RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF METFORMIN, VILDAGLIPTIN AND REMOGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Kedar A. Jagtap1, Vasudha S. Bavadekar2*, Pravin D. Chaudhari3, Geeta A. Anawade4, Akshata B. Birajdar5

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Abstract

A precise and robust method was developed for estimation of Metformin (MET), Vildagliptin (VDG) and Remogliflozin (RMG) in bulk and formulations by RP-HPLC technique. The Method used Agilent 1260 Infinity II model HPLC with DAD detector and Agilent Zorbax SB-Aq Column with dimension 250 x 4.6 mm, 5 µm. The Mobile phase combination used was Phosphate Buffer pH 3.3 and Acetonitrile (50:50). Flow rate at 1.0 ml/min and wavelength at 210 nm with run time of 15 minutes. The retention time of MET, VDG and RMG peaks was at 2.21, 3.68 and 8.14 minutes, respectively. The method was validated as per ICH guidelines. The instrument precision for MET, VDG & RMG had a %RSD of 0.75%, 0.72 and 1.04%, respectively. Method was linear and accurate for concentration range 400-600 µg/ml for MET, 40-60 µg/ml for VDG and 80-120 µg/ml for RMG with regression coefficient for MET, VDG & RMG of 0.9999, 0.9994 and 0.9996, respectively and % RSD for accuracy for MET at 80%, 100% and 120% was found to be 0.57%, 0.44% and 0.31%, respectively; for VDG at 80%, 100% and 120% was found to be 0.06%, 0.33% and 0.18% respectively; and for RMG at 80%, 100% and 120% was found to be 0.29%, 0.89% and 0.29% respectively.

Keywords: RP-HPLC, Metformin (MET), Vildagliptin (VDG) and Remogliflozin (RMG), Diabetes Mellitus.

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1. Introduction

Metformin Hydrochloride, Vildagliptin and Remogliflozin Etabonate are used to treat Diabetes Mellitus. Metformin is a biguanide drug that reduces blood glucose levels by decreasing glucose production in the liver, decreasing intestinal absorption, and increasing insulin sensitivity. Metformin decreases both basal and postprandial blood glucose levels. [1]

Vildagliptin is an orally active antihyperglycemic agent that selectively inhibits the dipeptidyl peptidase-4 (DPP-4) enzyme. It is used to manage type II diabetes mellitus, where GLP-1 secretion and insulinoic effects are impaired. [2]

Remogliflozin etabonate is SGLT2 inhibitor class of drugs that have been recently approved in India for the management of Type 2 Diabetes Mellitus. Remogliflozin is a potent and selective inhibitor of SGLT2 with the unique distinction of being administered as a prodrug, existence of active metabolites, and short half-life necessitating twice-daily dosing. [3]

The chemical name (IUPAC) of Metformin Hydrochloride is 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride (Figure 1).

![Figure 1: Chemical Structure of Metformin Hydrochloride](image1.png)

The chemical name (IUPAC) of Vildagliptin is (2S)-1-[2-[(3-hydroxy-1-adamantyl)amino]acetyl]pyrrolidine-2-carbonitrile (Figure 2).

![Figure 2: Chemical Structure of Vildagliptin](image2.png)

The chemical name (IUPAC) of Remogliflozin Etabonate is ethyl [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[5-methyl-1-propan-2-yl]-4-[(4-propan-2-yloxyphenyl) methyl] pyrazol-3-yl]oxyoxan-2-yl] methyl carbonate (Figure 3).
According to the literature review [7-23], there are no Liquid Chromatography analysis for Simultaneous estimation of MET, VDG and RMG in Combination pharmaceutical dosage form.. So, current study was planned for development and validation of method developed for Metformin Hydrochloride, Vildagliptin and Remogliflozin.

