A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM ITS VALIDATION AS PER ICH GUIDELINES

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Abstract:
A new RP-HPLC method for the quantitative determination of Ertugliflozin and Sitagliptin was developed and validated as per ICH guidelines. The drugs were injected into Std Azilent column (150×4.6, 5 μm), maintained at ambient temperature and effluent monitored at 240 nm. The mobile phase consisted of Buffer (Potassium di hydrogen Ortho Phosphate): Acetonitrile (70:30 V/V). The flow rate was maintained at 1.0 ml/min. The calibration curve for Ertugliflozin and Sitagliptin were linear from 3.75-22.5 μg/ml and 25-150 μg/ml respectively (r2 for Ertugliflozin = 0.9992, r2 for Sitagliptin = 0.9995). Retention time was 3.203 min (Ertugliflozin), 2.106 min (Sitagliptin ). Accuracy was in the range of 99.67-99.90% for both drugs. Precision was 0.1% and 0.2% for Ertugliflozin and Sitagliptin, LOD and LOQ are 0.43 μg/ml and 1.31μg/ml for Ertugliflozin and , 0.74 μg/ml and 2.24 μg/ml for Sitagliptin. The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Ertugliflozin and Sitagliptin in bulk and pharmaceutical dosage forms. When applied for tablet assay, drug content was within 99.18-99.13 % of labeled content. Forced degradation studies indicated the suitability of the method for stability studies.

Keywords: Ertugliflozin and Sitagliptin, RP-HPLC Method, Simultaneous estimation, Validation as per ICH guidelines, Forced degradation studies.

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INTRODUCTION:
Type 2 diabetes mellitus (T2DM) is a global pandemic, as evident from the global cartographic picture of diabetes by the International Diabetes Federation [1]. Diabetes mellitus is a chronic, progressive, incompletely understood metabolic condition chiefly characterized by hyperglycemia. Impaired insulin secretion, resistance to tissue actions of insulin, or a combination of both are thought to be the commonest reasons contributing to the pathophysiology of T2DM, a spectrum of disease originally arising from tissue insulin resistance and gradually progressing to a state characterized by complete loss of secretory activity of the beta cells of the pancreas. T2DM is a major contributor to the very large rise in the rate of non-communicable diseases [2-3].

Ertugliflozin is chemically known as ertugliflozin L-pyroglutamic acid is (1S,2S,3S,4R,5S)-5-(4-chloro-3-(4-ethoxybenzyl)phenyl)-1-(hydroxymethyl)-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol, compound with (2S)-5-oxopyrrolidine-2-carboxylic acid. The molecular formula is C_{27}H_{35}ClNO_{10} and the molecular weight is 566.00. Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (SGLT), more specifically the type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus[4]. Administration of ertugliflozin increases urinary glucose excretion which leads to a negative balance and osmotic diuresis. Thus, this antidiabetic agent has been reported to significantly reduce the body weight and blood pressure of diabetic patients [5].

Sitagliptin is chemically 3-amino-1-[3-(trifluoromethyl)6,8-dihydro-5H[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one; phosphoric acid hydrate. It has a molecular formula of C_{16}H_{15}F_{5}N_{3}O.H_{3}PO_{4}.H_{2}O with a molecular weight 523.324. Sitagliptin is potent and highly selective inhibitor of dipeptidyl peptidase-4 (DPP-4). Sitagliptin is the first of a new class of drugs for the treatment of type II diabetes, a well-known hypoglycemic drug in the present therapy. It reduces blood glucose concentration by enhancing the effect of incretins and there by leading to a significant increase in insulin secretion [6-7]. Indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus when treatment with both ertugliflozin and sitagliptin is appropriate [8-9].

Though several methods are reported in literature for the estimation of Sitagliptin with other drugs combination [10-17] and individually, no methods are reported for estimation of ertugliflozin and sitagliptin in combination. The objective of the present study is to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Ertugliflozin and Sitagliptin and validate the method with forced degradation studies according to ICH guidelines.

