

RESEARCH ARTICLE

Novel UHPLC DAD Method for Simultaneous determination of Vildagliptin and Metformin in Bulk and its Tablet formulation

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ABSTRACT:

A robust and rapid reversed-phase UHPLC method for routine determination of vildagliptin (VILDA) and metformin (MET) was developed and validated with DAD detection (207 nm). Chromatographic analysis was carried out with isocratic separation by C18 column (150mm x 2.1mm, 5 μ m) using a mobile phase with a mixture of 1.36 g phosphate buffer (pH 4.2) set to phosphoric acid and acetonitrile (80:20, v/v) with 0.6mL/min flow rate. The procedure was validated as per International Conference on Harmonization (ICH) guidelines for linearity (correlation coefficient $r^2 = 0.999$), accuracy, intermediate precision, and robustness. Accuracy was 99.79% $\pm$ 0.54% and 100.07 % $\pm$ 0.34% for VILDA and MET, respectively. For precision, the %RSD was founded to be less than 2% for three concentrations analyzed from three replicates. The proposed method was used for successful determination of VILDA and MET in a pharmaceutical preparation.

KEYWORDS: Vildagliptin, Metformin, UHPLC.

INTRODUCTION:

(DMT2) Diabetes mellitus type 2 is a liberal disease, and typically combination therapies are required to completely regulate hyperglycemia after initial monotherapy and life alteration to maintain glycemic control¹. Metformin hydrochloride (MET) (Fig. 1(b)) is the most widely used medication for treating DMT2 with a structure of dimethylbiguanide. Metformin-based combinations are found to be better than other single hypoglycemic agent therapy in efficacy and tolerance. The therapeutic properties of metformin, particularly gluconeogenesis, are due to its ability to suppress glucose production by the liver^{2,3}. Vildagliptin (VILDA), the other agent in this twofold analysis (Fig. 1(a)), is a pancreatitis linked to DPP-4 inhibitors has been documented in cases. One group at UCLA reported increased pre-cancer pancreatic changes in rats and donors of human organs treated with DPP-4 inhibitors^{4,5}. In type 2 diabetes mellitus, vildagliptin has been shown to reduce hyperglycemia⁶.

Since VILDA is a relatively new drug, there are no official methods for its pharmacopeia analysis, but MET has authentic analytical techniques in British Pharmacopeia (BP)⁷ and the United States Pharmacopeia (USP)⁸. In comparison to the literature, limited methods^{9,10,11} is available for the simultaneous determination of VILDA and MET in pharmaceutical preparations or in synthetic mixtures. To promote a clear understanding of the purpose of this study, an objective was set to develop a new and more efficient method compared to the existing ones. Very few methods for evaluating VILDA and MET alone were found, similarly in dosage form or in plasma¹²⁻¹⁵. Determination of the VILDA concentration by UV spectroscopy in bulk and its dosage form¹⁶⁻¹⁹ realized a more efficient process using reversed-phase HPLC technique²⁰⁻²⁴. ATR-FTIR has been used for quantification of VILDA and MET using chemometrics in pharmaceutical combinations with different concentration ranges²⁵. On the other hand, various methodologies are reported to analyze MET using both spectrophotometry and chromatography techniques. Using UV-spectrophotometry, MET was determined in the presence of recent empagliflozin in its dosage form^{26,27,28} and of trendy gliptins: Sitagliptin and linagliptin either in marketed dosage forms²⁹⁻³⁴. Currently, the highly sensitive and selective LC-MS

technique with diverse bioanalytical submissions has also been found for detection and quantification of MET³⁵.

However, there are no UHPLC-DAD method found for concurrent quantification of VILDA and MET in tablets. Furthermore, UHPLC is more economical than HPLC with lower organic solvent consumption and less running time and cost. Also, UHPLC provides higher nanogram-level sensitivity encouraging the progress of new analytical methods for the drugs being studied. This study attempts to develop new, yet more sensitive UHPLC DAD methods in bulk and their pharmaceutical preparation for simultaneous quantification of VILDA and MET. This is accompanied by complete process validation. The chemical structures of the studied drugs are given in (Fig. 1).

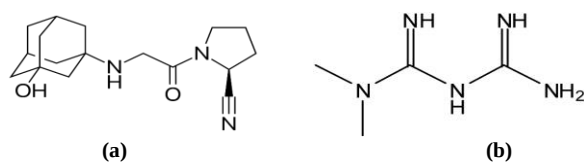


Fig. 1: Structures of vildagliptin (a) and metformin (b)

MATERIAL AND METHODS:

Instrumentation:

The chromatographic analysis was carried on Agilent 1290 series Ultra High-Performance Liquid Chromatography (US-CA). DAD Detector (DAD, G4212A), (G4226A, Agilent) autosampler with a thermostat (G1330B, Agilent), and (TCC, G1316C) thermo stated column compartment were employed. Separation and quantification were made on an Agilent Zorbax Eclipse Plus C18 (150×4.6mm, 5µm) column, while pH meter–EUTECH Instruments (Singapore) was used.