### Table No. 1: Quality Target Profile for HPLC Method development

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical Plates</td>
<td>Not less than 2000</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>Not More than 2.0 (Fairly at 1.0)</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>Not More than 2.0 (Fairly at 1.0)</td>
</tr>
<tr>
<td>Run time</td>
<td>Not More than 20 minutes</td>
</tr>
<tr>
<td>Resolution</td>
<td>Not Less than 2.0</td>
</tr>
</tbody>
</table>

### 2. Material and Method

#### 2.1. Chemicals and Reagents

Aadhaar Life Sciences Pvt. Ltd. provided a complimentary sample of Metformin Hydrochloride, Vildagliptin and Remogliflozin Etabonate. Acetonitrile was purchased from Merck in India and was of HPLC grade. Internal Milli-Q system provided water. All weighing was done using calibrated NABL scales. Samples were produced in Type A glassware and the analytical balance.

#### 2.2. Instrumentation

Agilent 1260 Infinity II with a DAD detector and quaternary pump was the tool utilized for development and validation. Agilent's open labs EzChrome software was employed. The labman ultrasonicator and the Aczet analytical balance were used for wet chemistry.

#### 2.3. HPLC Method Development

2.3.1. The table 2 and describes trials done during the development phase with the results and observations.

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Mobile Phase</th>
<th>Mobile phase Ratio</th>
<th>Diluent</th>
<th>Column</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol: Buffer</td>
<td>50-50</td>
<td>ACN-Water (50-50)</td>
<td>Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)</td>
<td>250</td>
</tr>
<tr>
<td>2</td>
<td>Methanol: Buffer</td>
<td>50-50</td>
<td>ACN-Water (50-50)</td>
<td>Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)</td>
<td>210</td>
</tr>
</tbody>
</table>
RP-HPLC Method Development and Validation of Metformin, Vildagliptin and Remogliflozin in Bulk and Pharmaceutical Dosage form

Table No. 3: Results of Method Development

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Metformin HCl</th>
<th>Vildagliptin</th>
<th>Remogliflozin Etabonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.54 76 13 1.10 0.00</td>
<td>No peak Observed</td>
<td>No peak observed</td>
</tr>
<tr>
<td>2</td>
<td>2.69 69 20 1.09 0.00</td>
<td>2.94 652 3 0.99</td>
<td>2.99 2.25 267 6 0.00</td>
</tr>
<tr>
<td>3</td>
<td>2.13 64 02 1.15 0.00</td>
<td>2.25 267 6 0.00</td>
<td>1.08 807 8 1.08</td>
</tr>
<tr>
<td>4</td>
<td>2.29 65 38 1.11 0.00</td>
<td>2.71 807 8 1.08</td>
<td>1.20 130 01 1.20</td>
</tr>
<tr>
<td>5</td>
<td>2.21 62 99 1.12 0.00</td>
<td>3.68 130 01 1.20</td>
<td>17.90 8.14 201 36 1.01 17.67</td>
</tr>
</tbody>
</table>

For all the above trials, wavelength was kept constant at 210 nm, as this was predetermined using HPLC DAD detector. Diluent was kept constant as 50-50 Acetonitrile-Water for all trails. Column used for all trails was Agilent Zorbax SB-Aq (250 x 4.6 mm, 5 micron). Based in the predetermined quality target profile for development work, the condition for trial 5 was finalized and individual Standard were run to confirm the retention times.
RP-HPLC Method Development and Validation of Metformin, Vildagliptin and Remogliflozin in Bulk and Pharmaceutical Dosage form

2.3.2. Final Chromatographic Conditions:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC Instrument</td>
<td>Agilent 1260 Infinity II</td>
</tr>
<tr>
<td>Column</td>
<td>Agilent Zorbax SB-Aq, 5µ, 100Å, 250 x 4.60 mm</td>
</tr>
<tr>
<td>Wavelength</td>
<td>210 nm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Buffer : Acetonitrile (50-50)</td>
</tr>
<tr>
<td>Diluent</td>
<td>Acetonitrile : Water (50:50) v/v</td>
</tr>
<tr>
<td>Run time</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 micro liters</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0 ml/min</td>
</tr>
<tr>
<td>Column oven Temperature</td>
<td>30°C (± 2°C allowed by Robustness)</td>
</tr>
</tbody>
</table>

2.3.3. Preparation of Mobile Phase

Preparation of 50 Mmol/L Potassium Phosphate Buffer

Weigh about 6.8 g of Potassium Dihydrogen Phosphate into a suitable container and add 1000 mL of water using graduated cylinder. Mix well. Adjust the pH of the solution to 3.3 with o-phosphoric acid.

