METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF VILDAGLIPTIN AND METFORMIN IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT
A RP-HPLC method was developed and validated for the simultaneous estimation of Metformin Hydrochloride (MET) and Vildagliptin (VIL) in pure and pharmaceutical dosage form. Chromatography was carried on Phenomex (kromosil-250 mm × 4.6 mm, 5 μm) column with mobile phase comprising of phosphate buffer and acetonitrile in the ratio 75:25 v/v. The flow rate was adjusted to 1.0 ml/min with UV detection at 260 nm. The retention times of MET and VIL were found to be 2.4 min, 3.4 min respectively. The different analytical parameters such as accuracy, linearity, precision, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the ICH-Q2B guidelines. The detector response was linear in the range of 25-250 μg/ml, 2.5-25 μg/ml for MET, VIL respectively. The proposed RP-HPLC method is sensitive, precise and accurate so it was successfully applied for the reliable quantification of drugs in the commercial dosage form.

KEYWORDS
Metformin hydrochloride, Vildagliptin, RP-HPLC and Simultaneous estimation.

INTRODUCTION
Metformin hydrochloride (MET), an oral antidiabetic drug which is the first line of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function. MET improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose (hepatic gluconeogenesis). MET activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body
energy balance, and the metabolism of glucose and fats; activation of AMPK is required for metformin’s inhibitory effect on the production of glucose by liver cells. MET is known chemically as 3- (diaminomethylidene) - 1, 1- dimethyl guanidine.\textsuperscript{1-4}

Vildagliptin (VIL) (S) – 1 - [N- (3- hydroxy- 1 - adamantyl) glycyl] pyrrolidine-2- carbonitrile is an oral anti-hyperglycemic agent of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drug. VIL 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.

VIL has been shown to reduce hyperglycemia in type 2 diabetes mellitus. Novartis has since withdrawn its intent to submit VIL to the FDA, as of July 2008. The Food and Drug Administration had demanded additional clinical data before it could approve VIL including extra evidence that skin lesions and kidney impairment seen during an early study on animals have not occurred in human trials. While the drug is still not approved for use in the US, it was approved in Feb 2008 by European Medicines Agency for use within the EU and is listed on the Australian PBS with certain restrictions. The EMEA has also approved a new oral treatment released by Novartis, called Eucreas, a combination of VIL and MET.\textsuperscript{5,6}

Literature survey shows that there are many methods for the quantitative estimation of MET separately and in combination VIL with other drugs.\textsuperscript{7-10} To our knowledge simple and economical analytical method for simultaneous estimation of MET and VIL has not been reported so far. So attempt was taken to develop and validate an economic, rapid reverse phase high performance liquid chromatographic method for the quality control of MET and VIL in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmental friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated as per ICH guidelines and found to be accurate, precise and reproducible.

**MATERIAL AND METHODS**

**Apparatus and chemicals**

Waters HPLC system connected with UV dual λ absorbance Detector 2487 and Empower-2 Software was used. MET and VIL pure drugs were kindly supplied as a gift sample by Dr. Reddys Laboratory, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade, collected from E.Merck, Mumbai. Potassium dihydrogen ortho phosphate, di-sodium hydrogen orthophosphate were analytical reagent grade supplied by Fischer Scientific Chemicals, India. Water HPLC grade was obtained from a Finar Limited, Ahmedabad, India.

**Commercial Formulation**

MET and VIL tablets available in the market in composition of Metformin HCL (500 mg), Vildagliptin (50 mg). The tablets were checked and stored properly.

**Preparation of solutions**

Preparation of mobile phase

Preparation of 0.1M Phosphate buffer (pH 6.8) was carried out by dissolving accurately weighed portion of 2.722g of potassium di hydrogen orthophosphate in 200 ml of HPLC water. Separately 700 mg of di-sodium hydrogen orthophosphate was weighed and dissolved in 20 ml of HPLC water, the pH adjusted to 6.8 using disodium hydrogen orthophosphate, and then the solution was filtered through a 0.22 µm filter membrane and stored in closed container. HPLC grade acetonitrile is filtered and store in a tightly caped container.