MATERIALS AND METHODS:
Chemicals and solvents:
The reference sample of Ertugliflozin and Sitagliptin was obtained as a gift sample from Merck & Co., and Steglujan tablet containing Ertugliflozin 15mg and Sitagliptin 10mg (Merck & Co.,) was procured from US market. Water (HPLC grade) purchased from Rankem and acetonitrile (HPLC grade), ortho phosphoric acid (AR grade), sodium hydroxide (pure), hydrogen peroxide (pure) was purchased from Merck Limited. 0.45µm Nylon filter was from Zodiac life sciences.

Instrumentation:
WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer, PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Ertugliflozin and Sitagliptin solutions, Electronics Balance-Denver p\textsuperscript{11} meter -BVK enterprises, India Ultrasonicator-BVK enterprises.
Chromatography conditions:
The chromatographic separation was performed on Standard Azilent (4.6 x 150mm, 5μm particle size) at an ambient column temperature. The samples were eluted using Buffer (Potassium dihydrogen Ortho Phosphate): Acetonitrile (70:30v/v) as the mobile phase at a flow rate of 1ml/min the mobile phase and samples were degassed by ultrasonication for 30 min and filtered through 0.45μm Nylon (N66) 47mm membrane filter. The measurements were carried out with an injection volume of 10μL, flow rate was set to 1 mL/min, and PDA detection was carried out at 240 nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Ertugliflozin and Sitagliptin were recorded under optimized chromatographic conditions.

Preparation of Buffer and Mobile Phase:
0.01N KH2PO4 Buffer: Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Orthophosphoric acid solution

0.1% OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water

Preparation of mobile phase:
700 ml (70%) of phosphate buffer and 300 ml of Acetonitrile (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Diluent: Based up on the solubility of the drugs, diluents was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard Solutions:
Stock solution of Ertugliflozin:
Standard stock solution of Ertugliflozin was prepared by dissolving 3.5 mg of Ertugliflozin in 25 ml of diluent (Acetonitrile and Water, 50:50v/v) in a 25 ml clean dry volumetric flask separately and the standard solutions was filtered through 0.45μm nylon membrane filter and degassed by sonicator to get the concentration of 150μg/ml of Ertugliflozin. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Ertugliflozin.

Stock solution of Sitagliptin:
Standard stock solution of Sitagliptin was prepared by dissolving 25 mg Sitagliptin in 25 ml of diluent (Acetonitrile and Water, 50:50v/v) in a 25 ml clean dry volumetric flask separately and the standard solutions was filtered through 0.45μm nylon membrane filter and degassed by sonicator to get the concentration of 1000 μg/ml of Sitagliptin respectively. The above standard stock solutions suitably diluted with diluents to obtain various concentrations of Ertugliflozin and Sitagliptin

Working Standard Solution of Ertugliflozin:
Working standard solution of Ertugliflozin was prepared by taking 1 ml of stock solutions of Ertugliflozin in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 15μg/ml of Ertugliflozin.

Working Standard Solution of Sitagliptin:
Working standard solution of Sitagliptin was prepared by taking 1 ml of stock solutions of Sitagliptin in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 100μg/ml of Sitagliptin.

Preparation of Sample Solutions of Ertugliflozin and Sitagliptin:
Five tablets were accurately weighed and powdered and tablet powder equivalent to 15mg of Ertugliflozin and 100mg of Sitagliptin was taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution was filtered and suitably diluted to get a concentration of 150μg/ml of Ertugliflozin and 1000μg/ml of Sitagliptin.

RESULTS AND DISCUSSION:
Optimization of chromatographic conditions:
During the optimization cycle, different columns with different lengths and internal diameters were tried namely, Discovery C18 column, Kromocil column, and Azilent column but finally satisfactory separation was obtained on Azilent (4.6 x 150mm, 5μm) column. Water, Methanol, Acetonitrile and different types of buffers were examined simultaneously as organic modifiers and Buffer (Potassium di hydrogen Ortho Phosphate): Acetonitrile (70:30 V/V) was found to be more suitable for better separation of Ertugliflozin and Sitagliptin under investigation. Isocratic mode of elution with different ratios of organic to aqueous phases was tried in order to achieve proper separation of the cited analytes in a reasonable run time. It was found that pH higher than 4.59 was not suitable as due to improper separation of the analyzed compounds. pH was adjusted at 3 for the best separation of Ertugliflozin in a reasonable run time (<10 min) and with good resolution between all peaks. Flow rate of 1 ml/ min was optimum. Quantization was achieved with UV-detection at 240nm. The column temperature...
was set at 30°C. Optimized method was providing
good resolution and peak shape for Ertugliflozin and
Sitagliptin. Under above described experimental
conditions, all the peaks were well defined and free
from tailing. The concern of small deliberate changes
in the mobile phase composition and flow rates on
results were evaluated as a part of testing for methods
robustness.

