DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF VILDAGLIPTIN FROM TABLET DOSAGE FORM
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ABSTRACT
A new simple, specific, precise and accurate reversed-phase liquid chromatography method has been developed for the determination of Vildagliptin (VLG) in pharmaceutical dosage form. The separation was achieved on a Xterra® Waters C_{18} column (150mm×4.6mm, 5µm) using mobile phase consisting of a mixture of aqueous phase (1 ml of 25% ammonium hydroxide was dissolved in 1000 ml of water for chromatography, pH of the solution was adjusted to the value of 9.5 using a 50% solution of phosphoric acid) and organic phase (methanol) in the ratio of 60:40 v/v at a flow rate of 1.0 ml/min. Detection was carried out at 210nm. The retention time of Vildagliptin was found to be 6.3 min. The calibration curve was found linear between 5-200µg/ml (r^2 = 0.9997). Limit of detection and limit of quantitation were 1.47 and 4.90 µg/mL, respectively. The percentage recoveries of Vildagliptin were found to be in the range of 99.11-100.62%. The method was validated in accordance with International Conference on Harmonization acceptance criteria for specificity, linearity, precision, accuracy, robustness and system suitability. The excipients did not interfere in the determination of VLG. The proposed method was successfully applied for the quantitative analysis of VLG in tablet dosage form, which will help to improve quality control.

Key Words: Vildagliptin, analytical method validation, HPLC

INTRODUCTION
Vildagliptin (VDG), S-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile (Fig.1) is an oral antihyperglycemic agent (anti-diabetic drug)
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of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Vildagliptin 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. Vildagliptin has been shown to reduce hyperglycemia in type 2 diabetes mellitus. Literature survey revealed that few analytical methods are used for estimation of Vildagliptin. But there is no analytical method for the determination of Vildagliptin from its pharmaceutical dosage form. Due to lack of published liquid chromatographic methods for VDG, so the aim of the present work was to develop a reversed-phase liquid chromatographic (RP-LC) method that would be suitable for the determination of VDG from its pharmaceutical dosage form. The proposed method is simple, accurate, reproducible and suitable for routine determination of Vildagliptin from its pharmaceutical dosage form.

MATERIAL AND METHODS
Chemicals and Reagents
Pharmaceutical grade Vildagliptin, certified to contain 99.72% and Galvus tablets nominally containing 50 mg Vildagliptin per tablet were kindly supplied from Novartis Europharm limited company (London, U.K.). HPLC-grade Methanol, ammonium hydroxide (25%) and orthophosphoric acid (85%) were obtained from Merck, Darmstadt, Germany. HPLC grade water was obtained through milli Q water purification system.

Instruments
The HPLC system consisted of a Schimadzu LC-20 AT Liquid Chromatograph (Japan) using a Xterra® Waters C18 column (150mm×4.6mm, 5µm) (Ireland). The system was equipped with a UV-visible detector (SPD-20A, Japan) and an autosampler (SIL-20A, Schimadzu, Japan). An Elma S100 ultrasonic processor model KBK 4200 (Germany) was used for the degassing of the mobile phases. In addition, an electronic balance (Shimadzu AX200), a pH meter (Systronics model EQMK VI), a
sonicator (Spectra Lab, model UCB 40) were used in this study.

**Chromatographic conditions**

Chromatographic separation was achieved on a Xterra® Waters C$_{18}$ column (150mm×4.6mm, 5µm) with UV detection at 210 nm. Aqueous phase was prepared by dissolving 1 ml of 25% ammonium hydroxide in 1000 ml of water and pH of solution was then adjusted to 9.5 with using a 50% solution of phosphoric acid. HPLC-grade Methanol was used as organic phase. Mobile phase was prepared by mixing aqueous phase and organic phase in the ratio of 60:40 v/v. The mobile phase was degassed for 30 min and filtered through 0.2µm Nylon 6, 6 membrane filters before use. The mobile phase was pumped through the column at a flow rate of 1 mL min⁻¹. Analyses were performed at 30°C and the injection volume was 25 µL.

**Preparation of Stock Solution**

About 100 mg of Vildagliptin WS was weighed accurately into a 100ml volumetric flask and dissolved and diluted to volume with mobile phase to obtain a concentration of 1000 µg/ml and this solution was used as stock solution.

**Calibration curve for Vildagliptin**

Appropriate aliquots of stock solution was taken in different 50 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 5-200µg/ml. The solutions were injected into HPLC with 25 µl inject volume and chromatograms were recorded. Calibration curve was constructed by plotting average peak areas versus concentrations and regression equation was computed for the drugs (Table 1).

