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DEVELOPMENT AND VALIDATED STABILITY- INDICATING ASSAY FOR SIMULTANEOUS ESTIMATION OF NEW ANTIDIABETIC DRUGS AS BULK & AMP; PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, precise and stability-indicating HPLC method was developed and validated for the simultaneous determination of metformin hydrochloride (MET) and vildagliptin (VLG) in pharmaceutical dosage forms. The method involves use of easily available inexpensive laboratory reagents. The separation was achieved on Grace Cyano column (250 mm×4.6 mm) 5 µm with isocratic flow. The mobile phase was pumped at a flow rate of 1.0 mL/min, consisted of 25 mM ammonium bicarbonate buffer and acetonitrile (65:35, v/v). The UV detection was carried out at 207 nm. A linear response was observed over the concentration range of 25-125 µg/mL for MET and 50-250 µg/mL for VLG respectively. Limit of detection and limit of quantification for MET were 0.36 µg/mL and 1.22 µg/mL, and for VLG were 0.75 µg/mL and 2.51 µg/mL respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria for specificity, linearity, accuracy, precision, robustness, and system suitability. Individual drugs (MET and VLG) were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions. The resultant stressed samples were analyzed by the proposed method. The method gave high resolution among the degradation products and the analytes. The peak purity of analyte peak in the stressed samples was confirmed by photo diode array detector. The proposed method was successfully applied for the quantitative analysis of MET and VLG in tablet dosage form, which will help to improve quality control and contribute to stability studies of pharmaceutical tablets containing these drugs.

KEYWORDS: RP-HPLC, metformin hydrochloride (MET), vildagliptin (VLG).

1.0. INTRODUCTION

Analytical Chemistry is defined as "The science and the art of determining the composition of materials in terms of the elements or compounds contained." This branch of chemistry, which deals with both theoretical, practical science and practiced in a large number of laboratories in many diverse ways. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species that is to find out what substance is composed and exactly how much. In quantitative analysis the question is how much is present. The research work in this thesis is based on this criterion. Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations. Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no "second quality" in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production.[1-4]

2.0. AIM AND OBJECTIVES

As per the literature review, there is no analytical methods reported for the estimation of Metformin hydrochloride and Vidagliptine in combined pharmaceutical dosage form by HPLC Chromatographic parameters. Various publications are available regarding the UV simultaneous estimation and RP-HPTLC method development of Metformin hydrochloride and Vidagliptine, either alone or in combination with other drugs in pharmaceutical dosage form. Hence,there is a need for suitable RP-HPLC and Method for routine analysis of Metformin hydrochloride and Vidagliptine in the combined formulation. The work was an attempt to develop simple, rapid, and sensitive analytical methods for the simultaneous estimation of Metformin hydrochloride and Vidagliptine in the combined formulation in accordance with ICH Q2B guidelines and to extend the method for routine analysis.

3.0. PLAN OF WORK

Present work is to develop and validate a new simple, rapid, and sensitive method for the simultaneous estimation of Metformin hydrochloride and Vidagliptine in the combined tablet dosage form by RP-HPLC method.

STEP-1: Study of physiochemical properties of the drug

STEP-2: Selection of chromatographic condition Wave length, mobile phase, column, flow rate

STEP-3: Optimization of the method for stability studies

STEP-4: Study of the system suitability parameters

STEP-5: Validation of the proposed method

MATERIALS AND METHODS FOR- HPLC

Chemicals and reagents

Table No.1: List of Chemicals and reagents.

