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NEW STABILITY INDICATING UFLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND VILDAGLIPTIN IN BULK AND SOLID DOSAGE FORM

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Keywords:

Stability indicating RP-HPLC, Metformin hydrochloride, Vildagliptin, Specificity studies

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ABSTRACT: A new stability indicating Ultra-Fast Liquid Chromatography (UFLC) method was validated for the simultaneous estimation of Metformin hydrochloride (MET) and Vildagliptin (VILDA) in bulk and tablet dosage form as per the International Council for Harmonization guidelines. Chromatography developed on Shimatzu UPLC, X-Bridge C18 column equipped with PDA detector. Mobile phase composed of phosphate buffer (25 mM) pH 3.0: acetonitrile: methanol (85:13:2). Sodium 1-octanesulfonate monohydrate (0.25 g/L) added in aqueous buffer. The effluent was passing through the column 1.0 ml/min at 40 ºC. The retention time of MET and VILDA were found to be 2.964 min and 6.358 min. The specificity study was determined by the addition of known impurities 1-cynoguanidine and vildagliptin amide of MET and VILDA, respectively. The forced degradation studies indicated that MET was stable while VILDA was susceptible to alkaline hydrolysis and peroxide oxidation. The degradation product peaks were well resolved from the MET and VILDA peaks. Purity angle and purity threshold of the MET and VILDA peak showed spectral homogeneity over the entire peak region indicated its peak purity. The proposed UFLC method can be employed for simultaneous estimation of MET and VILDA in bulk drugs and formulations.

INTRODUCTION: Chemically, Metformin hydrochloride (MET) is N, N-dimethyl-imido-dicarbonimidic diamide, monohydrochloride and Vildagliptin (VILDA) is (2S)-1-{2-[(3-hydro-xyadamantan - 1 - yl) amino] acetyl} pyrrolidine - 2 -carbonitrile [1]. MET is the first-line medication for the treatment of type-2 diabetes or non-insulin-dependent diabetes, particularly in people who are overweight. VILDA is a dipeptidyl peptidase-4 inhibitor.

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VILDA increases two incretin hormones *i.e.* glucagon-like peptide-1 and glucose-dependent insulin tropic peptide which stimulate a decrease in blood glucose level [2]. Literature survey revealed that the various analytical methods, such as high-performance liquid chromatography [3-10] and ultraviolet spectroscopy [11-12] had been reported for simultaneous determination of MET and VILDA while stability-indicating methods were limited for its determination.

As per International Conference on Harmonization (ICH) Q2 (R1) criteria for specificity, an analytical method has been unequivocally assessed the analyte in the presence of other components such as impurities, degradation products, and matrix components [13]. ICH guidelines Q1A and Q2A recommend interpreting the stability attribute of the

drug product under the stress conditions; acidic, alkaline, oxidative, UV, and photolytic [14-15]. Photo-diode array detector (PDA) facilitates the accurate detection of coeluting impurity within the analyte peak by measurement of its peak purity [16]. In the reported HPLC methods, effluents were not monitored by PDA detector; hence the interference of any impurities remained undetected. The literature showed that 1-cynoguanidine and vildagliptin amide is the main impurity associated with MET and VILDA, respectively [17-18]. Reported HPLC methods have not used these impurities for simultaneous determination of MET and VILDA, which may interfere in their subsequent analysis. Therefore, the present investigation deals with the development of stability indicative UFLC method to allow accurate, rapid, sensitive, and precise determinations of MET and VILDA in bulk and tablet dosage forms.

MATERIALS AND METHODS:

Materials: Pharmaceutical grade MET and VILDA in its bulk drugs and its impurities1-cynoguanidine and vildagliptin amide was generously gifted by Hetro Drugs Ltd., Hyderabad, India. HPLC grade acetonitrile, methanol, and water purchased from Rankem, Mumbai. Sodium dihydrogen phosphate, sodium hydroxide, hydrogen peroxide, triethyl-amine purchased from Merck, Mumbai. Sodium 1-octane sulfonate monohydrate purchased from Sigma Aldrich, Mumbai. Nylon membrane disc filter (0.45µ) and syringe filter procured from by mdi-Cat, Gurgaon. All other chemicals and reagents were of analytical grade.