Basis of Samples and Reagents:

Vildagliptin (VILDA) with purity (> 99.50%) and Metformin (MET) with purity (> 99.10%) were purchased from Clearsynth Labs Ltd. (Mumbai, India). INTAGLIP-M tablets containing 50mg VILDA and 500 mg MET were obtained from Intas Pharmaceuticals Ltd. (India). Orthophosphoric acid AR Grade, Potassium dihydrogen ortho-phosphate AR Grade, Acetonitrile HPLC Grade, and HPLC Grade Water were purchased from S. D. Fine-chem Ltd. (Ahmadabad, India)

Chromatography Separation Conditions:

The separation was carried out on an Agilent Zorbax Eclipse Plus C18 (150×4.6mm, 5µm) column. Isocratic elution was utilized, and the mobile phase was prepared as a mixture of Acetonitrile and 1.36g Potassium dihydrogen phosphate buffer (80:20, v/v). Phosphoric acid was set to pH 4.2. The wavelength was 207nm. The

mobile phase was initially filtered using a membrane filter of 0.45µm, and then degassed before use. A flow rate of 0.6mLmin⁻¹ was adapted. Separation was done at 30°C temperature with 5µL injection volume.

Standard Preparations:

For preparing the standard solutions, 25mg Vildagliptin (VILDA) and Metformin (MET) respectively were transferred into a 50mL volumetric flask, added 30mL of mobile phase and sonicated for 10 minutes. The solutions were allowed to come at the room temperature, mixed well and then diluted up to the mark with the diluents. The solutions were further diluted to volume diluents to furnish a 50µg mL⁻¹ solution.

Preparation of Tablet Samples:

Twenty INTAGLIP-M tablets containing 50mg VILDA and 500mg MET were weighed and crushed to a homogeneous, fine powder in a mortar pestle. Accurate weight of this powder equal to the one tablet product was weighted, transferred into a 100mL volumetric flask containing 70mL mobile phase, sonicated for approximately 40 minutes, and then completed with the same mobile phase to volume. This solution (2.5mL) was transferred to 25mL volumetric flasks made up to the mark mobile phase, and a further 2.5mL aliquot from Flask was transferred to 25mL volumetric flasks. The mobile phase was applied to the mark and filtered (Hydrophilic PVDF 0, 22µm) to achieve a final 50µg mL⁻¹ VILDA and 50µg mL⁻¹ MET concentration, respectively.

Applied Procedures:

Standardization curves:

Standard curves were constructed by making nine solutions of each drug using the mobile phase in the range of 20–200µg mL⁻¹ concentration for VILDA and MET, separately, and only 5µL was used injection. By plotting area under the peak of the corresponding drug against its concentration, the standardization curve was achieved.

Study of VILDA and MET in Laboratory Mixtures and INTAGLIP-M Tablets:

The measures stated earlier have been implemented for various VILDA and MET ratios and tablet samples, arranged under Section (Preparation of Tablet Samples). Each computed equation of regression obtained with different concentrations of the drugs was investigated.

Forced Degradation Study:

Studies of forced degradation were conducted to examine the stability-indicating properties and the process specificity. Intentional degradation was achieved by exposing the formulation to 5 different conditions of stress. The conditions set out in (Table 1) were observed

in the procedure for the stress test. Stressed samples were regularly analyzed, and the presence of associated peaks and peak purity was tested for the active ingredients.

Table 1: Forced degradation conditions

Sr. No.	Stress type	Conditions
1	Acid hydrolysis	50 μ g mL ⁻¹ in 1 N HCl at 60°C for 2hrs
2	Base hydrolysis	50 μ g mL ⁻¹ in 1 N NaOH at 60°C for 2hrs
3	Oxidative degradation	50 μ g mL ⁻¹ in 3 % H ₂ O ₂ at 60°C for 2hrs
4	Thermal degradation	50 μ g mL ⁻¹ in 60°C for 48hrs
5	Photo degradation	overall illumination of ≥ 210 Wh/m ² at 25°C for 48hrs with UV radiation at 320-400 nm

RESULTS AND DISCUSSION:

