Knowledge of molecular stability aids in the selection of appropriate formulation and packaging, as well as giving adequate storage conditions and shelf life, which is required for regulatory paperwork [21]. According to ICH guideline "Stability testing of new drug substances and products," stress testing is required to elucidate the inherent stability characteristics of the active substance, so the drugs were subjected to oxidation acid and alkaline degradation. The sample was injected under various stress conditions. The acidic degradation, base degradation, oxidative degradation, was performed as per procedure, and percent degradation was calculated from the chromatographic peaks. Metformin and Vildagliptin in standard as well as sample mixture in acid degradation, base degradation, oxidative degradation. It was found that the quantum degradation of both drugs in acidic and basic medium was reasonably acceptable (≤ 30 %) as per ICH guidelines. But the Vildagliptin is extremely sensitive to oxidative degradation and degraded more than 30 % in 3 %hydrogen peroxide and it is not acceptable as per ICH guidelines. No separate degrading peaks were co eluted with analyte peaks in the chromatograms in stressed studies. It indicates the method is capable of quantify both analyte in presence of their degradation products.

C**ONCLUSION**

A new simple and faster liquid chromatographic method was successfully developed and validated for simultaneous quantification of Vildagliptin and Metformin in bulk and Tablet formulation. The method was demonstrated to be stability-indicating, fast, sensitive, accurate, precise, and robust. Thus, the developed method can be easily adopted for the routine quality control of bulk and in the combination tablet dosage form.

ACKNOWLEDGEMENT

The authors thanks to the Director of Fine Cure Pharmaceuticals Limited for providing necessary facilities to develop and validate this method.

FUNDING

Nil

AUTHORS CONTRIBUTION

All authors have contributed equally.

CONFLICT OF INTERESTS

Author declares that there have been no conflicts of interest

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