Instruments: Shimadzu prominence Shim-pack GIST-HP C_{18} (150 × 4.6 mm, 3 µm), pump LC 20 AD gradient system, connected with SPD-M20A PDA detector empowered with LC solution software. Mettler Toledo XS205D0 analytical balance, pH Meter Orian Star A211.

Preparation of Standard MET Solution: Accurately weighed 50 mg standard MET was transferred to 100 ml volumetric flask. The content of the flask was dissolved in 70 ml of diluents with sonication, and volume was made up to mark with the diluents. Further, 20 ml of the resultant solution was pipetted and transferred to 100 ml of volumetric flask and diluted up to the mark with the diluents to get a concentration 100 µg/ml.

Preparation of Standard VILDA Solution: Accurately weighed 25 mg standard VILDA was transferred to 50 ml of volumetric flask. The content of the flask was dissolved in 25 ml of diluents with sonication, and volume was made up to mark with diluents. Further, 20 ml of the resultant solution was pipetted and transferred to a 50 ml volumetric flask and diluted up to the mark with the diluents to get a concentration 200 µg/ml.

Preparation of MET and VILDA Tablet Solution: The quantity equivalent to 100 mg was transferred to 100 ml volumetric flask. The content of the flask was dissolved in 70 ml of diluents with sonication for 30 min; intermittent shaking at 50 °C and diluted up to the mark with diluent. The solution was filtered through 0.45 µ Nylon membrane disc filter and further dilutions were made to obtain the concentration 100 and 200 µg/ml for MET and VILDA respectively.

Development UFLC Method: Chromatographic separation was performed on the UFLCC_{18} (150 x 4.6 mm, 3 µm) column. Isocratic elution carried by mobile phase comprised with phosphate buffer 25mM pH 3.0: acetonitrile: methanol 85:13:2 at flow rate 1 ml/min. The analysis was carried out at column temperature 40 ºC, and the injection volume was 10 µL.

Aqueous buffer (25 mM) was prepared by transferring 3.0 g sodium dihydrogen phosphate in 1L distilled water; pH 3 was adjusted by triethyl-amine. Sodium 1-octanesulfonate mono-hydrate added in buffer solution. The mobile phase was sonicated, degassed, and pass through a 0.45µ nylon membrane disc filter.

The stock solution and working standard prepared in diluents water: acetonitrile (85:15 v/v). The effluents are monitored by the PDA detector. The chromatographic peak of MET and VILDA is integrated to observe its λ_{max} of the UV spectrum by a PDA detector. Parameters like peak symmetry, peak purity, tailing factor, $R_{t,}$ and area under curve measured by LC solution software.

Method Validation: The method validation parameter such as specificity, linearity, accuracy, precision and robustness were evaluated as per the ICH guidelines Q2B and USP [19-20].

Specificity: Specificity study was performed by analyzing standard working solution of MET, and VILDA spiked with drug impurities, placebo (excipients), and blank (diluents).

Preparation of Standard 1-cyanoguinadine Impurity Solution: Accurately weighed 2.0 mg of standard 1-cyanoguinadine impurity was transferred into 20 mL volumetric flask. The content of the flask was dissolved in 10 mL of diluents with sonication for 10 min, and the volume was made up to the mark with diluents. Further, 1.0 ml resultant solution was pipetted and transferred to 100 mL volumetric flask and diluted up to the mark with the diluents to obtain the concentration 1µg/ml.

Preparation of Standard Vildagliptin Amide Impurity Solution: Accurately weighed 2.0 mg of standard vildagliptin amide impurity was transferred into 20 ml of volumetric flask.

The content of the flask was dissolved in 10 ml of diluents with sonication for 10 min, and the volume made up to the mark with diluents. Further, 1.0 ml resultant solution was pipetted and transferred to 50 ml volumetric flask and diluted up to the mark with the diluent to obtain the concentration 2 µg/ml.