It should be noted that the recommended methods are the first approaches to use UHPLC techniques to estimate the drugs studied, rather than the HPLC method. UHPLC is more beneficial in terms of resisting the high back pressure of the device, increased performance, shorter running times, and requires less consumables. The projected methods have also been recognized with a simple mobile phase, which allows their additional application on various detectors. The results from the validated methods exhibited better resolution and sharp peak shapes. Using a Zorbax Eclipse Plus C18 (150 $\times$ 4.6

mm, 5 μ m) showed better results than the commonly used Symmetry C18 column (50 $\times$ 4.6mm, 1.8 μ m). Upon use of UHPLC with 1.8 μ m particles, sharp peaks were observed, especially VILDA which has much lower proportion in the pharmaceutical mixture.

Chromatographic examinations related to conditions of separation.

A UHPLC Zorbax Eclipse Plus C18 (150 $\times$ 4.6mm, 5 μ m) was used to achieve optimal discovery of the studied drugs. With water, the mobile phase of methanol or acetonitrile results in no isolate. Potassium di-hydrogen ortho-phosphate buffer (1.36g) has been tested in growing ratios up to 80% of the total mobile phase with acetonitrile pumped into the stationary phase put on isocratic mode; this results in separation with well-dressed peak shapes. Just be sure, mobile phase pH optimization was a must. It was required to be in the acidic area (3.5 and 4.5) to ensure its value by more than two units below the pKa of the drugs studied; thus, the best results were obtained by changing pH to 4.2 with phosphoric acid. The detection wavelength was also 207 nm for completing optimum consideration for two drugs. Additionally, the percentage of acetonitrile was necessary for this method to progress the determination between the two eluted peaks found at 30°C temperature with better shape and flat outline. The flow rate was 0.6 mLmin⁻¹, and the injection volume was 5 μ L.

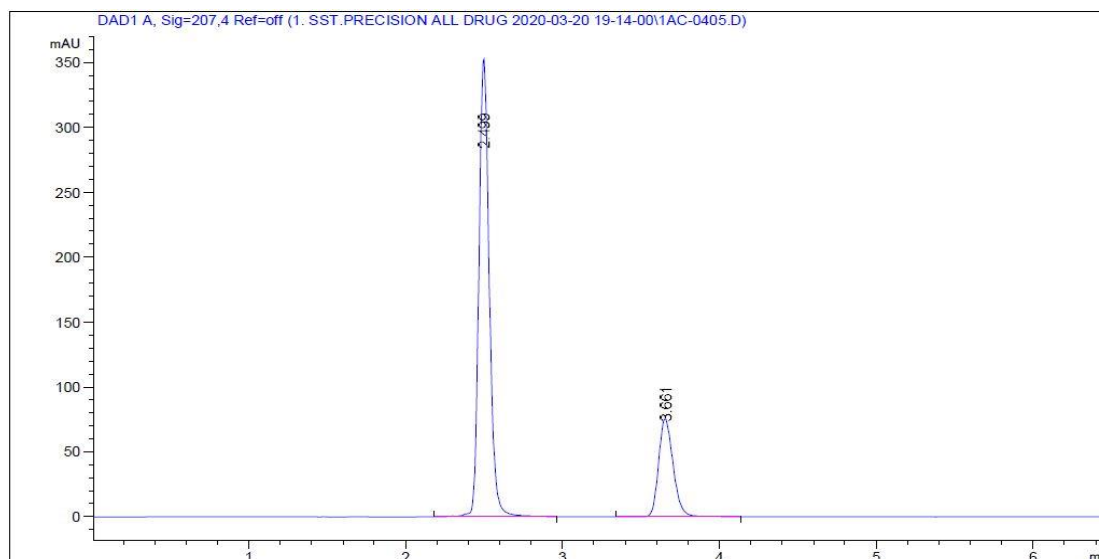


Fig. 2: UPLC chromatogram obtained during simultaneous separation of VILDA and MET. Chromatographic conditions: Zorbax Eclipse Plus C-18 (150 mm $\times$ 4.6 mm i.d., particle size 5 μ m); mobile phase phosphate buffer (1.36 g Potassium dihydrogen phosphate, pH adjusted to 4.2 $\pm$ 0.02 with ortho phosphoric acid) and acetonitrile (80:20 v/v); flow rate of 0.6 mLmin⁻¹; and UV detection at 207 nm.

Methods Validation:

Validation was complete in harmony with ICH guidelines

Linearity:

VILDA and MET were analyzed by studying their equal concentrations under the optimum conditions. Acceptable linearity between the concentration range and the corresponding absorption of each product was

achieved; regression coefficients were found with a high value of the correlation coefficient as seen in (Table 2).

