A NEW STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND ALOGLIPTIN IN BULK AS WELL AS IN PHARMACEUTICAL FORMULATION BY USING PDA DETECTOR

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ARTICLE INFO

ABSTRACT

APPROACH: Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Alogliptin is a selective, orally-bioavailable inhibitor of enzymatic activity of dipeptidyl peptidase-4. A new reversed-phase High Pressure Liquid Chromatographic (RP-HPLC) method was developed for the determination of Metformin & Alogliptin (ALG) based on isocratic elution using a mobile phase consisting of potassium dihydrogen phosphate buffer [pH 4.0] and Acetonitrile [HPLC Grade] (70:30, v/v) at a flow rate of 1 mL min⁻¹ with UV detection at 235nm.

SUMMARY: The chromatographic separation was achieved on a X Terra column (250 mm × 4.6 mm, 5 µm). The run time was maintained for 8mins. The Inter day and intraday precision was found to be within the limits. The Accuracy values were within specified limits (98-102%) The calibration curve for Metformin was linear from (300-700 µg mL) and for Alogliptin from (7.5-17.5 µg /ml). The Limit of Detection for Metformin and Alogliptin was found to be 0.175 and 0.050 µg/ml respectively. The Limit of Quantification for Metformin and Alogliptin was found to be 0.57 and 0.20 µg/ml respectively.

CONCLUSION: The proposed method was adequate sensitive, reproducible, and specific for the determination of Metformin and Alogliptin bulk as well as in its tablet dosage forms. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. The drug was exposed to Thermal, Hydrolytic and oxidative stress conditions and the stressed samples were analyzed by the proposed method. The peak homogeneity data for the drugs Metformin & Alogliptin were obtained by using Photodiode Array detector in the stressed sample chromatograms which demonstrated the specificity of the method for the estimation in the presence of degradants. The present work was undertaken with the aim to develop and validate a rapid and consistent stability indicating RP-HPLC in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, Accurate and precise method for the Quantification of drug in the dosage form, bulk drug as well as for routine analysis in Quality control. Overall the proposed method was found to be suitable and Accurate for the Quantitative determination and stability study of the drug in Pharmaceutical dosage form. The method was effectively separated the drug from its degradation product and it was employed as a stability-indicating one.

Keywords
Metformin, Alogliptin, ICH Guideline, Validation, LOD, LOQ, Accuracy, Stability Indicating Method, Precision.

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INTRODUCTION
As the number of individuals affected by diabetes is continuing to increase worldwide, the need for effective management assumes ever greater urgency. Newer classes of medications, particularly those which work via the incretin pathway, achieve glucose lowering and minimizing risks associated with more traditional therapies. Ideally, combination therapies should be well tolerated, convenient to take, have few contraindications, have a low risk of hypoglycemia and weight gain, and be reasonably effective over both the short and long term such as the combination of Metformin (MF) and the dipeptidyl peptidase-4 (DPP-4) inhibitor Alogliptin (ALG). The chemical structure of the drugs was represented in Fig. no.1 & 2 respectively.

Fig. No. 1 Chemical Structure of Metformin

Fig. No. 2 Chemical Structure of Alogliptin
Alogliptin is a selective, orally-bioavailable inhibitor of enzymatic activity of dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose dependent insulin release and reduce glucagons levels. This is done through inhibition of the inactivation of in cretins, particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). Alogliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretin glucose-dependent insulin tropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1), thereby improving glycemic control [1-3]. Several analytical methods based on UV [4-6], Spectrofluorimetry [6], RP-HPLC [7-8], LC-MS/MS [9-11] was reported for the determination of Sitagliptin phosphate monohydrate in plasma and urine of humans, rats and dogs. Metformin hydrochloride (MTF) (C₄H₁₁N₅.HCl) is 1:1 dimethylbiguanidine monohydrchloride is an anti-diabetic drug from the biguanide class of oral Hypoglycaemic agents, given orally in the treatment of non–insulin-dependent diabetes mellitus[12].Major action of Metformin HCl in increasing glucose transport across the cell membrane in skeletal muscle[13-14]. Several analytical methods based on UV [15-18], Spectrofluorimetry [15], Reverse Phase-HPLC [19-27], HPTLC [28] and LC-MS/MS [29] was reported for the determination of Metformin. Although literature survey reveals that various methods were reported for Metformin (MTF) and Alogliptin (ALG) both for single estimation and in combination with others drugs, but no method was reported for the analysis of these drugs in combination.
OBJECTIVE OF THE WORK: This paper describes a new RP-HPLC method for the simultaneous estimation of MF & ALG in combined dosage form using simple mobile phase. However no one has reported for the Simultaneous estimation of all these drugs together till dated.