S. No.	Name	Manufacturer	Grade
1.	Metformin hydrochloride working standard	Jivanta life sciences	-
2.	Vidagliptine and HPTLC working standard	Chemisol	-
3.	Oratil LZ	Macleods pharmaceuticals. Ltd	-
4.	Potassium dihydrogenphosphate	Merck	GR
5.	Sodium perchlorate	Merck	GR

6.	Perchloric acid	Merck	GR
7.	Ortho phosphoric acid	Merck	GR
8.	Methanol	Merck	HPLC
9.	Acetontrile	Merck	HPLC
10.	Water	Merck	HPLC
11.	0.45 µm Nylon filter	Axivia	S0761009
12.	0.45µm PVDF filter	Rankem	D004A07

Equipment/Instrument details

Table No.2: List of Equipment/Instrument details

S. No.	Instrument Name	MODL
1.	HPLC system	Agilent 1220 InfinityLC(G4288C)
2.	Analytical balance	Shimadzu
3.	pH Meter	Thermo electron corporationorion 2 star
4.	Sonicator	Ultrasonic cleaner power sonic 420
5.	Vacuum oven	Wadegati
6.	Constant temperature water bath	Thermolab GMP

Analytical method development for the simultaneous estimation of Metformin hydrochloride and Vidalgliptine by RP-HPLC

A. Selection of wavelength

A solution of 100µg/mL of Metformin hydrochloride and Linezolid were prepared in methanol. The resulting solutions were scanned individually from 190 to 400 nm in UV-Visible spectrophotometer. Spectrums obtained are shown in Fig.No.9-11.

B. Selection of chromatographic condition

Proper selection of the method depends up on the nature of the sample (ionic/ ionisable / neutral molecule), its molecular weight and solubility. The drugs selected in the present study, were polar in nature. Thus reverse phase HPLC was selected forthe initial separation because of its simplicity, suitability, ruggedness and its wider usage.

C. Initial separation condition

The mobile phase selected to elute the drug from the stationary phase was acetonitrile and phosphate buffer, because of its favorable UV transmittance, low viscosity and low back pressure.

D. Effect of buffer

Potassium di-hydrogen phosphate buffer was selected because better and higher intensity of response was obtained.

E. Effect of pH

The mobile phase pH was optimized using different pH, ranging from 2.0 to 3.0 (pH is adjusted with Ortho phosphoric acid), at a flow rate of 0.5 mL/min and symmetry Xterra C18 column as the stationary phase. The peak shape and resolution was observed at different pH.

F. Effect of ionic strength

The phosphate buffer was prepared in different strengths such as 0.01M, 0.025M, 0.05M of Potassium di-hydrogen phosphate at pH 2.8. The retention time wasdecreased by increasing the buffer strength. For the present study, the optimizedmobile phase composition phosphate buffer of pH 2.8: acetonitrile (35:65v/v) wasselected, because of the

retention times of Metformin hydrochloride and Linezolid wereeffected due to slight change of ionic strength during analysis.

G. Effect of nature of stationary phase

The following stationary phases were used and the chromatograms were recorded.

1. Agilent zorbax SB C18 (4.6 x 150mm, 5mm)
2. Symmetry C8 (4.6 x 100mm, 5mm)
3. Symmetry XterraC18 column.

With Agilent zorbax SB C_{18} and Phenomex-kinetex-XDB-C18 the results obtained were not satisfactory because peak tailing was observed and also the resolution between the peaks was comparatively lesser than that with symmetry XterraC18. With XterraC18 column the peak shape and resolution observed were good. Therefore, Xterra C18 column was used for further studies.

Preparation of Placebo

The amount of powdered inactive ingredient supposed to be present in 10 tablets were accurately weighed and transferred in to 100 ml volumetric flask, 70 ml of diluent was added and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 0.6 ml of upper clear solution was transferred to a 100 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 mm filter before injecting into HPLC system.

Preparation of Ammonium bicarbonate buffer

7.0 grams of NH_4CO_3 was weighed into a 1000ml beaker, dissolved and diluted to1000ml with HPLC water. The flask was shaken until the particles get dissolved andvolume was made up to the mark with Water. The pH was adjusted to 2.8 with orthophosphoric acid.

Solution preparation

MET and VLG standard stock solution MET (1 mg/mL) and VLG (1 mg/mL) standard stock solution was prepared by transferring approximately 100 mg of MET and 100 mg of VLG reference standards to a 100 mL volumetric fl ask. About 50 mL MilliQ water was added. It was then sonicated for 10 min and water was added to make up the volume in the fl ask. Each stock solution was further diluted 10 times, with the diluent, to produce reference standard and test solutions containing MET (100 µg/mL) and VLG (100 µg/mL).