Mobile Phase: 50% Buffer: 50% Acetonitrile

Mix separately measured 500 mL of Potassium Phosphate Buffer and 500 mL of Acetonitrile into a suitable container. Filter the mobile phase through 0.45 µm nylon membrane filter. Briefly sonicate to degas.

2.3.4. Preparation of Diluent
Mix separately measured 500 mL of Water with 500 mL of Acetonitrile into a suitable container and mix well. Mixture is to be filtered through 0.45 μm nylon membrane filter. Briefly sonicate to degas.

2.3.5. Preparation of Standard Solution

A. Working Standard:
1. Remogliflozin Etabonate Stock Solution-I (RSS-I):
Prepare a Remogliflozin Etabonate Stock Solution (RSS-I) by adding 10 mg of Remogliflozin Etabonate in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Remogliflozin Etabonate = 1000 µg/ml).

2. Vildagliptin Stock Solution-I (VSS-II):
Prepare a Vildagliptin Stock Solution (VSS-II) by adding 5 mg of Vildagliptin in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Vildagliptin = 500 µg/ml).

3. Metformin Hydrochloride Stock Solution-I (MSS-III):
Prepare a Metformin Hydrochloride Stock Solution (MSS-III) by adding 50 mg of Metformin Hydrochloride in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Metformin Hydrochloride = 5000 µg/ml).

4. Add 1.0 ml of RSS-I, 1.0 ml of VSS-II and 1.0 ml of MSS-III in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Remogliflozin Etabonate =100 µg/ml, Vildagliptin = 50 µg/ml, Metformin HCl = 500 µg/ml).

B. Preparation of Sample for Assay
1. Weigh 10 tablets and calculate average weight of 1 tablet, transfer tablets into mortar and pestle and crush them. Weigh powder equivalent to 500 mg of metformin, 100 mg of Remogliflozin and 50 mg of Vildagliptin and transfer to 100 ml volumetric flask & add 50-70 ml diluent, mix for 5 minutes and make the volume to 100 ml with diluent. (Conc. of Remogliflozin Etabonate =1000 µg/ml, Vildagliptin = 500 µg/ml, Metformin HCl = 5000 µg/ml).

2. Then add 1.0 ml of above stock solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Remogliflozin Etabonate =100 µg/ml, Vildagliptin = 50 µg/ml, Metformin HCl = 500 µg/ml).

2.4. Method validation

2.4.1. Specificity
Individual injections of Remogliflozin, metformin and Vildagliptin were prepared of 100 µg/ml, 500 µg/ml and 50 µg/ml, respectively and peaks were identified from Retention Time. Blank was injected to ensure there is no blank peak interfering with the main analyte peaks.

2.4.2. System Suitability
Using a series of tests, the suitability and performance of the system were examined. Theoretical Plate count, tailing factor, and resolution are all found to be within allowed ranges for the ICH guideline system.

2.4.3. Accuracy
To determine the accuracy of a technique, one must examine how closely its test findings correspond to the actual value. In the recovery studies, three distinct concentration levers were evaluated. At each level, three replicate injections were performed and the amount of drug present, the percentage of recovery, and the related standard deviation were calculated.

2.4.4. Repeatability
Analytical precision is determined by the degree of concordance between individual test results. Multiple samples of a uniform sample were examined. A single sample was prepared as described and 6 injections were made from same sample and checked for system suitability. Instrument precision was performed as Instrument precision (how good the instrument preforms back-to-back replicate injection of same concentration).