JUSTIFICATION OF THE WORK: In this communication we reported a Stability Indicating RP-HPLC method for the development as well as the validation for the simultaneous estimation of MF & ALG in combined dosage form using simple mobile phase.

MATERIALS & METHOD [30-31]
Chemicals and Reagents Used:
The following chemicals were procured for the process Water [HPLC Grade], Acetonitrile [HPLC Grade], Metformin and Alogliptin [Working standards] & ortho phosphoric acid all the chemicals were procured from STANDARD SOLUTIONS, HCL [LR Grade] procured from FINAR CHEMICAL LIMITED, NaOH [L R Grade] procured from S D FINE-CHEM LIMITED & H2O2 procured from ALPHA PHARMA LIMITED and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions:
Equipment : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column : XTerra column (250 mm × 4.6 mm, 5 μm)
Flow rate : 1.0 mL per min
Wavelength : 235 nm
Injection volume : 20 μl
Column oven : Ambient
Run time : 8min
Detector : Photo diode array [For Force Degradation Studies]
Soft ware : Empower 2
Model No : 2996
MFD by : WATERS

Preparation of Phosphate buffer:
The buffer solution was prepared by dissolving accurately weighed 2.5 grams of potassium dihydrogen ortho phosphate into a 1000ml volumetric flask, dissolved and diluted with 1000ml water [HPLC Grade]. The final pH of the buffer was adjusted to 4.0 by using Orthophosphoric acid.

Preparation of mobile phase
The Mobile phase was prepared by mixing the above buffer 700 mL (70%) and 300 mL of acetonitrile (30%) [HPLC Grade] and degas in ultrasonic water bath for 5 minutes. The resultant solution was filtered through 0.45 μ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as Diluent.

Preparation of the Metformin & Alogliptin Standard & Sample Solution:
Preparation of Stock solution:
The Stock solution was prepared by weighing accurately 10mg of Metformin and Alogliptin [working standard] and transferred into 10ml and 100ml clean dry volumetric flask respectively. About 7ml and 70 ml of the diluent was added to the individual flask and sonicated to dissolve it completely and the final volume was made up to the mark with the same solvent. From the above prepared Stock Solution pipette out 5.0 ml and 1.25 ml of Metformin & Alogliptin respectively into a 10ml volumetric flask and the volume was made up to the mark with the diluent.

Sample Solution Preparation:
The Sample solution was prepared by weighing accurately the weight equivalent to 10 mg of Metformin & Alogliptin [Sample] and transferred into a 10ml and 100ml clean dry volumetric flask. About 7ml & 70ml of Diluent was added to the individual flask and sonicated to dissolve it completely and the final volume was made up to the mark with the same solvent. From the above prepared Stock Solution pipette out 5.0 ml and 1.25 ml of Metformin & Alogliptin respectively into a 10ml volumetric flask and the volume was made up to the mark with the diluent. 20μL of the standard sample was injected into the chromatographic system and measured the areas for the Metformin & Alogliptin peaks and calculate the % Assay by using suitable formulae.

System Suitability:
The Tailing factor for the peaks due to Metformin & Alogliptin in Standard solution should not be more than 1.5
The Theoretical plates for the Metformin & Alogliptin peaks in Standard solution should not be less than 2000.

Calculation for Metformin:
Assay % = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg Wt.}{Label Claim} \times 100
Where
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT = Weight of test taken in mg.
DS = Dilution of standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

System Suitability Results for Metformin:
1) The Tailing factor obtained from the standard injection was 1.91
2) The Theoretical Plates obtained from the standard injection was 2814

Assay Result for Metformin:
\[ \frac{5690870}{5689593} \times \frac{10}{10} \times \frac{5.0}{17.93} \times \frac{10}{5} \times \frac{99.9}{100} \times \frac{896.5}{500} \times 100 = 99.92\% \]

Calculation for Alogliptin:
Assay % = \[ \frac{4T}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{\text{Label Claim}} \times 100 \]
Where:
AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT = Weight of test taken in mg.
DS = Dilution of standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

System Suitability Results for Alogliptin:
1) The Tailing factor obtained from the standard injection was 1.41
2) The Theoretical Plates obtained from the standard injection was 2685