Method development

A variety of mobile phase were investigated in the development of a stability indicating LC method for the analysis of MET and VLG in tablet dosage form. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay, stability studies and separation among degradants formed during forced degradation studies. Inorder to avoid poor resolution column was changed and flow rate was decreased.

Mobile phase: Ammonium bicarbonate buffer and acetonitrile (80:20, 75:25, 70:30, 60:40 and 55:45; v/v)
Diluent: Methanol.

Chromatographic conditions

Flow rate	**:**	1.00 ml per min
Column	**:**	Symmetry C8(4.6 x 100mm, 5mm)
Detector wavelength	**:**	207 nm
Column oven	**:**	Ambient
Injection volume	**:**	20 ml.

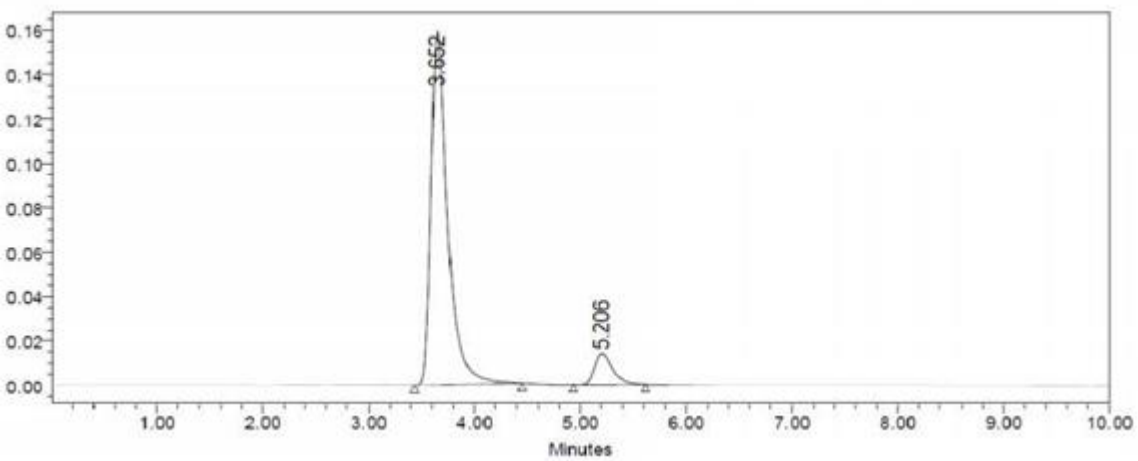


Fig. no. 1: Chromatogram of MET and VTG.

Observation: peaks were eluted with less resolution. The chromatogram of simultaneous estimation of MET and VTG Fig.No-2.

Forced degradation studies

Forced degradation study was conducted on samples containing individual drugs. Intentional degradation was carried out by exposing 20 mg of reference standard dissolved in 20 mL of 2 N hydrochloric acid for 6h at 80 °C using a water bath. The solutions were withdrawn in a 10 mL volumetric flask allowed to attain room temperature and then neutralized with base. For alkaline degradation 20 mg reference standard dissolved in 20 mL of 0.1 M NaOH for 30 min at ambient temperature. The solutions were withdrawn in a 10 mL volumetric flask and neutralized with acid. For oxidative degradation 10 mg of reference standard dissolved in 0.3 % hydrogen peroxide in 10 mL volumetric flask at ambient temperature. For thermal stress study, the solid drug and tablets were kept in dry oven at 60 °C for 10 days. Photolytic study was carried out on solid drugs, their combination and their dosage form. The sample in a petri-plate was spread as a thin layer (1 mm) and exposed to light in a photo stability chamber. Blank solutions were prepared by aforementioned procedure where in stock solutions were replaced with diluent.

Method validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1).

Linearity

Standard stock solutions of drugs were diluted in the concentration range of 25–125 µg/mL MET and 50–250 µg/mL VLG respectively for the determination of linearity. 3 sets of such solutions were prepared. Each set was analyzed to

plot a calibration curve. Standard deviation (SD), slope, intercept and coefficient of determination of the calibration curves were calculated to ascertain linearity of the method.