**Validation of Method Developed:**
The proposed method was validated according to the
ICH guidelines for system suitability, specificity,
recovery, precision, linearity, robustness, limit of
detection (LOD) and limit of quantification (LOQ).
Under the validation study, the following parameters
were studied.

**System suitability test:**
The system suitability parameters were determined
by preparing standard solutions of Ertugliflozin
(15ppm) and Sitagliptin (100ppm) and the solutions
were injected six times and the parameters like peak
tailing, resolution and USP plate count were
calculated.

**Specificity:**
The specificity of the method was carried out to check
whether there is any interference of any impurities
with the retention time of analyte peaks. The
specificity was performed by the injecting blank,
Placebo and standard solutions of drugs.

**Precision:**
Precision is expressed as the closeness of agreement
between a series of measurements obtaining from
multiple sampling of the same homogeneous sample.
Six replicate injections of a known concentration of
Ertugliflozin (15µg/mL) and Sitagliptin (100µg/mL),
have been analyzed by injecting them into a HPLC
column on the same day. The intermediate precision
was estimated by injecting samples prepared at the
same concentrations on three different days by
different operators. The peak area ratios of all
injections were taken and standard deviation, %
relative standard deviation (RSD) was calculated.

**Accuracy:**
Accuracy is tested by the standard addition
method at different levels: 50, 100 and 150%. A
known amount of the standard drug was added to
the blank sample at each level. Each sample was
injected thrice The mean recovery of
Ertugliflozin and Sitagliptin were calculated and
accepted with 100±2%.

**Linearity:**
The linearity of the method was established by
determining the absorbance of different
concentrations of Ertugliflozin and Sitagliptin over a
range of 3.75-22.5µg/ml and 25-0.150µg/ml
respectively. Six replicates of each concentration
were independently prepared and injected in to HPLC
system. The linearity was determined by calculating
a regression line from plot of peak area ratio of drug and
is versus concentration of the drug. Regression
analysis was computed for Ertugliflozin and
Sitagliptin. The method was evaluated by
determination of correlation coefficient and intercept
values according to ICH guidelines.

**Limit of Detection and Limit of Quantification:**
Limit of detection (LOD) and limit of quantification
(LOQ) of Saxagliptine and Dapagliflozin were
determined by calibration curve method. Solutions of
Saxagliptine and Dapagliflozin were prepared in
linearity range and injected in triplicate. Average
peak area of three analyses was plotted against
concentration. LOD and LOQ were calculated by
using the following equations:

\[
LOD = 3Xn/B \\
LOQ = 10X N/B
\]

Where N is residual variance due
to regression; B is the slope.

**Robustness:**
HPLC conditions were slightly modified to evaluate
the analytical method robustness. These changes
included the flow rate, column temperature and the
Acetonitrile proportion in the mobile phase.

**Degradation Study:**
Alkaline, acidic, oxidative stress, thermal, water and
direct exposure to UV were carried out. No internal
standard was added in the forced degradation study.

**Acid Degradation Studies:**
To one ml of stock s solution Ertugliflozin and
Sitagliptine, one ml of 2N Hydrochloric acid
was added and refluxed for 30mins at 600C. The
resultant solution was diluted to obtain 15µg/ml
& 100µg/ml solution and 10 µl solutions were
injected into the system and the chromatograms
were recorded to assess the stability of sample.