Assay Results for Alogliptin:
\[ \frac{341359}{339647} \times \frac{10}{10} \times \frac{1.25}{17.93} \times \frac{10}{5} \times \frac{896.5}{12.5} \times \frac{99.9}{100} \times 100 = 100.40\% \]

VALIDATION DEVELOPMENT [32-36]
1. PRECISION: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. (Table no.1)

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Injection</th>
<th>Metformin Area</th>
<th>Alogliptin Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Injection-1</td>
<td>5530014</td>
<td>343164</td>
</tr>
<tr>
<td>2</td>
<td>Injection-2</td>
<td>5594763</td>
<td>344316</td>
</tr>
<tr>
<td>3</td>
<td>Injection-3</td>
<td>5539942</td>
<td>340852</td>
</tr>
<tr>
<td>4</td>
<td>Injection-4</td>
<td>5529536</td>
<td>343508</td>
</tr>
<tr>
<td>5</td>
<td>Injection-5</td>
<td>5530129</td>
<td>348148</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>5544877</td>
<td>343997.6</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>28226</td>
<td>2653.14</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.51</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Acceptance Criteria: The % RSD for the area of all the five standard injections should not be more than 2%.

2. INTERMEDIATE PRECISION/RUGGEDNESS: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. (Table no 2)

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Injection</th>
<th>Area</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Injection-1</td>
<td>5631161</td>
<td>332181</td>
</tr>
<tr>
<td>2</td>
<td>Injection-2</td>
<td>5630764</td>
<td>338764</td>
</tr>
<tr>
<td>3</td>
<td>Injection-3</td>
<td>5610497</td>
<td>337903</td>
</tr>
<tr>
<td>4</td>
<td>Injection-4</td>
<td>5630533</td>
<td>331134</td>
</tr>
<tr>
<td>5</td>
<td>Injection-5</td>
<td>5681177</td>
<td>335835</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>5636826</td>
<td>335163</td>
<td></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>26309</td>
<td>3393</td>
<td></td>
</tr>
<tr>
<td><strong>%RSD</strong></td>
<td>0.47</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

Acceptance Criteria: The % RSD for all the five standard injections results should not be more than 2%

3. ACCURACY: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found.

For preparation of 50% solution (With respect to target Assay concentration):
The solution was prepared by weighing accurately 5mg of Metformin and 5mg of Alogliptin [working standard] and transferred into a 10 ml and 100mL clean dry volumetric flask respectively. About 7ml and 70ml diluent were added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. From the above prepared solution pipette out 5.0 ml & 1.25ml of Metformin & Alogliptin respectively into a10ml volumetric flask and diluted up to the mark with the diluent.

For preparation of 100% solution (With respect to target Assay concentration):
The solution was prepared by weighing accurately 10mg of Metformin and 10mg of Alogliptin [working standard] and transferred into a 10 ml and 100mL clean dry volumetric flask respectively. About 7ml and 70ml diluent were added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. From the above prepared solution pipette out 5.0 ml & 1.25ml of Metformin & Alogliptin respectively into a10ml volumetric flask and diluted up to the mark with the diluent.

For preparation of 150% solution (With respect to target Assay concentration):
The solution was prepared by weighing accurately 15mg of Metformin and 15mg of Alogliptin [working standard] and transferred into a 10 ml and 100mL clean dry volumetric flask respectively. About 7ml and 70ml diluent were added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. From the above prepared solution pipette out 5.0 ml & 1.25ml of Metformin & Alogliptin respectively into a10ml volumetric flask and diluted up to the mark with the diluent. The standard solution with Accuracy -50%, Accuracy -100% and Accuracy -150% were injected into chromatographic system and calculated the amount found and amount added for Metformin & Alogliptin and further calculated the individual recovery and mean recovery values. (Table no. 3 & 4)
Table no.3: The Accuracy result was summarized for the drug Metformin.