2.4.5. Linearity
Methodological linearity is the capacity of an analytical method to yield results proportionate to analyte concentrations within a given range. There were five sets of standard solutions used to determine linearity. On the calibration curve, the peak area against concentration of the
standard solution was plotted, and the regression equation was developed. The least-squares method was utilized to determine the slope, intercept, and correlation coefficient.

2.4.6. LOD and LOQ
The LOD and LOQ are denoting ability of the method to detect and quantify smallest amount of analyte, respectively. The LOD and LOQ were calculated by using standard deviation and slope of regression line by using following equations.

2.4.7. Robustness
The Robustness was performed by changing the mobile phase A concentration by ± 2% and column temperature by ± 2°C.

Table No. 5: Robustness Trials

<table>
<thead>
<tr>
<th>Condition</th>
<th>Increased</th>
<th>Normal</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase Conc (A:B)</td>
<td>A52:B48</td>
<td>A50:B50</td>
<td>A48:B52</td>
</tr>
<tr>
<td>Column Oven Temperature</td>
<td>32°C</td>
<td>30°C</td>
<td>28°C</td>
</tr>
</tbody>
</table>

2.4.8. Inter-day & Intraday Precision:
The prepared working standard was analyzed in morning and at evening and % RSD was calculated to identify the stability of solution for intraday precision. The same solution was injected on second day and compared with morning results of intraday precision and % RSD was calculated.

3. Results and Discussion

3.1. Specificity
Specificity was performed to check if there was any interaction between the peaks from blank or the APIs.

Table no. 6: Specificity and ID of MET, VDG and RMG

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Metformin</th>
<th>Vildagliptin</th>
<th>Remogliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metformin ID</td>
<td>2.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vildagliptin ID</td>
<td>-</td>
<td>3.68</td>
<td>-</td>
</tr>
<tr>
<td>Remogliflozin ID</td>
<td>-</td>
<td>-</td>
<td>6.45</td>
</tr>
<tr>
<td>MIX WS</td>
<td>2.15</td>
<td>3.68</td>
<td>6.45</td>
</tr>
<tr>
<td>Drug Product</td>
<td>2.15</td>
<td>3.68</td>
<td>6.45</td>
</tr>
</tbody>
</table>

a. Diluent
b. Metformin Hydrochloride ID
c. Vildagliptin ID
d. Remogliflozin Etabonate ID
3.2. Instrument Precision and System suitability

The HPLC Instrument was tested for its suitability to perform the validation. Based on the limits mentioned in table 1, the equipment was found to be suitable for continuing the validations. Instrument precisions of all 3 drugs were performed after system suitability and the reported data in below shows the relative standard deviation for Instrument precision of MET, VDG & RMG are 0.75%, 0.72% and 1.04% respectively. This %RSD shows the method is very much precise with respect to multiple sample preparation for same concentration. The data is shown in table 7-10.

Table 7: System suitability for MET

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>100% Rep 1</td>
<td>2.15</td>
</tr>
<tr>
<td>100% Rep 2</td>
<td>2.15</td>
</tr>
<tr>
<td>100% Rep 3</td>
<td>2.15</td>
</tr>
<tr>
<td>100% Rep 4</td>
<td>2.15</td>
</tr>
<tr>
<td>100% Rep 5</td>
<td>2.15</td>
</tr>
<tr>
<td>100% Rep 6</td>
<td>2.15</td>
</tr>
<tr>
<td>Average</td>
<td>2.15</td>
</tr>
<tr>
<td>STDEV</td>
<td>0</td>
</tr>
</tbody>
</table>
### RP-HPLC Method Development and Validation of Metformin, Vildagliptin and Remogliflozin in Bulk and Pharmaceutical Dosage form