Better results were observed for VILDA and MET, respectively, in 20–100 µg mL⁻¹ range.

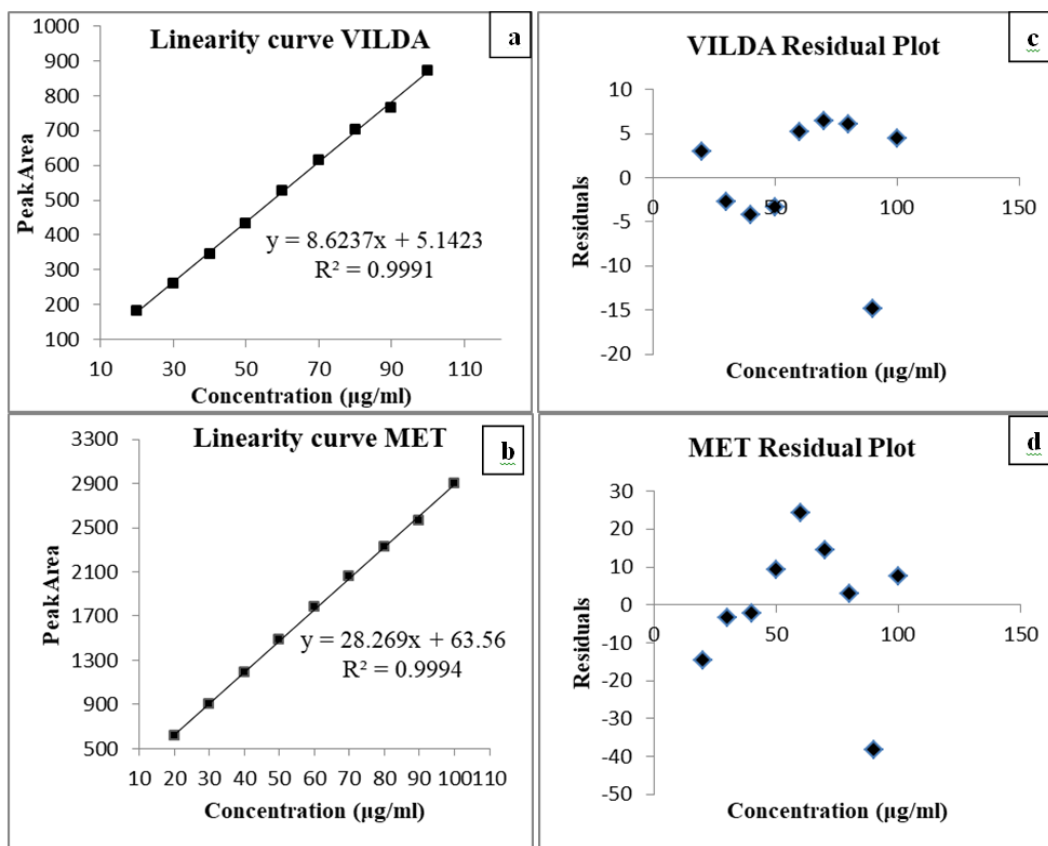


Fig. 3: Linearity plots for VILDA (a) and MET (b) with corresponding residual plots for the VILDA (c) and MET (d)

Table 2: Linearity parameters for the VILDA and MET

Linearity Parameter	VILDA	MET
Range (µg mL ⁻¹)	20-100	20-100
Slope	8.62	28.26
Intercept	5.14	63.56
Regression coefficient (r ²)	0.999	0.999
Standard error of Intercept	6.32	27.56
Standard deviation of intercept	18.96	49.12
Confidence limit of the slope	8.62±0.82	28.26±1.21
Confidence limit of the intercept	5.14±2.87	63.56±7.43

Accuracy and Precision:

The accuracy of the results was verified by analyzing percentage recovery at three concentrations for both the drugs. In fact, the percentage recovery of each drug was determined in a lab-prepared mix. The results with standard deviations values are listed in (Table 3) as the mean recovery.

Precision was tested three times by investigating three concentrations of both VILDA and MET, within the same day to test the intraday repeatability. Further, three separate analyte concentrations were analyzed on three following days using the procedures previously declared, to test inter-day precision and confirm reproducibility. The corresponding level of RSD was found to be less

than 2% in the three concentrations, as shown in (Table 4).