<table>
<thead>
<tr>
<th>% Concentration (at Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>2864550</td>
<td>5.0</td>
<td>5.08</td>
<td>101.56 %</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>5656807</td>
<td>10.0</td>
<td>10.03</td>
<td>100.27 %</td>
<td>99.95%</td>
</tr>
<tr>
<td>150%</td>
<td>8295366</td>
<td>15.0</td>
<td>14.7</td>
<td>98.03%</td>
<td></td>
</tr>
</tbody>
</table>

Table no.4: The Accuracy result was summarized for the drug Alogliptin

<table>
<thead>
<tr>
<th>% Concentration (at Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>165004</td>
<td>5.0</td>
<td>4.92</td>
<td>98.40%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>334196</td>
<td>10.0</td>
<td>9.96</td>
<td>99.65%</td>
<td>99.36%</td>
</tr>
<tr>
<td>150%</td>
<td>503246</td>
<td>15.0</td>
<td>15.01</td>
<td>100.03%</td>
<td></td>
</tr>
</tbody>
</table>

Acceptance Criteria: The % Recovery for each level should be in between 98.0 to 102.0

4. LINEARITY: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. It is determined by series of three to six injections of five or more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. (Table no. 5 & 6)

Table no.5: Linearity results for Metformin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>300 µg/ml</td>
<td>3088551</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>400 µg/ml</td>
<td>4457852</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>500 µg/ml</td>
<td>5647585</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>600 µg/ml</td>
<td>6847525</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>700 µg/ml</td>
<td>7854689</td>
</tr>
</tbody>
</table>

Correlation Coefficient: 0.999
Table no.6: Linearity results for Alogliptin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>7.5 µg/ml</td>
<td>194146</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>10 µg/ml</td>
<td>272195</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>12.5 µg/ml</td>
<td>344643</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>15 µg/ml</td>
<td>408293</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>17.5 µg/ml</td>
<td>476342</td>
</tr>
</tbody>
</table>

Correlation Coefficient: 0.999

Acceptance Criteria: The Correlation coefficient should not be less than 0.999.

5. LIMIT OF DETECTION: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

   a. Limit of Detection for Metformin:

   Calculation of S/N Ratio:
   
   Average Baseline Noise obtained from Blank: 41 µV
   Signal Obtained from LOD solution: 124 µV
   
   S/N = 124/41 = 3.02

   b. Limit of Detection for Alogliptin:

   Calculation of S/N Ratio:
   
   Average Baseline Noise obtained from Blank: 41 µV
   Signal Obtained from LOD solution: 121 µV
   
   S/N = 121/41 = 2.95

   Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

6. LIMIT OF QUANTIFICATION: It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio.

   a. Limit of Quantification for Metformin:

   Calculation of S/N Ratio:
   
   Average Baseline Noise obtained from Blank: 41 µV
   Signal Obtained from LOQ solution: 409 µV
   
   S/N = 409/41 = 9.98

   b. Limit of Quantification for Alogliptin:

   Calculation of S/N Ratio:
   
   Average Baseline Noise obtained from Blank: 41 µV
   Signal Obtained from LOQ solution: 411 µV
   
   S/N = 411/41 = 10.02

   Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

7. ROBUSTNESS: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

   a) The flow rate was varied at 0.9 ml/min to 1.1ml/min.: The Standard solution of Metformin & Alogliptin was prepared and analysed using the varied flow rates along with method developed flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it was indicated that the method was robust even by change in the flow rate. (Table no 7&8)

Table no.7: The Robustness result was summarized for Metformin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>2830</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>2814</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>2804</td>
</tr>
</tbody>
</table>
Table no.8: The Robustness result was summarized for Alogliptin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>2747</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>2685</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>2703</td>
</tr>
</tbody>
</table>

b) The Organic composition in the Mobile phase was varied from 20% to 40%: The Standard solution for the drug Metformin & Alogliptin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition. On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase does not affect the method significantly. Hence it was indicated that the method was robust even by change in the Mobile phase ±10.

Table no.9: The Robustness result was summarized for Metformin after changing the organic composition

<table>
<thead>
<tr>
<th>S.No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>2807</td>
</tr>
<tr>
<td>2</td>
<td>Actual</td>
<td>2814</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>2831</td>
</tr>
</tbody>
</table>

Table no. 10: The Robustness result was summarized for Alogliptin after changing the organic composition

<table>
<thead>
<tr>
<th>S.No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>2769</td>
</tr>
<tr>
<td>2</td>
<td>Actual</td>
<td>2685</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>2705</td>
</tr>
</tbody>
</table>

8. DEGRADATION STUDIES [37-41]: The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Metformin and Alogliptin using the proposed method.