Recovery

Recovery of the method was determined by spiking the marketed sample with 80 %, 100 %, and 120 % standard solutions. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (% RSD), of spiked drug were calculated.

Precision the precision of the proposed method was evaluated by carrying out 6 replicate independent assays of test sample. RSD (%) of 6 assay values obtained was calculated.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ for MET and VLG was estimated by considering signal to noise ratio of 3:1 and 10:1, which was determined by injecting a series of 6 diluted solutions with known concentrations.

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ± 0.1 mL/min), buffer pH (altered by ± 0.2) and buffer concentration by ± 10 mM. These chromatographic variations were evaluated for resolution between MET and VLG peaks.

Solution stability

To assess the solution stability, standard and test solutions were kept at 25 °C (laboratory temperature) for 24 h. These solutions were compared with freshly prepared standard and test solutions.

System suitability

The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between MET peak and VLG peak were defined.

STABILITY STUDIES OF METFORMIN AND VIDAGLIPTINE

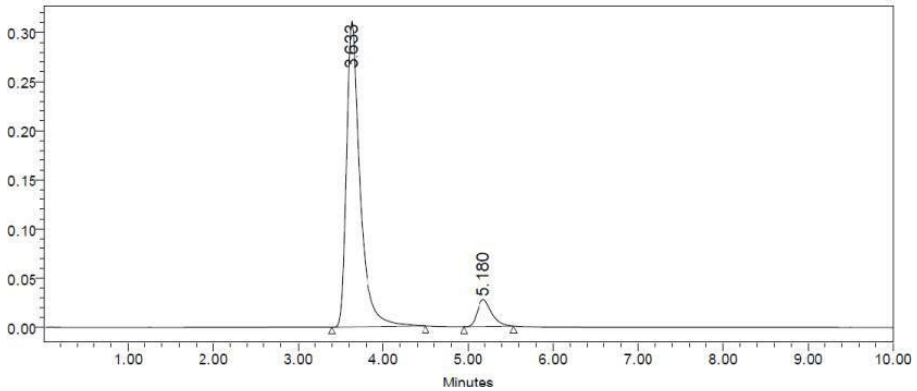
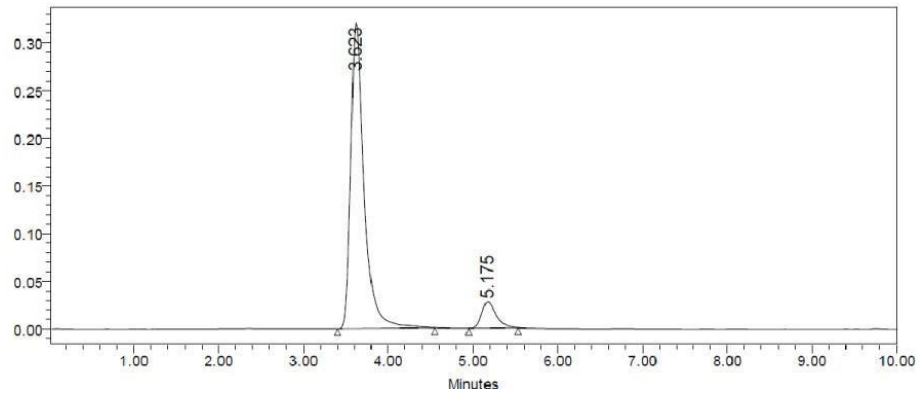
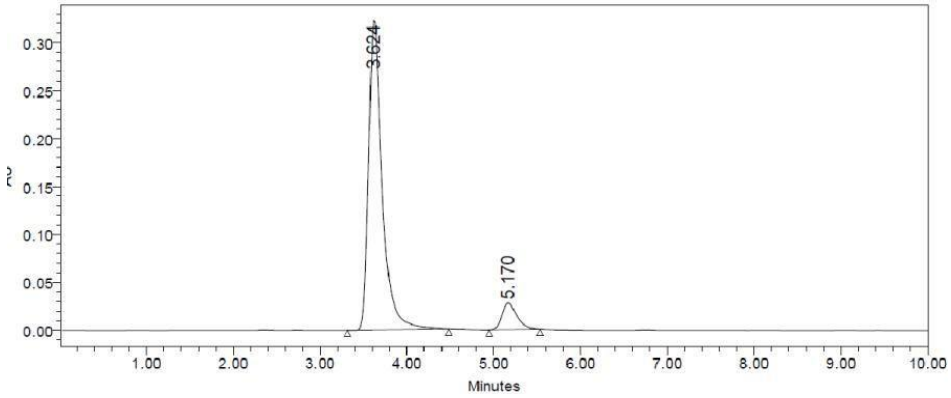