Table 3: Percent recovery data VILDA and MET

Drug	% Simulated dosage nominal	% Mean (n=3)	RSD (%)	RE%
VILDA	50	99.81±0.96	0.96	-0.19
MET	50	100.14±0.35	0.35	0.14
VILDA	100	99.77±0.33	0.33	-0.23
MET	100	100.71±0.36	0.36	0.71
VILDA	150	99.81±0.36	0.36	-0.19
MET	150	99.64±0.16	0.16	-0.36

Table 4: Precision data and Intermediate precision (Assay)

Analysis Date	Day 1		Day 2		Day 3	
Assay	Vilda	Met	Vilda	Met	Vilda	Met
% Assay Mean	100.42	99.86	100.65	99.79	99.75	99.88
% RSD	0.56	0.48	0.68	0.60	0.66	0.40

Robustness and Ruggedness:

The peak area consistency of the analytes was tested for robustness after small but deliberate adjustments in chromatographic conditions. This was performed by affecting small changes in the investigational parameters for the determination of VILDA and MET. For the

UHPLC method with the 5-micron C18 column, the flow rate was changed from 0.4 mL min^{-1} to 0.8 mL min^{-1} , which was tested at lower and upper wavelengths of 205 nm and 209nm, and also by varying the temperature (± 5

unit) on either side from 25°C and 35°C . The results showed no substantial changes, suggesting good robustness and ruggedness of the method.

Table 5: Robustness and Ruggedness data of TEN and MET

Parameter	conditions	% RSD (n=3)		tR (min)		N		AS	
		VILDA	MET	VILDA	MET	VILDA	MET	VILDA	MET
Change in λ_{max} 207 ± 2 nm	205	0.32	0.3	3.66	2.51	7265	6564	0.96	0.98
	209	0.41	0.32	3.67	2.5	7333	6594	0.99	0.95
Change in flow rate 0.6 ± 2 mL/min	0.4	0.32	0.22	4.97	3.38	8502	7954	1.02	0.97
	0.8	0.55	0.62	2.48	1.69	5857	4882	1.04	0.98
Change in Temp. 30 ± 5 $^{\circ}\text{C}$	25	0.59	0.35	2.96	1.9	7214	6203	0.99	0.94
	35	0.35	0.32	3.3	2.25	7171	6551	1.01	0.97
Ruggedness									
Different analyst	Analyst 1	0.31	0.25	3.68	2.51	7735	7162	1.02	0.97
	Analyst 2	0.53	0.79	3.66	2.50	7750	7139	1.01	0.98

(tR: retention time; N: number of theoretical plates; AS: Symmetric factor)

Specificity:

In the presence of placebo matrix from common tablet excipients, the analytical method was able to detect and evaluate VILDA and MET. The representative chromatogram of standard, placebo, VILDA and MET, and usual VILDA and MET mixture chromatogram are

shown in (Fig. 4). The specificity of the method was verified where the optimized conditions for detecting VILDA and MET (from the manufacturer's excipients) were applied in INTAGLIP-M tablets, respectively. The representative VILDA and MET chromatograms in tablets are shown in (Fig. 4).