a. Hydrolytic degradation under acidic condition: From the prepared Stock Solution pipette out 5.0 ml of Metformin & 1.25 ml of Alogliptin into a 10ml clean and dry volumetric flask, then 3 ml of 0.1N HCl was added. The volumetric flask was kept at normal condition for 90 minutes and further it was neutralized with 0.1 N NaOH and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

b. Hydrolytic degradation under alkaline condition: From the prepared Stock Solution pipette out 5.0 ml of Metformin & 1.25 ml of Alogliptin into a 10ml clean and dry volumetric flask, then 3 ml of 0.1N NaOH was added. The volumetric flask was kept at normal condition for 90 minutes and further it was neutralized with 0.1 N HCl and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

c. Thermal induced degradation: From the prepared Stock Solution pipette out 5.0 ml of Metformin & 1.25 ml of Alogliptin into a 10ml clean and dry volumetric flask, then 3 ml of diluent was added. Then, the volumetric flask was kept at reflux condition for 60 minutes and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

d. Oxidative degradation: From the prepared Stock Solution pipette out 5.0 ml of Metformin & 1.25 ml of Alogliptin into a 10ml clean and dry volumetric flask, then 1 ml of 3 % w/v of hydrogen peroxide was added. Then the volumetric flask was kept at room temperature for 15 min and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.
RESULT & DISCUSSION
The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of the drug in pharmaceutical dosage forms. In order to develop a method under isocratic conditions, mixtures of Phosphate Buffer (pH was adjusted to 4 by using Orthophosphoric acid) and Acetonitrile [HPLC grade] in different combinations were tested as mobile phase on a Symmetry C₈ (4.6 x 250mm, 5 µm) column. A binary mixture of Phosphate Buffer (pH 4) and Acetonitrile [HPLC grade] in 70:30v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for the drug Metformin was around 2.73min and for the drug Alogliptin was 4.45. (Fig. no. 3)
The Precision data was represented in Table no. 1 and the chromatograph was represented in Fig. no. 6.
When Metformin & Alogliptin was analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed. It was represented in Table no. 2 and the chromatogram was represented in Fig. no. 7 which shows that the developed RP-HPLC method was highly precise.

The Accuracy data were summarized in Table no. 3 & 4 and the chromatograms for Accuracy 50%, 100% & 150% were represented in Fig. no. 8, 9 & 10.
In order to test the linearity of the method, five dilutions of the working standard solutions of Metformin & Alogliptin in the range of 300 to 700 µg per mL and 7.5 to 17.5 µg per mL respectively were prepared. The data were represented in Table no. 5 and 6. Each of the dilution was injected into the column and the Linearity Curve was represented in Fig. no.11 & 12.
Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope(s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for Metformin and Alogliptin was found to be 0.175 & 0.050 µg/ml respectively. The LOQ for Metformin and Alogliptin was found to be 0.57 & 0.20µg/mL respectively. The drug content formulations were quantified by using the proposed analytical method. The chromatograms were represented in Fig. no. 13 & 14.
Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate buffer and Acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table7, 8, 9 & 10 and chromatograms were represented in Fig. no. 15, 16, 17 &18.
Fig. no. 15 Typical chromatogram of Metformin & Alogliptin for Robustness (Less flow rate)

Fig. no. 16 Typical chromatogram of Metformin & Alogliptin for Robustness (More flow rate)
The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms. It was concluded that the proposed RP-HPLC method was sufficiently sensitive and reproducible for the analysis of Metformin and Alogliptin. In order to evaluate the stability of Metformin, Alogliptin and ability of the method to separate Metformin and Alogliptin from its degradation products, the drug was subjected to various stress conditions such as Hydrolytic degradation under acidic condition (using 0.1N HCl & 0.1 N NaOH), Hydrolytic degradation under alkaline condition (using0.1N NaOH & 0.1N HCL), Thermal induced degradation (Reflex Condition for 60 mins), Oxidative degradation (by using 3 % w/v of hydrogen peroxide). The following chromatograph represents the degradation studies for the drug Metformin and Alogliptin which were represented in table no. 11 & 12 and Fig no 19, 20 21, 22 & 23.
Fig. no. 19 The chromatograph represents the Thermal induced degradation

Fig. no. 20 The chromatograph represents the Hydrolytic degradation under acidic condition
Fig. no. 21 The chromatograph represents the Hydrolytic degradation under alkaline condition.

Fig. no. 22 The chromatograph represents the Oxidative degradation.

Fig. no. 23 The chromatogram represents for standard Drugs.
CONCLUSION
It was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Metformin and Alogliptin in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Metformin and Alogliptin depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Metformin and Alogliptin. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Metformin and Alogliptin from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of drug in bulk and pharmaceutical dosage forms.

FUTURE ASPECT: The proposed method can be use in future for the clinical, biological and pharmacokinetic studies of Metformin and Alogliptin.

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