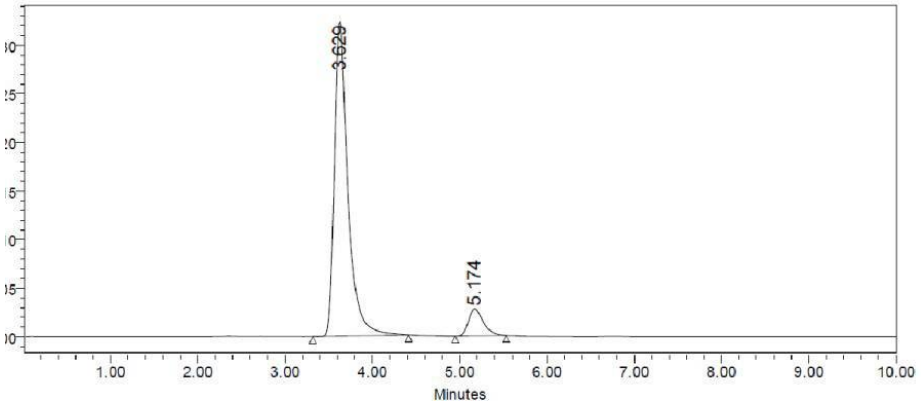
Fig-2: Chromatograms of forced degradation solutions a) Neutral hydrolysis.



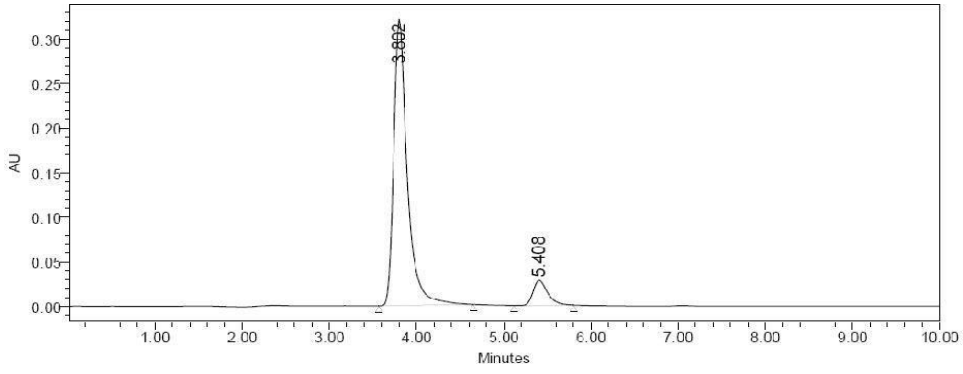
Acid hydrolysis



Base hydrolysis



Oxidative hydrolysis



Thermal degradation

METHOD VALIDATION PARAMETERS

Table 3: Intra-day Precision and Inter-day precision; (n = 6).

Precision type	Intraday precision		Interday precision	
	Vildagliptin	Metformin	Vildagliptin	Metformin
1.	2175365	7589753	2165971	7593989
2.	2147408	7586891	2144059	7649052
3.	2146444	7626857	2127770	7606898
4.	2153337	7592300	2157460	7584748
5.	2137383	7631293	2145282	7578001
6.	2140040	7643444	2165564	7561633
MEAN	2149996	7611756	2151018	7595720
STDEV	13654	24878	14822	30227
% RSD	0.635	0.326	0.689	0.397

Table 4a: Recovery study results for Metformin. HCl; (n = 6).