Preparation of MET and VILDA Solution Spiked with Impurity: In 50 ml of the volumetric flask, 1 ml of each standard 1-cyanoguinadine (1 µg/ml) and vildagliptin amide (2 µg/ml) impurity solution were transferred with further addition of 10 ml MET and 20 ml VILDA standard working solution. The volume was made up to the mark with the diluents.

Linearity: MET working standard 50, 80, 110, 130 and 160 µg/ml and VILDA working standard 100, 160, 220, 260, and 320 µg/ml were analyzed by UFLC. A calibration curve constructed against concentration (µg/ml) and its corresponding area under the curve (AUC). The linear regression equation and correlation coefficient (r^2) calculated from the calibration curve.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the following formulae:

$$LOD = 3.3 (SD) / S \text{ and } LOQ = 10(SD) / S$$

Where, SD = Standard deviation of response (peak area) and S = average of the slope of the linear curve.

Accuracy: The accuracy of the method was determined by recovery studies. The stock solution MET, and VILDA, each containing 1000µg/ml spiked separately with an equivalent quantity of placebo (excipients). The appropriate aliquots of MET and VILDA stock solutions were made with the diluent to obtain concentrations 50,100 and 150µg/ml for MET and 100, 200, and 300µg/ml for VILDA. The solutions were analyzed in triplicate.

Precision: The precision was determined to ensure the closeness of the data values to each other for the number of measurements under the same analytical conditions.

System Precision: The system precision measured by six replicate injections of a homogeneous sample of standard MET (100 µg/ml) and VILDA (200 µg/ml).

Method Precision: Method precision was measured by six replicate injections of standard MET (100 µg/ml) and VILDA (200 µg/ml) on 3 different UFLC systems.

Intermediate Precision: Intermediate precision was determined to notice typical variations in an analytical method on different days, analysts. The sample solution MET (100 µg/ml) and VILDA (200 µg/ml) were used in the analysis.

Robustness: The standard solution MET (100 µg/ml) and VILDA (200 µg/ml) were injected six times for each varied conditions of flow rate (1 ± 0.1ml/min), column temperature (40 ± 5 °C), and wavelength (λ_{max} ± 2 nm). The absolute difference in % assay value at each modified condition was determined compared to the optimized condition.

Forced Degradation Studies: The forced degradation studies were carried out in acid, base hydrolysis, peroxide oxidation and humidity, thermal and photolysis condition.

In 100 ml volumetric flask, quantity equivalent to 100 mg MET dissolved in 70 ml diluent with intermittent swirling at 50 °C. Similarly, dilutions were prepared for VILDA and tablet.

In resultant solution, 10 ml of 1N HCl (acid degradation), 1N NaOH (base degradation), 30% H_2O_2 (peroxide degradation) added in subsequent volumetric flasks. It was kept at room temperature for 24 h.

The acid and base stock solution was neutralized with each 10 ml 1N NaOH and 1N HCl, respectively. The volume was made up to mark with diluents to obtain the concentration of 1000 µg/ml. The stock solution of the MET, VILDA, and tablet (1000 µg/ml) were exposed to UV and white light for 1.2 million lux h and integrated near the ultraviolet energy not less than 200 w/sq. Thermal degradation study of MET and VILDA tablet (1000 µg/ml) performed by exposing stock solution at 70 ºC in a water bath for a period of 24 h. Humidity degradation of the drug product was determined by exposing the tablet in petri plate at 25 ºC and 90% relative humidity chamber for 2 days. Forced degradation of placebo (excipients) was prepared similar to MET and VILDA.

All the above-mentioned stock solutions of the forced degradation studies were filtered through a 0.45 µ Nylon membrane disc filter. It was further diluted to obtain the concentrations 10 and 20 µg/ml for MET and VILDA, respectively.

RESULTS AND DISCUSSION:

Method Development: The UFLC method was optimized on C_{18} (150 x 4.6 mm, 3 µm) column with the mobile phase comprising of phosphate buffer 25 mM (pH3.0): acetonitrile: methanol 85:13:2 at isocratic mode. Sodium 1-octane-sulfonate monohydrate (0.25 g) was added in 1.0 L buffer solution. The flow rate was maintained at 1.0 ml/min, and the column temperature was kept at 40^0 C. The proposed analytical method has fulfilled the USP criteria of tailing factor, HETP count, and resolution factor. MET and VILDA peak eluted at R_t 2.964 min and 6.358 min respectively with well-resolved chromatogram depicted in Fig.1.Assay of MET and VILDA showed 98.3 ± 0.80 and 98.5 ± 1.25%, respectively.