**Section A-Research paper**

**Table 8: System suitability for VDG**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Vildagliptin</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>TP</td>
<td>Asymmetry</td>
<td>Resolution</td>
</tr>
<tr>
<td>100% Rep 1</td>
<td>3.68</td>
<td>10120</td>
<td>1.16</td>
<td>12.01</td>
</tr>
<tr>
<td>100% Rep 2</td>
<td>3.68</td>
<td>10385</td>
<td>1.15</td>
<td>12.01</td>
</tr>
<tr>
<td>100% Rep 3</td>
<td>3.68</td>
<td>10225</td>
<td>1.13</td>
<td>12.01</td>
</tr>
<tr>
<td>100% Rep 4</td>
<td>3.68</td>
<td>10397</td>
<td>1.17</td>
<td>12.01</td>
</tr>
<tr>
<td>100% Rep 5</td>
<td>3.68</td>
<td>10455</td>
<td>1.14</td>
<td>12.01</td>
</tr>
<tr>
<td>100% Rep 6</td>
<td>3.68</td>
<td>10193</td>
<td>1.17</td>
<td>12.01</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>3.68</strong></td>
<td><strong>10193</strong></td>
<td><strong>1.17</strong></td>
<td><strong>12.01</strong></td>
</tr>
<tr>
<td><strong>STDEV</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td>0.00</td>
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</table>

**Table 9: System suitability for RMG**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Remogliflozin</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>TP</td>
<td>Asymmetry</td>
<td>Resolution</td>
</tr>
<tr>
<td>100% Rep 1</td>
<td>6.45</td>
<td>13675</td>
<td>1.06</td>
<td>15.11</td>
</tr>
<tr>
<td>100% Rep 2</td>
<td>6.45</td>
<td>13978</td>
<td>1.03</td>
<td>15.11</td>
</tr>
<tr>
<td>100% Rep 3</td>
<td>6.45</td>
<td>13551</td>
<td>1.05</td>
<td>15.11</td>
</tr>
<tr>
<td>100% Rep 4</td>
<td>6.45</td>
<td>13256</td>
<td>1.02</td>
<td>15.11</td>
</tr>
<tr>
<td>100% Rep 5</td>
<td>6.45</td>
<td>13589</td>
<td>1.02</td>
<td>15.11</td>
</tr>
<tr>
<td>100% Rep 6</td>
<td>6.45</td>
<td>13566</td>
<td>1.05</td>
<td>15.11</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>6.45</strong></td>
<td><strong>13589</strong></td>
<td><strong>1.03</strong></td>
<td><strong>15.11</strong></td>
</tr>
<tr>
<td><strong>STDEV</strong></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 10: Instrument Precision for MET, VDG & RMG**

<table>
<thead>
<tr>
<th>Repeatability</th>
<th>Met Area</th>
<th>Vilda Area</th>
<th>Remo Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Rep 1</td>
<td>23344798</td>
<td>2546218</td>
<td>2394339</td>
</tr>
<tr>
<td>100% Rep 2</td>
<td>23419452</td>
<td>2532451</td>
<td>2352545</td>
</tr>
<tr>
<td>100% Rep 3</td>
<td>23215458</td>
<td>2547895</td>
<td>2368733</td>
</tr>
<tr>
<td>100% Rep 4</td>
<td>23222145</td>
<td>2567845</td>
<td>2319493</td>
</tr>
<tr>
<td>100% Rep 5</td>
<td>23545871</td>
<td>2514136</td>
<td>2356857</td>
</tr>
<tr>
<td>100% Rep 6</td>
<td>23654854</td>
<td>2553245</td>
<td>2368745</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>23400430</td>
<td>2543632</td>
<td>2360119</td>
</tr>
<tr>
<td><strong>STDEV</strong></td>
<td>176262.074</td>
<td>18431.324</td>
<td>24649.93009</td>
</tr>
<tr>
<td><strong>% RSD</strong></td>
<td>0.75</td>
<td>0.72</td>
<td>1.04</td>
</tr>
</tbody>
</table>
3.3. Linearity of MET, VDG and RMG
Linearity was performed at different levels. The graph plotted between peak area and concentration showed linearity with correlation coefficient as shown in table below. The linearity data in shown in table 11 and graph in figure 7.