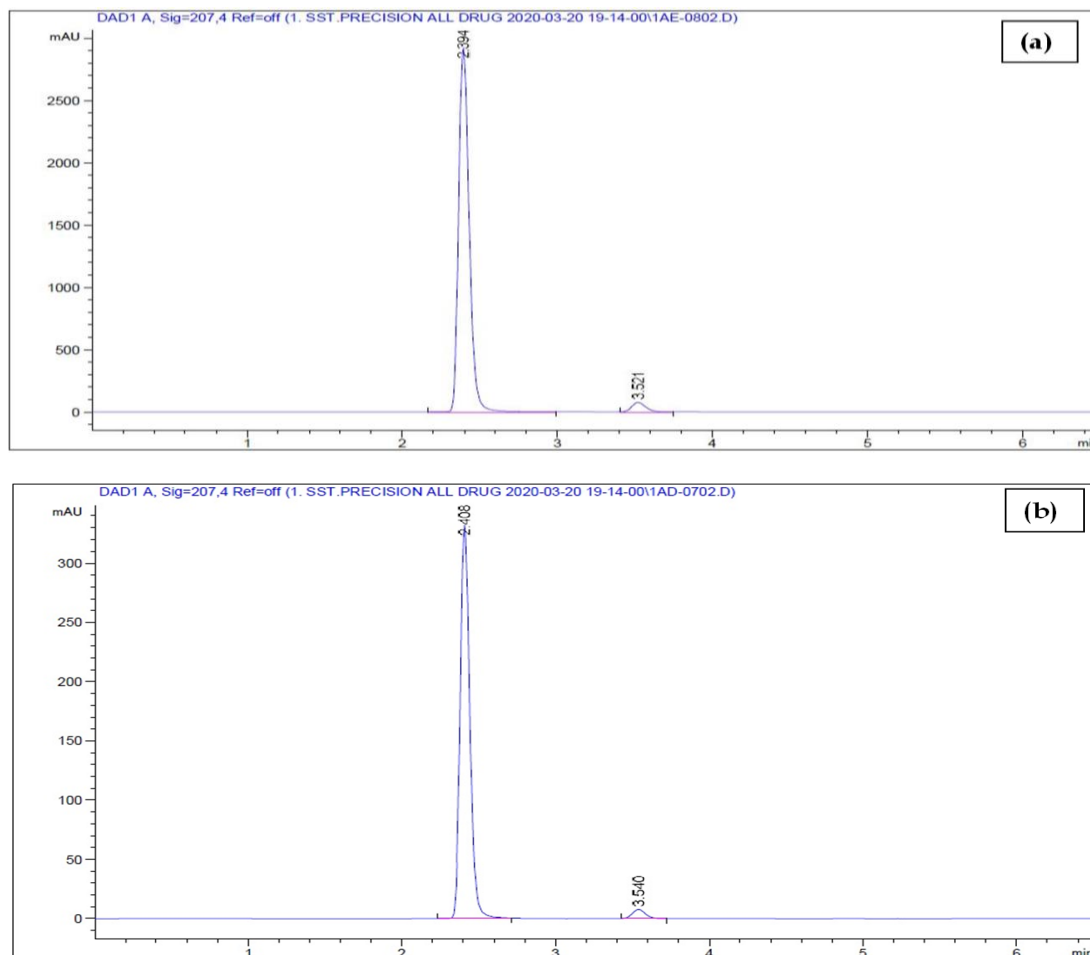


Fig. 4: A typical UHPLC chromatogram of INTAGLIP-M capsule sample solution containing (a) $50\text{ }\mu\text{g mL}^{-1}$ VILDA and (b) $50\text{ }\mu\text{g mL}^{-1}$ MET

LOD and LOQ for the Proposed Methods.

Detection limit (LOD) and quantification limit (LOQ) were estimated and are indicated in (Table 6). LOD is any analyte's concentration of 3.3 at the signal/noise ratio; while LOQ was evaluated at the signal/noise ratio of 10.

Table 6: The Values of LOD and LOQ

Drug	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
VILDA	2.20	7.33
MET	1.74	5.79

System Suitability Tests:

These tests were conducted to ensure sufficient repeatability of the chromatographic process, which mainly involves column performance, chromatographic peak, tailing factor and peak resolution. The results for both the drugs are listed in (Table 7).

Table 7: System suitability data for VILDA and MET

Parameters	VILDA	MET
Peak area (A) mAs	489.06 $\pm$ 3.50	1624.80 $\pm$ 4.50
Relative standard deviation (RSD)	0.71%	0.28%
Retention time (tR)	3.67	2.5
Theoretical plates (N)	7743	7151
Symmetry factor (AS)	1.02	0.98
Retention factor K'	3.42	2.01
Resolution	8.16	-

Standard Addition Technique for Claim on Dosage Forms:

The optimized chromatographic conditions were used to evaluate a pharmaceutical dosage form (Fig. 4). Standard addition technique was employed by adding various identified amounts of the pure drug to various previously prepared identified concentrations of the drug material using the above-described method. The concentrations were calculated using the regression equation and the data is depicted in (Table 8).

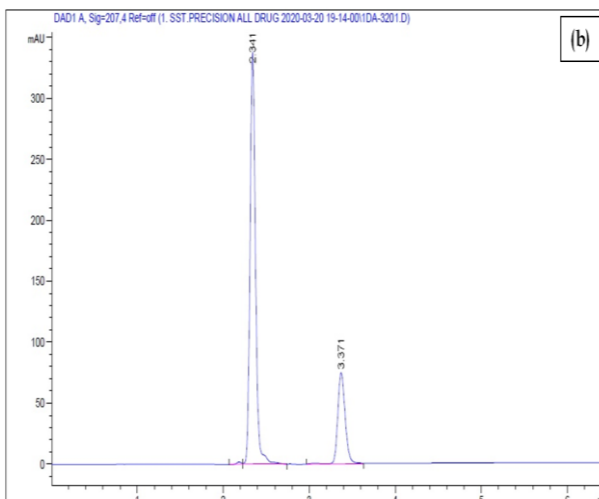
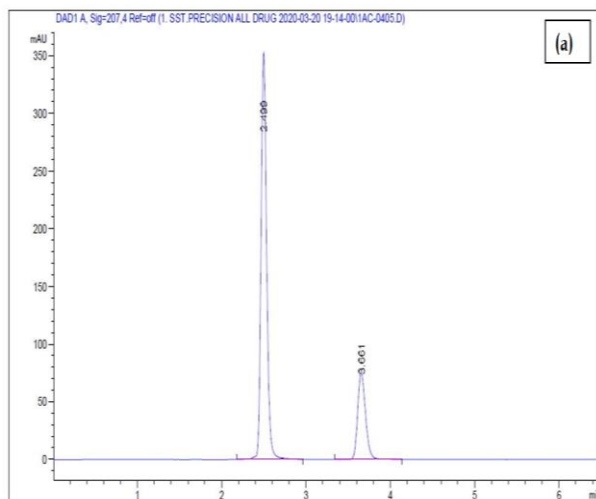
Forced degradation study:

VILDA and MET were stressed under various conditions, and separation of the samples was subjected to UHPLC analysis. Significant peaks corresponding to drug degradation were observed under basic and neutral (H_2O_2) conditions. The stability studies were conducted as shown in (Table 1). The samples were neutralized after the time of analysis, except for samples treated with thermal, Ultraviolet, and peroxide, and diluted with diluent. The samples were filtered through membrane filters with 0.45- μm Millipore. VILDA and MET have been found stable under conditions of acid, thermal, and photolysis. (Fig. 5 (b), (c), (d), (e) and (f)) showed the chromatograms of pure drug and its stressed samples. (Table 9 and 10) report the peak retention time, recovery percentage degradation of the VILDA and MET under various stress conditions.

Table 8: Marketed Tablet Review

Tablet (Teniza M-500) Replicate number	tR (min)		Aa (mAs)		AS		N		% assay	
	Vilda	Met	Vilda	Met	Vilda	Met	Vilda	Met	Vilda	Met
1	3.663	2.497	491	1626	1.01	0.98	7768	7167	100.43	100.25
2	3.662	2.499	486	1628	0.99	0.98	7762	7164	99.74	100.10
3	3.667	2.497	488	1623	1.03	0.97	7761	7159	100.50	99.66
4	3.666	2.499	487	1631	1.02	0.97	7764	7156	99.48	100.35
5	3.668	2.496	487	1630	1.02	0.98	7764	7157	99.26	99.64
6	3.668	2.496	487	1622	1.03	0.98	7764	7156	99.35	100.42
Mean $\pm$ SD	3.666 $\pm$ 0.00	2.497 $\pm$ 0.00	487 $\pm$ 1.66	1626 $\pm$ 3.57	1.02 $\pm$ 0.01	0.98 $\pm$ 0.01	7764 $\pm$ 2.40	7160 $\pm$ 4.62	99.79 $\pm$ 0.54	100.07 $\pm$ 0.34
%RSD	0.07	0.05	0.34	0.22	0.88	0.53	0.03	0.06	0.50	0.34

(tR: retention time; A^a Area; AS: Symmetric factor; N: number of theoretical plates)