S. No	Excess drug added to the analyte (%)	Mean theoretical area	Actual area	Added area	Percent-age recovery	% RSD
1.	80	5408622	5882916	474293	99.42	0.09
2.	100	6514147	6690598	176451	101.68	0.19
3.	120	7881720	7825454	56266	99.03	0.14

Table 4b: Recovery study results for Vildagliptin; (n = 6).

S. No	Excess drug added to the analyte (%)	Mean theoretical area	Actual area	Added area	Percent-age recovery	% RSD
1.	80	1926431	1888403	38028	99.08	0.08
2.	100	2140479	2118454	22025	100.42	0.09
3.	120	2568574	2546004	22570	101.09	0.51

Table 5: Results for Robustness.

Description	Condition	Retention time (min)		Tailing factor		Resolution	Plate count	
		VLG	MET	VLG	MET		VLG	MET
Flow rate (mL/min)	0.8	6.13	8.45	1.353	1.563	7.21	5576	6485
	1.2	4.92	6.84	1.341	1.572	7.06	5464	6671
Buffer conc. (mM)	20	5.52	8.05	1.362	1.593	6.23	5523	6586
	30	5.35	7.72	1.358	1.565	8.24	5556	6550
pH of buffer	6.8	5.36	7.65	1.299	1.559	7.17	5682	6464
	7.2	5.41	7.73	1.347	1.552	7.08	5521	6524

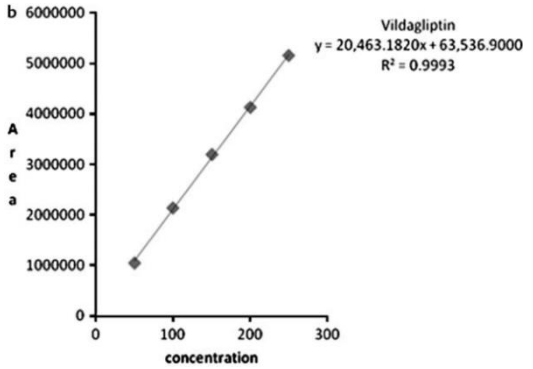


Fig. 6a: Linearity curve of Metformin.

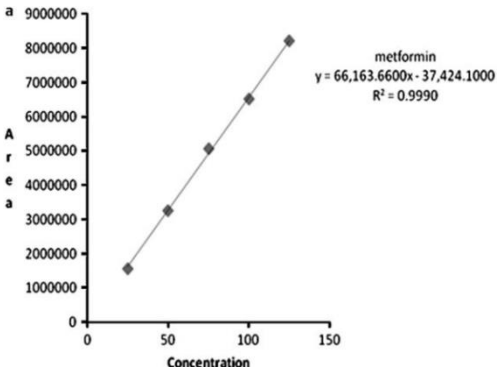


Fig. 6b: Linearity curve of Vildagliptin.