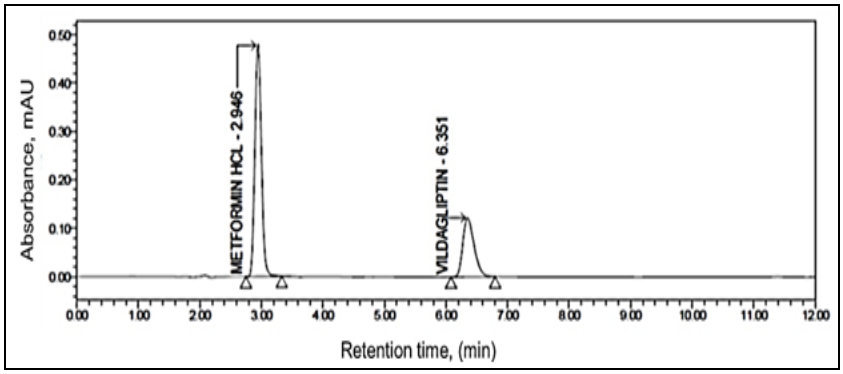

FIG. 1: CHROMATOGRAM OF MET AND VILDA TEST SAMPLE

Method Validation:

Specificity: The specificity studies were performed by spiking known impurities 1-cynoguanidine and vildagliptin amide associated with MET and VILDA, respectively.

The chromatogram of specificity study has shown in **Fig. 2** and **3**. The results demonstrated that the optimized analytical method was unaffected by the presence of these impurities and placebo.

