VALIDATED STABILITY-INDICATING HPTLC METHOD DEVELOPMENT FOR DETERMINATION OF VILDAGLIPTIN AS BULK DRUG AND IN TABLET DOSAGE FORM

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ABSTRACT
A new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for estimation of Vildagliptin in tablet dosage form. The chromatographic separation was achieved by using Ethyl acetate: Methanol (8.5: 1.5, v/v) as mobile phase and UV detection at 217 nm. The retention factor for Vildagliptin was found to be 0.37 ± 0.003. The method was validated with respect to linearity, accuracy, precision and robustness as per ICH guidelines. The drug was subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Results found to be linear in the concentration range of 200-1000 ng band. The method has been successfully applied for the analysis of drug in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 100.61 ± 1.32. The developed method can be used for monitoring the stability of Vildagliptin in bulk drug and pharmaceutical dosage form.

KEYWORDS: Vildagliptin, HPTLC, Forced degradation, Validation.

INTRODUCTION
Vildagliptin (VILDA), chemically, (2S)-1-[(2-[(3-hydroxyadamantyl)amino]acetyl)pyrrolidin-2-carbonitrile is a new oral anti-hyperglycemic agent of the new dipeptidyl peptidase-4 inhibitor class of drugs which inhibits the inactivation of GLP-1 and GIP by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of langerhans in the pancreas. [1]

Extensive review of literature reveals High Performance Liquid Chromatographic (HPLC)[2-14], LC-MS[15] methods for determination of VILDA in human plasma and pharmaceutical formulations either as single or in combination with other drugs. Stability indicating HPLC method for determination of VILDA with metformin in solid dosage form is also reported. [16]

To best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of VILDA in tablet dosage form.

This paper describes simple, precise, accurate stability indicating HPTLC method development and validation for determination of VILDA as per International Conference on Harmonisation Guidelines. [17,18]

MATERIALS AND METHODS
Chemicals and reagents
Pharmaceutical grade working standard of VILDA was kindly supplied by Spectrum Labs, (Hyderabad, India). The pharmaceutical dosage form used in this study was GALVUS tablets labeled to contain 50 mg of VILDA was procured from the local market. Ethyl acetate, Methanol (HPLC grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions
Chromatographic separation of drug was performed on precoated silica gel aluminium plate 60 F254 (10 x10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using ethyl acetate: methanol (8.5: 1.5, v/v) as mobile phase. The mobile phase was saturated in chamber for 15 min.

After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 217 nm for all
developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

**Preparation of Standard Stock Solution**

Standard stock solution of VILDA was prepared by dissolving 10 mg of drug in 10 mL of methanol to get working standard solution of concentration 1 mg mL⁻¹ from which 1 mL was further diluted to 10 mL with methanol to get solution of 100 ng µL⁻¹.

**Selection of Detection Wavelength**

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 217 nm. So, 217 nm was selected as the wavelength for detection.

**Analysis of Tablet Formulation**

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 50 mg of VILDA was weighed and transferred to a 100 mL volumetric flask containing 50 mL of methanol and the content was sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 500 ng band⁻¹.

Two millilitre volume of above solution was diluted with methanol to obtain final concentration of 100 ng band⁻¹. Four µL volume of this solution was applied on TLC plate to obtain final sample concentration of 400 ng band⁻¹.

After chromatographic development peak areas of the bands were measured at 217 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

**Stress degradation studies of bulk drug**

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid/base hydrolytic, oxidative, thermolitic, and photolytic stress conditions.

**Acid treatment**

1 mL working standard solution of VILDA (1000 ng µL⁻¹) was mixed with 1 mL of 5 N methanolic NaOH and the volume was made with solvent. Solution was kept at room temperature for 3 h.

The 6 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. The drug was found to be degraded in acid condition with degradation product at Rf 0.50. The representative densitogram obtained after acid treatment is shown in Figure 1.

1 mL working standard solution of VILDA (1000 ng µL⁻¹) was mixed with 1 mL of 5 N methanolic NaOH and the volume was made with solvent. Solution was kept at room temperature for 3 h. The 6 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. The degradation was observed with decrease in the area but no peak for product of degradation appeared.

**Neutral Hydrolysis**

1 mL working standard solution of VILDA (1000 ng µL⁻¹) was mixed with 1 mL of water and 8 mL methanol. The solution was refluxed for 72 h. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. No degradation was observed for the drug in neutral hydrolytic condition.