<table>
<thead>
<tr>
<th>Sample Conc (µg/ml)</th>
<th>Metformin HCl Peak Area</th>
<th>Vildagliptin Sample Conc (µg/ml) Peak Area</th>
<th>Remogliflozin Etabonate Sample Conc (µg/ml) Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>18888553</td>
<td>40</td>
<td>2025607</td>
</tr>
<tr>
<td>450</td>
<td>21050550</td>
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<td>2278841</td>
</tr>
<tr>
<td>500</td>
<td>23344798</td>
<td>50</td>
<td>2546218</td>
</tr>
<tr>
<td>550</td>
<td>25573828</td>
<td>55</td>
<td>2790633</td>
</tr>
<tr>
<td>600</td>
<td>27906852</td>
<td>60</td>
<td>3023372</td>
</tr>
<tr>
<td>R²</td>
<td>0.9999</td>
<td>R²</td>
<td>0.9994</td>
</tr>
</tbody>
</table>

Figure No. 6: Instrument Precision of MET, VDG & RMG
RP-HPLC Method Development and Validation of Metformin, Vildagliptin and Remogliflozin in Bulk and Pharmaceutical Dosage Form

**Section A: Research paper**

**RP-HPLC Method Development and Validation of Metformin, Vildagliptin and Remogliflozin in Bulk and Pharmaceutical Dosage Form**


### Figure No. 7: Linearity Plot for MET, VDG & RMG

#### 3.4. LOD and LOQ for MET, VDG & RMG

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined for MET, VDG and RMG. The results of analysis are shown in table 12.

#### Table No. 12: LOD and LOQ for MET, VDG & RMG

<table>
<thead>
<tr>
<th>Name</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>11.66</td>
<td>35.33</td>
</tr>
<tr>
<td>Vildagliptin</td>
<td>2.40</td>
<td>7.28</td>
</tr>
<tr>
<td>Remogliflozin Etabonate</td>
<td>3.65</td>
<td>11.07</td>
</tr>
</tbody>
</table>

The LOD and LOQ were significantly low, implying the method to be very efficient in determining low concentration of drug. This value of LOD and LOQ can be used during cleaning validation in industry which can help companies know if the manufactured vessel or equipment is free from APIs stains.

#### 3.5. Accuracy

Accuracy for MET was performed in triplicates and it was observed that the method was accurate for the range 80%, 100% and 120%. The relative standard deviation for 80%, 100% and 120% were 0.57%, 0.44% and 0.31% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately. The accuracy data is shown in table 13.

#### Table No.13: Accuracy data for Metformin HCl

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Reps</th>
<th>Spiked Conc (µg/ml)</th>
<th>Area</th>
<th>Amount Recovered (µg/ml)</th>
<th>% Recovery</th>
<th>AVG</th>
<th>STDEV</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>Rep 1</td>
<td>400</td>
<td>18888553</td>
<td>403.59</td>
<td>100.90</td>
<td>100.81</td>
<td>0.573905</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Rep 2</td>
<td></td>
<td>18756213</td>
<td>400.77</td>
<td>100.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rep 3</td>
<td></td>
<td>18968987</td>
<td>405.31</td>
<td>101.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>Rep 1</td>
<td>500</td>
<td>23344798</td>
<td>498.81</td>
<td>99.76</td>
<td>99.68</td>
<td>0.441066</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Rep 2</td>
<td></td>
<td>23419452</td>
<td>500.41</td>
<td>100.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rep 3</td>
<td></td>
<td>23215458</td>
<td>496.05</td>
<td>99.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>Rep 1</td>
<td>600</td>
<td>27906852</td>
<td>596.29</td>
<td>99.38</td>
<td>99.73</td>
<td>0.305564</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Rep 2</td>
<td></td>
<td>28069541</td>
<td>599.77</td>
<td>99.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rep 3</td>
<td></td>
<td>28035485</td>
<td>599.04</td>
<td>99.84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Accuracy for VDG was performed in triplicates and it was observed that the method was accurate for the range 80%, 100% and 120%. The relative standard deviation for 80%, 100% and 120% were 0.57%, 0.44% and 0.31% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately. The accuracy data is shown in table 14.