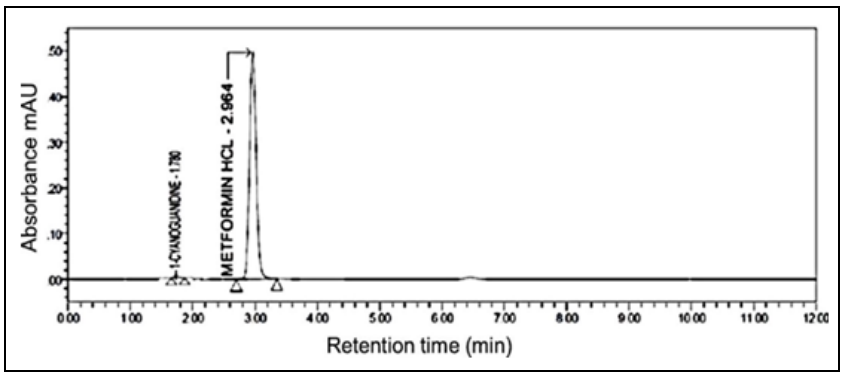

FIG. 2: CHROMATOGRAM OF MET SPIKE WITH 1-CYNOGUANIDINE IMPURITY

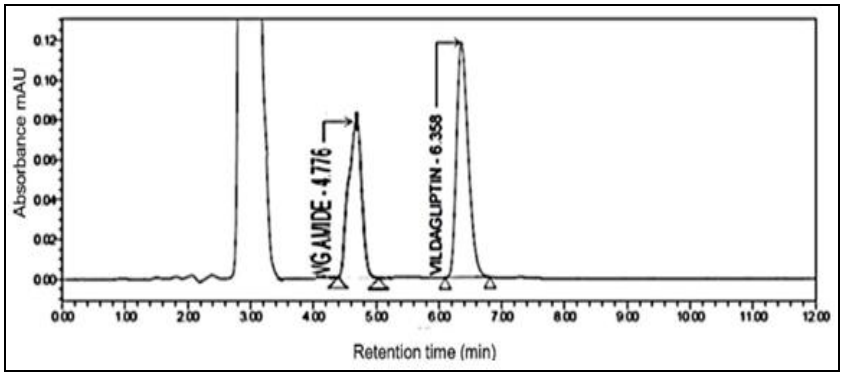

FIG. 3: CHROMATOGRAM OF VILDA SPIKE WITH VILDAGLIPTIN AMIDE IMPURITY

Linearity: The linearity study of standard MET and VILDA was studied in the concentration range 50-160 µg/ml and 100-320 µg/ml, respectively. The calibration function (AUC Vs. concentration) was linear with five-point calibration used for quantitation by linear regression. The regression equation and coefficient of variance (r^2) was found to be y = 31735x + 36316; r^2 = 0.9999 for MET and y = 6906x + 18607; r^2 0.9999 for VILDA. The LOD and LOQ of MET were found to be 0.215 and 0.625 µg/ml, respectively. The LOD and LOQ of VILDA were found to be 0.0045 and 0.0148 µg/ml, respectively.

Accuracy: The recovery study was performed by spiking placebo (excipient) into standard MET and VILDA at three different levels 50, 100, and 150% (n=3). The recovery of MET and VILDA was found to be 100.5 ± 0.47% and 98.8 ± 0.30%, respectively, which indicating that the method was accurate.

Precision: The system precision was found to be precise for six replicates of standard MET and VILDA. The % RSD of MET and VILDA was 0.09% and 0.18%, respectively, which was well within the acceptance criteria. The % RSD of MET and VILDA (n=3) was 0.37% and 1.35%, respectively, for the method precision studies. In intra-day (n=3) studies, % RSD of MET and VILDA was found to be 0.24% and 0.40%, respectively. In intermediate precision (n=3) studies, % RSD of MET and VILDA were found to be 0.61% and 0.36%, respectively.

Robustness: The results of robustness studies were expressed relative to control. The relative deviation caused by deliberate variation in flow rate (1 ± 0.1 ml/min), wavelength (210 & 232 ± 5 nm), and column temperature (40 ± 5 ºC) was within the acceptable criteria ≤2%.

TABLE 1: SUMMARY OF VALIDATION

Parameter (Units)	MET	VILDA
Linearity range (µg/ml)	50-150	100-300
Correlation coefficient	0.9999	0.9999
LOD (µg/ml)	0.215	0.0045
LOQ µg/ml	0.625	0.0148
Recovery (%)	100.5±0.47	98.8±0.30
Precision (%RSD)		
System precision (n=6)	0.18	0.09
Method precision (n=3)	0.37	1.35
Intraday (n=3)	0.24	0.40
Interday (n=3)	0.61	0.36
Robustness		
	MET	**VILDA**
Parameter	**Specification**	**Mean ± SD**	**Mean ± SD**
Control	Original	98.7 ± 0.32	100.0 ± 0.20
Flow rate (1 ± 0.1 mL/min)	0.9	98.4 ± 0.29	100.6 ± 0.21
	1.1	98.3 ± 0.47	100.8 ± 0.25
Wavelength (210 ± 5nm)	205 nm	98.7 ± 0.61	100.1 ± 0.15
	215 nm	98.7 ± 0.39	100.1 ± 0.13
Column temperature (30 ± 5°C)	35°C	98.0 ± 0.25	99.6 ± 0.27
	45°C	98.1 ± 0.42	99.1 ± 0.12

Average of three determinations (n=3)

Hence, the proposed method was found to be robust for six replicates of MET and VILDA. The summary of validation parameters of MET and VLDA have shown in **Table 1.**

Forced Degradation: The degradation of the drug was indicated by a decrease area in the peak of the drug and additional peak of the degraded products when compared with the peak concentration of the non-degraded drug (control). The degradation studies showed that MET was relatively stable in forced degradation conditions. VILDA was susceptible to base hydrolysis and peroxide oxidation while it was stable in acidic, peroxide, photolytic (open petri plate & closed covered with aluminum foil), thermal and humidity condition. The VILDA gets degraded into four degradation products in the stress condition of alkaline hydrolysis. The maximum deviation of 11.9% was estimated in the basic hydrolysis compared to control. Chromatogram of base degradation showed t_R at 1.780, which coincide with t_R of 1-cynoguanidin impurity. Other peaks of degraded products showed at t_R 1.920, 2.458 and 3.825 min **Fig. 4.** The deviation of 10.7% was estimated in the peroxide oxidation compared to control. Three degradation products were observed in peroxide degradation. Chromatogram of peroxide oxidation showed t_R at.1.450, 1.651, and 2.236 min **Fig. 5.**