**Oxidative degradation**

1 mL working standard solution of VILDA (1000 ng µL⁻¹) was mixed with 1 mL of 30 % solution of H₂O₂ and the volume was made with solvent. Solution was kept at room temperature for 1 h. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. 23.05 % degradation was observed with degradation products at Rf 0.18, 0.34. The representative densitogram obtained after oxidative degradation is shown in Figure 2.
0.70 RESULTS AND DISCUSSION

Optimization of chromatographic conditions
The primary objective in developing this stability indicating HPTLC method is to achieve the resolution of VILDA and its degradation products. The chromatographic separation was achieved using Ethyl acetate: Methanol (8.5: 1.5, v/v) as mobile phase and detection was carried out at 217 nm. The retention factor for VILDA was found to be 0.37 ± 0.003. Representative densitogram of standard solution of VILDA is shown in Figure 4.

![Figure 4: Representative densitogram of standard solution of VILDA (600 ng/band, RF = 0.37 ± 0.003).](image)

Stress degradation studies
The drug was found to be susceptible to acid and base catalysed hydrolysis, thermal stress as well as oxidation and was found to be stable in neutral hydrolytic and photolytic condition. The results obtained indicate that the method is highly specific and the degraded products were well separated the drug. The amount of drug recovered after degradation studies and the RF values of degradation products are given in Table 1.

<table>
<thead>
<tr>
<th>Stress conditions/duration</th>
<th>% Assay of active substance</th>
<th>RF values of degraded products</th>
</tr>
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<tbody>
<tr>
<td>Acidic / 5 N HCl/ Kept at RT for 3 h</td>
<td>89.58</td>
<td>0.50</td>
</tr>
<tr>
<td>Alkaline /5 N NaOH/ Kept at RT for 3 h</td>
<td>91.95</td>
<td>---</td>
</tr>
<tr>
<td>Oxidative /30 % H2O2 / Kept at RT for 1 h</td>
<td>76.94</td>
<td>0.18, 0.34</td>
</tr>
<tr>
<td>Neutral/H2O/ Kept at RT for 72 h</td>
<td>Stable</td>
<td>---</td>
</tr>
<tr>
<td>Photolysis</td>
<td>Stable</td>
<td>---</td>
</tr>
<tr>
<td>Dry heat/ 105°C/ 48 h</td>
<td>79.04</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Method Validation
The method was validated for linearity, accuracy, intra-

![Figure 3: Representative densitogram after dry heat degradation with degradation peak at RF](image)
day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines.\textsuperscript{[17, 18]}

**Preparation of Calibration Curve**

Standard stock solutions of VILDA (100 ng \( \mu L^{-1} \)) were applied by on TLC plate in range of 2, 4, 6, 8, 10 \( \mu L \). Straight-line calibration graphs were obtained in the concentration range 200-1000 ng band\(^{-1} \) with high correlation coefficient.

**Precision**

Set of three different concentrations in three replicates of standard solutions of VILDA were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.77 to 1.47. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.69 to 1.34. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

**Limit of detection (LOD) and Limit of quantitation (LOQ)**

LOD and LOQ were calculated as 3.3 \( \sigma / S \) and 10 \( \sigma / S \), respectively, where \( \sigma \) is the standard deviation of the response (y-intercept) and \( S \) is the slope of the calibration plot. The LOD and LOQ were found to be 61 ng band\(^{-1} \) and 102 ng band\(^{-1} \), respectively.

**Recovery Studies**

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 400 ng band\(^{-1} \) from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are shown in Table 2.

<table>
<thead>
<tr>
<th>Table 2: Recovery Studies of VILDA</th>
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<tbody>
<tr>
<td>Drug</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>VILDA</td>
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</table>

*Average of three determinations

**Robustness Studies**

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, wavelength was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results are given in Table 3.

<table>
<thead>
<tr>
<th>Table 3: Robustness Data in Terms of Peak Area (% RSD)</th>
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<tbody>
<tr>
<td>Sr. No.</td>
</tr>
<tr>
<td>---------</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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*Average of three determinations

**CONCLUSION**

The HPTLC method has been developed and validated for the determination of VILDA as bulk drug and in tablet dosage form. The developed method is simple, precise, accurate, reproducible, and stability-indicating without interference from the excipients or from degradation products and can be used for quantitative analysis of VILDA in pharmaceutical dosage form. As the method is stability indicating one it may be extended to study the degradation kinetics of drug.

**ACKNOWLEDGEMENTS**

The authors express their gratitude to Spectrum Labs, (Hyderabad, India) for the gift sample of pure VILDA.

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