**Alkali Degradation Studies:**
To one ml of stock solution Ertugliflozin and
Sitagliptine, one ml of 2N sodium hydroxide was
added and refluxed for 30mins at 60°C. The
resultant solution was diluted to obtain
15µg/ml&100µg/ml solution and 10 µl were injected.
into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative Studies:
To one ml of stock solution of Ertugliflozin and Sitagliptine, one ml of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 15µg/ml & 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation:
To one ml of stock solution of Ertugliflozin and Sitagliptine, 1 ml of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 15µg/ml & 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:
The standard drug solution was placed in oven at 105°C for 1 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 15µg/ml&100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 150µg/ml and 1000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m² in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 15µg/ml and 100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 15µg/ml and 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Validation of Method Developed:
The proposed method was validated according to the ICH guidelines31 for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System Suitability:
The Retention time of Ertugliflozin and Sitagliptine using optimum conditions was 3.203 min and 2.106 min respectively. For two of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Ertugliflozin and Sitagliptine was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in Table 1.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Ertugliflozin</th>
<th>Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT(min)</td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>3.188</td>
<td>6022</td>
</tr>
<tr>
<td>2</td>
<td>3.188</td>
<td>6051</td>
</tr>
<tr>
<td>3</td>
<td>3.197</td>
<td>5944</td>
</tr>
<tr>
<td>4</td>
<td>3.201</td>
<td>5867</td>
</tr>
<tr>
<td>5</td>
<td>3.204</td>
<td>5974</td>
</tr>
<tr>
<td>6</td>
<td>3.210</td>
<td>6032</td>
</tr>
<tr>
<td>MEAN</td>
<td>3.199</td>
<td>MEAN</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.2</td>
<td>%RSD</td>
</tr>
</tbody>
</table>

Table 1: System suitability parameters for Ertugliflozin and Sitagliptin
Specificity:
The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Ertugliflozin and Sitagliptine is shown in Fig.4 clearly shows the ability of the method to assess the analyte in the presence of other excipients.

![Blank Chromatogram](image1)

![Optimized Chromatogram](image2)

Precision:
**System Precision:**
One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2).

**Method Precision (Repeatability):**
Six replicate injections of a known concentration of sample preparation of Ertugliflozin (15μg/mL) and Sitagliptin (100μg/mL) have been analyzed by injecting them into a HPLC column on the same day. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in Table 2.
Table 2: System precision data of Ertugliflozin and Sitagliptin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Injection number</th>
<th>Retention time of Ertugliflozin</th>
<th>Area of Ertugliflozin</th>
<th>Retention time of Sitagliptin</th>
<th>Area of Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Injection 1</td>
<td>3.167</td>
<td>373573</td>
<td>2.084</td>
<td>4256686</td>
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<tr>
<td>2</td>
<td>Injection 2</td>
<td>3.203</td>
<td>374129</td>
<td>2.107</td>
<td>425437</td>
</tr>
<tr>
<td>3</td>
<td>Injection 3</td>
<td>3.206</td>
<td>373730</td>
<td>2.108</td>
<td>4259188</td>
</tr>
<tr>
<td>4</td>
<td>Injection 4</td>
<td>3.207</td>
<td>373943</td>
<td>2.113</td>
<td>4254120</td>
</tr>
<tr>
<td>5</td>
<td>Injection 5</td>
<td>3.211</td>
<td>373933</td>
<td>2.116</td>
<td>4257632</td>
</tr>
<tr>
<td>6</td>
<td>Injection 6</td>
<td>3.215</td>
<td>375047</td>
<td>2.119</td>
<td>4276343</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>374059</td>
<td>2.119</td>
<td>4259721</td>
</tr>
</tbody>
</table>

Ruggedness:
Intermediate precision was accessed injecting sample preparation of Ertugliflozin (15μg/mL) and Sitagliptin (100µg/mL) in six replicates into HPLC column on the same day and on consecutive days in different laboratories by different analysts. Results were found within the acceptance limits (RSD<2) as shown in the Tables 3, 4.