### Table No.14: Accuracy data for Vildagliptin

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Reps</th>
<th>Spiked Conc (ug/ml)</th>
<th>Area</th>
<th>Amount Recovered (ug/ml)</th>
<th>% Recovery</th>
<th>AVG</th>
<th>STDEV</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>40</td>
<td>Rep 1</td>
<td>2025607</td>
<td>39.82</td>
<td>99.54</td>
<td>99.47</td>
<td>0.062576</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 2</td>
<td>2023215</td>
<td>39.77</td>
<td>99.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 3</td>
<td>2023654</td>
<td>39.78</td>
<td>99.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>50</td>
<td>Rep 1</td>
<td>2546218</td>
<td>50.05</td>
<td>100.10</td>
<td>99.94</td>
<td>0.333149</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 2</td>
<td>2532451</td>
<td>49.78</td>
<td>99.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 3</td>
<td>2547895</td>
<td>50.08</td>
<td>100.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>60</td>
<td>Rep 1</td>
<td>3023372</td>
<td>59.43</td>
<td>99.05</td>
<td>98.88</td>
<td>0.175363</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 2</td>
<td>3018453</td>
<td>59.33</td>
<td>98.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 3</td>
<td>3012678</td>
<td>59.22</td>
<td>98.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Accuracy for RMG was performed in triplicates and it was observed that the method was accurate for the range 80%, 100% and 120%. The relative standard deviation for 80%, 100% and 120% were 0.57%, 0.44% and 0.31% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately. The accuracy data is shown in table 15.

### Table No.15: Accuracy data for Remogliflozin Etabonate

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Reps</th>
<th>Spiked Conc (ug/ml)</th>
<th>Area</th>
<th>Amount Recovered (ug/ml)</th>
<th>% Recovery</th>
<th>AVG</th>
<th>STDEV</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>80</td>
<td>Rep 1</td>
<td>1916948</td>
<td>81.22</td>
<td>101.53</td>
<td>101.56</td>
<td>0.297823</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 2</td>
<td>1923541</td>
<td>81.50</td>
<td>101.88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 3</td>
<td>1912354</td>
<td>81.03</td>
<td>101.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>100</td>
<td>Rep 1</td>
<td>2394339</td>
<td>101.45</td>
<td>101.45</td>
<td>100.50</td>
<td>0.892884</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 2</td>
<td>2352545</td>
<td>99.68</td>
<td>99.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 3</td>
<td>2368733</td>
<td>100.36</td>
<td>100.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>120</td>
<td>Rep 1</td>
<td>2865348</td>
<td>121.41</td>
<td>101.17</td>
<td>100.85</td>
<td>0.290818</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 2</td>
<td>2854225</td>
<td>120.94</td>
<td>100.78</td>
<td></td>
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<td></td>
<td>Rep 3</td>
<td>2849264</td>
<td>120.73</td>
<td>100.60</td>
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<td></td>
</tr>
</tbody>
</table>

### 3.5. Inter and Intraday Precision

Intra and inter day precision study was performed and reported the % RSD change in peak area of the APIs at different time points. The acceptance criteria are to have %RSD of peak area <2%. The Results are given in Table 16.
Table No. 16. Inter and Intraday Precision

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Metformin</th>
<th>Vildagliptin</th>
<th>Remogliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT Area</td>
<td>RT Area</td>
<td>RT Area</td>
</tr>
<tr>
<td>Morning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td>23344798</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2546218</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>2394339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td>23157854</td>
<td>3.68</td>
</tr>
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<td></td>
<td></td>
<td>2515895</td>
<td>6.45</td>
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<td></td>
<td>2356785</td>
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<td></td>
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<tr>
<td>RSD</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>1.12</td>
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</table>

Inter-day Precision

<table>
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<tr>
<th>Sample ID</th>
<th>Metformin</th>
<th>Vildagliptin</th>
<th>Remogliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT Area</td>
<td>RT Area</td>
<td>RT Area</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td>23344798</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2546218</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>2394339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td>23035545</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
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<td>2484456</td>
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</tr>
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</tr>
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<td>RSD</td>
<td>-</td>
<td>0.94</td>
<td>-</td>
</tr>
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<td></td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.67</td>
</tr>
</tbody>
</table>

3.6. Robustness

Robustness is done to check how deviating the method is with respect to its critical parameters. All over the world, the equipment is calibrated before use, but to know if the method is robust, changes were done in column temperature and Mobile phase as shown in table 17 and 18.