Since peak purity angle was less than the threshold angle of the MET and VILDA peak in all the degradation conditions, which indicates that the method was stability-indicating. Summary of degradation studies of the MET and VILDA tablet has shown in **Table 2.**

TABLE 2: SUMMARY OF DEGRADATION STUDIES

Parameter	MET			VILDA		
	% Assay	Purity Angle	Purity Threshold	% Assay	Purity Angle	Purity Threshold
Control	97.9	0.101	0.272	100.0	0.058	0.262
Acid degradation	97.7	0.208	0.275	91.1	0.183	0.386
Base degradation	95.4	0.068	0.275	84.2	0.200	0.398
Peroxide degradation	95.4	0.214	0.275	85.4	0.203	0.399
Heat degradation	97.0	0.213	0.277	88.8	0.185	0.390
Humidity degradation	93.4	0.103	0.269	102.6	0.054	0.257
Photolytic (Closed)	98.2	0.104	0.273	93.2	0.057	0.263
Photolytic (Open)	97.4	0.103	0.273	95.9	0.056	0.263

Average of three determinations (n=3)

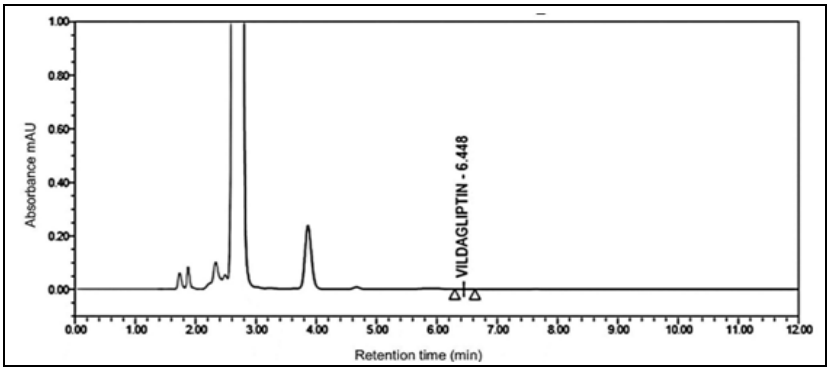

FIG. 4: CHROMATOGRAM OF VILDA IN BASE DEGRADATION

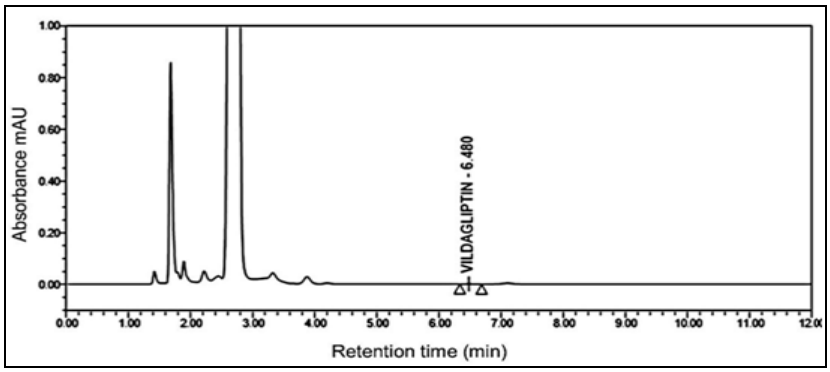

FIG. 5: CHROMATOGRAM OF VILDA IN PEROXIDE DEGRADATION

CONCLUSION: Statistical analysis proved that the proposed method was simple, sensitive, accurate, precise, and repeatable for the determination of MET and VILDA. The developed method was unaffected by the presence of its major impurities and excipients. The proposed method was found to be suitable for estimation of MET and VILDA under various force degradation conditions as the degraded products were well resolved from the pure drug peak. Hence, the proposed method can be successfully employed for simultaneous quantitative analysis of MET and VILDA in bulk drugs and formulations.

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