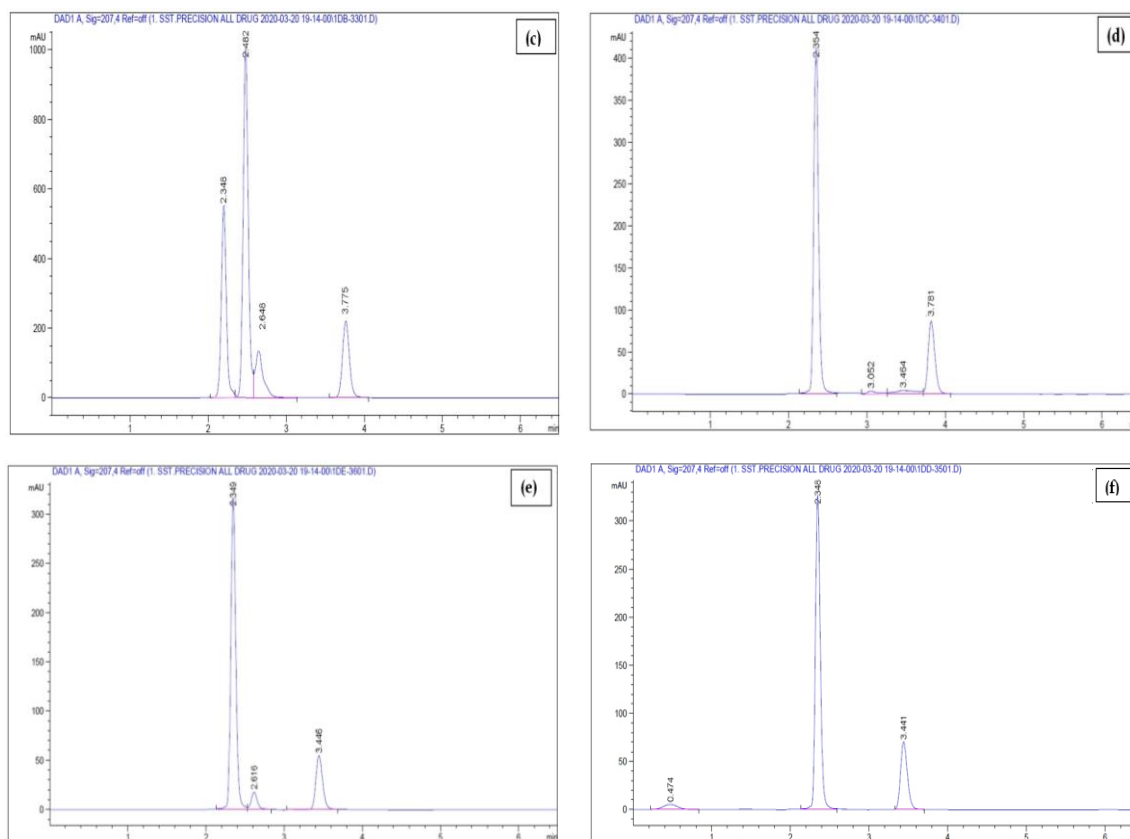


Fig. 5: (a) A typical UHPLC chromatogram of a standard sample solution containing VILDA and MET. UHPLC chromatogram of VILDA and MET obtained from degradation studies, (b) Acid hydrolysis (1 N HCl at 60°C for 1 hrs); (c) Base hydrolysis (1 N NaOH at 60°C for 1 hrs); (d) Oxidative degradation (3% H₂O₂ at 80°C for 1 hrs); (e) Thermal degradation (80°C for 48hrs); (f) Photo degradation (at 25°C for 7 days with UV radiation at 320-400 nm)

Table 9: Degradation study of VILDA

Sr. No.	Condition	Rt (min)	Recovery $\pm$ SD	%RSD	% Drug degraded
1	Acid hydrolysis	3.37	91.58 $\pm$ 0.27	0.3	7.94
2	Base hydrolysis	3.78	75.44 $\pm$ 0.52	0.69	23.74
3	Oxidative degradation	3.78	74.24 $\pm$ 0.41	0.55	24.32
4	Thermal degradation	3.45	96.07 $\pm$ 0.27	0.28	3.28
5	Photo degradation	3.44	97.93 $\pm$ 0.89	0.92	2.28

Table 10: Degradation study of MET

Sr. No.	Condition	Rt (min)	Recovery $\pm$ SD	%RSD	% Drug degraded
1	Acid hydrolysis	2.34	98.33 $\pm$ 0.23	0.23	1.37
2	Base hydrolysis	2.48	75.36 $\pm$ 0.46	0.62	23.71
3	Oxidative degradation	2.35	89.51 $\pm$ 0.34	0.38	11.33
4	Thermal degradation	2.35	98.50 $\pm$ 0.24	0.25	3.39
5	Photo degradation	2.35	98.86 $\pm$ 0.27	0.27	1.19

Remarks about the Procedure:

The lack of UHPLC-DAD methods to evaluate VILDA and MET simultaneously in tablets has inspired us to develop an efficient and improved method. Based on our expected future studies, this analysis is considered a key phase in determining which approach may be used further to evaluate and measure the drugs tested, with their numerous degradation products and also in organic solutions. In addition, the developed UHPLC technique has other significant outcomes which include detection of the studied drugs at the most sensitive wavelength and a mobile phase that consisted of higher proportion of the

organic phase compared to the buffer solution. As a result, better resolution was possible between the peaks, with lower LOD and LOQ values, and a dynamic linear range from 20 $\mu\text{g mL}^{-1}$ to 100 $\mu\text{g mL}^{-1}$.

CONCLUSION:

The proposed chromatographic method has revealed that UHPLC-DAD is more sensitive. In comparison, it not only provides more benefits, with baseline separation of the drugs but also provides a shorter analysis time compared to HPLC-UV.

The method developed overwhelms the failings of previous methods with respect to linearity, sensitivity and ease of use. It is the first method based on UHPLC-DAD for concurrent determination of VILDA and MET. The method developed is quick, effective and appropriate for quality control.

CONFLICTS OF INTEREST:

All authors have none to declare.

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