**Preparation of standard solution**

500 mg of MET and 50 mg of VIL was weighed accurately and dissolved in HPLC water in 50 ml volumetric flask, which gave 10000 µg/ml of MET and 1000 µg/ml of VIL.

From the above solution 1 ml was diluted to 10ml (1000 µg/ml MET and 100 µg/ml of VIL). From this into a series of five 10 ml volumetric flasks 0.25, 0.5, 1, 1.5, 2, 2.5 ml were transferred and diluted to 10 ml with HPLC water, that gave 2.5, 5, 10, 15, 20,
and 25 µg/ml of VIL and 25, 50, 100, 150, 200 and 250 µg/ml of MET.

Preparation of test solution
20 tablets of combined formulation of MET and VIL were weighed, average weight was calculated and triturated in a mortar with pestle from that, powder equivalent to 500 mg of MET and 50 mg of VIL was weighed and dissolved in HPLC water and test concentration was prepared by further dilution with same.

Chromatographic Conditions
The mobile phase, a mixture of 0.1M phosphste buffer pH 6.8 and acetonitrile (75:25 v/v) pumped at a flow rate of 1.0 ml/min through the column (Phenomex kromosil 5μ, 4.6 × 250 mm). The mobile phase was degassed prior to use under vacuum by filtration through a 0.22 μ membrane filter. Both drugs showed good absorbance at 260 nm, which was selected as wavelength for further analysis.

DEVELOPMENT AND VALIDATION OF HPLC METHOD
Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of MET and VIL in tablet dosage form. The experiment was carried out according to the official specifications of ICH. The method was validated for the parameters like system suitability, specificity, linearity, precision, accuracy, LOD, LOQ and robustness.

System Suitability
System suitability study of the method was carried out by six replicate analysis of solution containing 100% test concentration of MET and VIL. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Specificity
Specificity test determines the effect of excipients on the assay result. To determine the specificity of the method, standard solution of MET and VIL were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another. No any interference at retention time of drugs was observed.

Linearity
Linearity of the method was determined by constructing calibration curves. Standard solutions of MET and VIL of different concentrations level (25, 50, 100, 150, 200 and 25 µg/ml) were used for this purpose. Each measurement was carried out in 6 replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Accuracy
Accuracy is the percentage of analyte recovered by assay from a known added amount. To check the degree of accuracy of the method, recovery studies were performed in six times by standard addition method at 50%, 100% and 150%. Known amounts of standard MET and VIL were added to pre-analyzed samples and were subjected to the proposed HPLC method.

Precision
Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Limit of detection (LOD) and Limit of quantification (LOQ)
LOD and LOQ were calculated for the sensitivity of the method. They were quantified based on the signal to noise ratio. LOD is lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines.

\[
\text{LOD} = 3.3 \times \text{SD/SLOPE}
\]
\[
\text{LOQ} = 10 \times \text{SD/SLOPE}
\]

Robustness of Method
To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate and absorbance (nm) on the
retention time and tailing factor were studied. The method was found to be unaffected by small changes 1±0.1ml change in flow rate and small changes in absorbance as 260±2nm.