RESULTS AND DISCUSSION

HPLC method development the effect of the column (CN, C6, C8, and C18) and mobile phase composition on the retention time of MET and VLG and its chromatographic parameters was initially investigated. The best chromatographic conditions were chosen after the column test where CN, compared to C6, C8 and C18, presented appropriate chromatographic parameters. Different mobile phases were tested in distinct proportions of organic solvent, ammonium bicarbonate buffer and acetonitrile (80:20, 75:25, 70:30, 60:40 and 55:45; v/v), adjusted with glacial acetic acid, at different pH values of aqueous phases (6.8, 7.0, 7.5 and 10.0). As these component are volatile, it can be concluded as the mobile phase is mass compatible. The adequacy of mobile phase was decided on the basis of assay selectivity and sensitivity, stability studies and the separation between degradation products formed during stress studies. The system suitability test was appropriate using 25 mM ammonium bicarbonate pH 7.0 adjusted with dilute glacial acetic acid in combination with acetonitrile (65:35, v/v) at room temperature, which resulted in a retention time of 3.652 min for VLG and 5.206 min for MET, as shown in **Fig. 2.** MET and VLG were determined at 207 nm and no interference was observed, therefore, this wavelength was utilized. The optimized conditions of the RP-HPLC method was validated for the analysis of MET and VLG in pharmaceutical dosage forms, due to capability and application for quality control. Specificity and forced degradation studies Forced degradations were performed to show the stability indicating properties of the analytical method, particularly when there is no information available about the potential degradation products. MET and VLG were found to be stable under acid, neutral, photolytic stress and thermal stress conditions. Significant degradation was observed during alkaline and oxidative stress conditions (Fig. 3). For MET and VLG, the oxidative and basic conditions resulted in a significant decrease in the area with the additional peaks. Under the basic conditions, a significant decrease of the peak area of VLG was observed within 30 min, with one additional peak detected at 3.10 min respectively. Under acid condition no significant decrease in the area of the MET and VLG was exhibited. The acid and photolytic conditions resulted in non significant reduction of the peak area with no additional peak detected. Under oxidative condition, a significant decrease of the area of VLG was detected and 2 small degradation peaks were seen approximately at 3.1 and 4.1 min. Under the thermal condition, no significant decrease in area was observed for solid samples. No interference from formulation excipients was found, showing that the peaks were free from any co eluting peak, thus demonstrating that the proposed method is specific for the analysis of MET and VLG. The stressed samples were respectively analyzed and compared to the MET and VLG reference solution spectrum and with the help of PDA the peak purity of MET and VLG was verified in the degraded samples.

Linearity

The analytical curves constructed for MET and VLG were found to be linear in the 25–125 µg/mL and 50–250 µg/mL range respectively. The value of correlation coefficient calculated for MET (R 2 = 0.9990, y = 66 163.66x−37 424.10, where x is concentration and y is the peak absolute area) were shown in (Fig. 4a) and VLG correlation coefficient (R 2 = 0.9993, y = 20 463.18x + 63 536.90) were shown in **(Fig. 4b).**

Precision

The precision evaluated at the repeatability of the method was studied by calculating the relative standard deviation (RSD) for 6 determinations of 100 µg/mL performed on the same day and under the same experimental conditions. The RSD value for MET and VLG was 0.32 and 0.63 respectively. The inter-day precision was assessed by analyzing 6 samples on 3 diff erent days. The RSD values obtained for MET and VLG was 0.39 and 0.68 respectively. These results were summarized in **Table 1**.

Accuracy

Accuracy was evaluated by determining the analyte in solutions prepared according to the standard addition method and expressed in terms of percentage recoveries of MET and VLG from the real samples. The mean recovery data of MET and VLG were 100.04 % (Table 2a) and 100.09 % (Table 2b), demonstrating that the method is accurate within the desired range.

Limits of detection and quantitation

The LOD and LOQ were obtained by using the signal to noise ratio calculations. The experimental LOD and LOQ were 0.75 µg/ mL and 2.51 µg/mL for VLG, 0.36 µg/mL and 1.22 µg/mL for MET respectively.

Robustness

No significant changes were found in the chromatographic pattern when the modifications were made under experimental conditions, thus showing the method to be robust (Table-3). The stability of the sample solutions were studied and the data obtained showed that the sample were stable for 24 h in the auto sampler and for 72 h when maintained at 2–8 °C. System suitability the experimental results showed that the parameters tested were within the acceptable range, indicating that the system is suitable for the intended analysis (Table 4).

5.0. CONCLUSIONS

A new method of analysis is developed for simultaneous estimation of Metformin hydrochloride and Vidagliptine drugs in pharmaceutical tablet dosage form by RP- HPLC method. The analytical procedure is validated as per ICH Q2B guidelines and shown to be simple, accurate, precise and specific. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

Results of the validation studies show that the stability-indicating RP-LC method is simple, mass compatible, accurate robust and specific without any interference from the excipients and degradation products. The proposed method was successfully applied for the quantitative analysis of MET and VLG in tablets. The method may thus be used for routine analysis, quality control and stability studies of the pharmaceutical tablets containing these drugs

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