Table No. 17: Robustness data for MEG, VDG & RMG with changes in Mobile Phase Composition

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample ID</th>
<th>Metformin</th>
<th>Vildagliptin</th>
<th>Remogliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT Area</td>
<td>RT Area</td>
<td>RT Area</td>
</tr>
<tr>
<td>Increase</td>
<td>WS</td>
<td>2.15</td>
<td>2348515</td>
<td>3.68</td>
</tr>
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<td>6.45</td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>2.15</td>
<td>2319745</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>254987</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98.77</td>
<td>100.3</td>
<td>6.45</td>
</tr>
<tr>
<td>Normal</td>
<td>WS</td>
<td>2.15</td>
<td>2334479</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>254621</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.29</td>
<td>100.3</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>2.15</td>
<td>2317844</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>255458</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.29</td>
<td>100.3</td>
<td>6.45</td>
</tr>
<tr>
<td>Decrease</td>
<td>WS</td>
<td>2.15</td>
<td>2355654</td>
<td>3.68</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>100.0</td>
<td>99.79</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>2.15</td>
<td>2356855</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>100.0</td>
<td>99.67</td>
<td>6.45</td>
</tr>
</tbody>
</table>

Table No. 18: Robustness data for MEG, VDG & RMG with changes in Mobile Phase Composition

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample ID</th>
<th>Metformin</th>
<th>Vildagliptin</th>
<th>Remogliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT Area</td>
<td>RT Area</td>
<td>RT Area</td>
</tr>
<tr>
<td>Increase</td>
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</tr>
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<td>3</td>
<td>256144</td>
<td>6.45</td>
</tr>
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<td>DP</td>
<td>2.15</td>
<td>232115</td>
<td>3.68</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>254598</td>
<td>99.40</td>
</tr>
</tbody>
</table>

There was a small change in retention time of MET, VDG & RMG peak with lowering in mobile phase A, the peak tends to elute late. With increase in mobile phase A the peak tends to elute early. This is due to the polarity difference between Acetonitrile and drug causing the change in retention time.

### 3.7. % Assay:

Based on the validated method, assay was carried out on marketed formulation. The assay results are mentioned in table no. 19

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Metformin</th>
<th>Vildagliptin</th>
<th>Remogliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>Area %</td>
<td>RT</td>
</tr>
<tr>
<td>Blank</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metformin ID</td>
<td>2.1(^5)</td>
<td>2385985(^7)</td>
<td>-</td>
</tr>
<tr>
<td>Vildagliptin ID</td>
<td>-</td>
<td>-</td>
<td>254621(^8)</td>
</tr>
<tr>
<td>Remogliflozin ID</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MIX WS</td>
<td>2.1(^5)</td>
<td>2334479(^5)</td>
<td>3.6(^8)</td>
</tr>
<tr>
<td>Drug Product</td>
<td>2.1(^5)</td>
<td>2317844(^5)</td>
<td>99.29(^5)</td>
</tr>
</tbody>
</table>

The assay was found to be between 99 to 101% for market formulations.

### 4. Conclusion

In this research article, a precise and accurate method was developed based on method developed technique for estimation of MET, VDG & RMG in bulk drugs and formulation by RP-HPLC technique. The developed method was validated for accuracy, precision and robustness. As there was no published method for analysis of these drug in a single method by RP-HPLC, therefore this method can be employed for the simultaneous analysis of Metformin, Vildagliptin and Remogliflozin.

### 5. References

22. 5.12. Praveen A. et al (2013)51, had described RP-HPLC method for two drugs have been developed and validated for simultaneous determination of Alogliptin and Metformin Hydrochloride in Tablet dosage form.