RESULTS AND DISCUSSION
The developed method has been validated as per ICH guidelines. Every 20 μL of the working standard solution of VIL in the concentration range of 2.5 to 25 μg/mL, and that for MET in the concentration range of 25 to 250 μg/mL were injected into the chromatographic system. The chromatograms were recorded and the peak area was determined for each concentration of the drug solution. Calibration curves of VIL and MET were obtained by plotting the peak area versus concentrations of VIL and MET. System suitability and precision study are shown in Table No.1. Precision study of the developed method is in Table No.2. Standard chromatogram and marketed formulation chromatogram are in Figure No.1 and 2. Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, Limit of detection (LOD) and limit of quantification (LOQ) were calculated. Results of accuracy study are presented in Table No.3. The measured value was obtained by recovery test. Spiked amount of both the drugs were compared against the recovery amount. All the results indicate that the method is highly accurate. The results of robustness of the present method showed that small changes were made in the flow rate did not produce significant changes in analytical results, we can say that the method is robust. Results of robustness are presented in Table No. 4.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>MET</th>
<th>VIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity range</td>
<td>25-50 μg/ml</td>
<td>2.5-25 μg/ml</td>
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<tr>
<td>2</td>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
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<tr>
<td>3</td>
<td>Slope</td>
<td>2796</td>
<td>650.8</td>
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<tr>
<td>4</td>
<td>Intercept</td>
<td>-26058</td>
<td>-13643</td>
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<tr>
<td>5</td>
<td>Retention time(min)</td>
<td>2.8 min</td>
<td>3.9 min</td>
</tr>
<tr>
<td>6</td>
<td>USP plate count</td>
<td>3850</td>
<td>7700</td>
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<tr>
<td>7</td>
<td>Tailing factor</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>8</td>
<td>Limit of Detection (LOD)</td>
<td>0.219 μg/ml</td>
<td>0.0053 μg/ml</td>
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<tr>
<td>9</td>
<td>Limit of quantification (LOQ)</td>
<td>0.669 μg/ml</td>
<td>0.0159 μg/ml</td>
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Table No.2: Precision Study

<table>
<thead>
<tr>
<th>S.No</th>
<th>Repeatability (% RSD) (n=6)</th>
<th>Intermediate precision (% RSD) (n=6)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Analyst 1</td>
</tr>
<tr>
<td>1</td>
<td>MET</td>
<td>0.6413</td>
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<tr>
<td>2</td>
<td>VIL</td>
<td>1.0830</td>
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</table>
Table No.3: Accuracy (% recovery) results of MET and VIL

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Spiked Amount (mg)</th>
<th>Recovered Amount (mg)</th>
<th>% Recovered</th>
<th>Average Recovery</th>
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<tbody>
<tr>
<td>1</td>
<td>VIL</td>
<td>5</td>
<td>5.02</td>
<td>100.7</td>
<td>100.06</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>15</td>
<td>15.01</td>
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<td>2</td>
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<td>50</td>
<td>49.76</td>
<td>99.54</td>
<td>99.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>100.03</td>
<td>100.03</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>150</td>
<td>150.01</td>
<td>100.006</td>
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Table No.4: Results for robustness test of VIL and MET

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug</th>
<th>Parameters count</th>
<th>Changes</th>
<th>RT(min)</th>
<th>USP Tailing</th>
<th>USP Plate</th>
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<tbody>
<tr>
<td>1</td>
<td>VIL</td>
<td>Flow rate (ml/min)</td>
<td>0.9</td>
<td>3.885</td>
<td>1.2</td>
<td>3856</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.1</td>
<td>3.252</td>
<td>1.3</td>
<td>3789</td>
</tr>
<tr>
<td>2</td>
<td>MET</td>
<td>Flow rate (ml/min)</td>
<td>0.9</td>
<td>2.789</td>
<td>1.3</td>
<td>3850</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.1</td>
<td>2.338</td>
<td>1.2</td>
<td>3758</td>
</tr>
</tbody>
</table>

Figure No.1: Typical Chromatogram of standard MET and VIL
Figure No.2: Typical chromatogram of MET and VIL in marketed formulation

Figure No.3: Linearity of MET

\[ y = 2796.5x + 26058 \]  
\[ R^2 = 0.9994 \]
CONCLUSION
The new RPHPLC method developed and validated for simultaneous estimation of MET and VIL in pure and in pharmaceutical dosage form and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation. The developed method was validated in terms of accuracy, repeatability, and precision. The assay experiment showed that the contents of VIL and MET estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of VIL and MET simultaneously, from its pharmaceutical formulations and pure drug.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

REFERENCES


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