**Analysis of Marketed Formulation**

Twenty tablets were weighed. An accurately weighed amount of the finely powdered Galvus tablets equivalent to 100 mg of VDG was taken and transferred into a 100 ml volumetric flask; 60ml of mobile phase was added and sonicated with occasional shaking for 10 min. The solution was cooled to room temperature and diluted to volume with the mobile phase. The resultant solution was filtered through Whatman 1
filter paper. 5 ml of this solution was diluted to 50 ml with mobile phase. The final solution was filtered through 0.20µm PTFE membrane filter. 25µl volume of final sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions.

**Procedure**

**Method Validation**
The method of analysis was validated as per the recommendations of ICH\(^4\) and USP\(^5\) for the parameters like accuracy, linearity, precision, detection limit, quantitation and robustness. The accuracy of the method was determined by calculating percentage recovery of Vildagliptin. For the drug, recovery study was carried out by applying the method to drug sample to which known amount of Vildagliptin corresponding to 80%, 100% and 120% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared. Intraday and interday precision study of Vildagliptin was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of 100 µg/ml of Vildagliptin. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve. System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level of 100µg/ml and the results are shown in Table 2. For robustness evaluation of HPLC method a few parameters like flow rate, percentage of organic phase in the mobile phase and pH of aqueous phase (buffer) were deliberately changed. One factor was changed at one time to estimate the effect. The flow rate of the mobile phase was changed from 1.0 to 0.9 and 1.1 ml/min. The organic strength of mobile phase was varied by ± 10% while pH of buffer was varied by ± 0.2
RESULTS AND CONCLUSION
The proposed method was optimized which gave sharp peak with minimum tailing factor for Vildagliptin (Figure 2). The retention time for Vildagliptin was 6.3 min. UV overlain spectra of Vildagliptin showed that it absorbed appreciably at 210nm, so this wavelength was selected as the detection wavelength. The calibration curve for Vildagliptin was found to be linear over the range of 5-200µg/ml. The data of regression analysis of the calibration curves is shown in Table 1. The proposed method was successfully applied to the determination of Vildagliptin in tablet dosage form. The developed method was also found to be specific, since it was able to separate other excipients present in sample solution (Figure 2). The LOD for Vildagliptin was found to be 1.47µg/ml, while LOQ 4.90µg/ml. The results for validation and system suitability test parameters are summarized in Table 2.

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REFERENCES
2. Ramzia I. El-Bagary, Ehab F. Elkady, Bassam M. Ayoub. Liquid Chromatographic Methods for the Determination of Vildagliptin in the tablet dosage form. Results for robustness evaluation for Vildagliptin are presented in Table 3. The proposed RP-HPLC method has the advantages of simplicity, precision, accuracy and convenience for the separation and quantization of VDG in tablet dosage form. The method was validated showing satisfactory data for all the method validation parameters tested. The developed methods can be conveniently used by quality control laboratories.


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FIGURE AND TABLE

Figure 1: Chemical Structures of Vildagliptin
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Figure 2: A typical LC chromatogram of 25 µL injector of 100 µg /ml standard solution

Figure 3: A typical LC chromatogram of 25 µL injector of 100 µg /ml Galvus sample solution

Table 1: Linear Regression Data for Calibration Curves.

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Vildagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>5-200</td>
</tr>
<tr>
<td>$R^2 \pm SD$</td>
<td>0.9997±0.00032</td>
</tr>
<tr>
<td>Slope ± SD</td>
<td>0.35234±0.0101</td>
</tr>
<tr>
<td>Intercept ± SD</td>
<td>0.788532±0.07712</td>
</tr>
<tr>
<td>Avg. of SE of estimation</td>
<td>1.182054</td>
</tr>
</tbody>
</table>
### Table 2: Summary of Validation and System Suitability Test Parameters.

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Vildagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>2-200</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9994±0.00038</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>1.47</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>4.90</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>99.94%</td>
</tr>
</tbody>
</table>

#### Precision (%RSD)
- Interday (n=3): 1.3
- Intraday (n=3): 1.2
- Robustness: Robust
- Retention Time ± allowable time (min.): 6.2±0.2
- Theoretical Plates: 6250
- Tailing Factor (asymmetry factor): 1.12

### Table 3: Summary of Robustness Studies for Vildagliptin

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>% mean assay</th>
<th>% RSD</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of aqueous phase (buffer)</td>
<td>9.3</td>
<td>99.7</td>
<td>0.39</td>
<td>6.189</td>
</tr>
<tr>
<td></td>
<td>9.7</td>
<td>100.1</td>
<td>1.28</td>
<td>6.288</td>
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<tr>
<td>Flow rate (ml/min)</td>
<td>0.9</td>
<td>99.6</td>
<td>1.03</td>
<td>5.589</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>99.8</td>
<td>0.65</td>
<td>6.821</td>
</tr>
<tr>
<td>% of organic phase in the mobile phase</td>
<td>36</td>
<td>99.3</td>
<td>0.95</td>
<td>7.524</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>100.1</td>
<td>1.04</td>
<td>5.245</td>
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