Table 3: Ruggedness data of Ertugliflozin

<table>
<thead>
<tr>
<th>Concentration (15 μg/mL)</th>
<th>Analyst-1</th>
<th>Analyst-2</th>
<th>Laboratory -1</th>
<th>Laboratory-2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>Area</td>
<td>RT</td>
<td>Area</td>
</tr>
<tr>
<td>Injection 1</td>
<td>3.197</td>
<td>368398</td>
<td>3.188</td>
<td>377112</td>
</tr>
<tr>
<td>Injection 2</td>
<td>3.198</td>
<td>368857</td>
<td>3.188</td>
<td>377288</td>
</tr>
<tr>
<td>Injection 3</td>
<td>3.199</td>
<td>370732</td>
<td>3.197</td>
<td>374762</td>
</tr>
<tr>
<td>Injection 4</td>
<td>3.201</td>
<td>369155</td>
<td>3.201</td>
<td>375094</td>
</tr>
<tr>
<td>Injection 5</td>
<td>3.204</td>
<td>367556</td>
<td>3.204</td>
<td>378661</td>
</tr>
<tr>
<td>Injection 6</td>
<td>3.204</td>
<td>370177</td>
<td>3.210</td>
<td>377650</td>
</tr>
<tr>
<td>Mean</td>
<td>369146</td>
<td>376761</td>
<td>3.237</td>
<td>371239</td>
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<tr>
<td>Standard deviation</td>
<td>1161.9</td>
<td>1521.6</td>
<td>981.3</td>
<td>937.9</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
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Table 4: Ruggedness data of Sitagliptin

<table>
<thead>
<tr>
<th>Concentration (100 μg/mL)</th>
<th>Analyst-1</th>
<th>Analyst-2</th>
<th>Laboratory -1</th>
<th>Laboratory-2</th>
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<tr>
<td>Injection 1</td>
<td>2.100</td>
<td>4264949</td>
<td>2.100</td>
<td>370544</td>
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<tr>
<td>Injection 2</td>
<td>2.102</td>
<td>4243876</td>
<td>2.102</td>
<td>370442</td>
</tr>
<tr>
<td>Injection 3</td>
<td>2.104</td>
<td>4264228</td>
<td>2.104</td>
<td>369183</td>
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<tr>
<td>Injection 4</td>
<td>2.105</td>
<td>4278501</td>
<td>2.105</td>
<td>369269</td>
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<tr>
<td>Injection 5</td>
<td>2.106</td>
<td>4249209</td>
<td>2.112</td>
<td>371673</td>
</tr>
<tr>
<td>Injection 6</td>
<td>2.106</td>
<td>4243174</td>
<td>2.115</td>
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<tr>
<td>Mean</td>
<td>4257323</td>
<td>370151</td>
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<tr>
<td>Standard deviation</td>
<td>368398</td>
<td>937.9</td>
<td>14476.2</td>
<td>8674.6</td>
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<tr>
<td>% RSD</td>
<td>368857</td>
<td>0.3</td>
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<td>0.2</td>
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</table>
Robustness:
The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (75:25), mobile phase plus (65:35), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. The result of robustness study of the developed assay method was established in Table 5.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Condition</th>
<th>% RSD of Ertugliflozin</th>
<th>% RSD of Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flow rate (-) 1.1ml/min</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>Flow rate (+) 1.3ml/min</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>Mobile phase (-) 75B:25A</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>Mobile phase (+) 65B:35A</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>Temperature (-) 25°C</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>Temperature (+) 35°C</td>
<td>1.7</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Accuracy:
A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Ertugliflozin and Sitagliptin was achieved between 99.67% and 99.90% respectively. The results are given in Tables 6,7.

<table>
<thead>
<tr>
<th>S NO</th>
<th>Accuracy level</th>
<th>Amount Spiked (μg/mL)</th>
<th>Amount recovered (μg/mL)</th>
<th>% Recovery</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50%</td>
<td>7.5</td>
<td>7.484061</td>
<td>99.79</td>
<td>Mean = 99.87 SD = 0.19 %RSD = 0.19</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>7.5</td>
<td>7.497311</td>
<td>99.96</td>
<td>Mean = 99.29 SD = 0.5 %RSD = 0.5</td>
</tr>
<tr>
<td>3</td>
<td>100%</td>
<td>15</td>
<td>14.90247</td>
<td>99.35</td>
<td>Mean = 99.94 SD = 0.07 %RSD = 0.07</td>
</tr>
<tr>
<td>4</td>
<td>150%</td>
<td>15</td>
<td>14.88807</td>
<td>99.25</td>
<td>Mean = 99.94 SD = 0.07 %RSD = 0.07</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>22.5</td>
<td>22.49854</td>
<td>99.99</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>22.5</td>
<td>22.49191</td>
<td>99.96</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>22.5</td>
<td>22.46683</td>
<td>99.85</td>
<td></td>
</tr>
</tbody>
</table>
Table 7: Accuracy table of Sitagliptin

<table>
<thead>
<tr>
<th>S NO</th>
<th>Accuracy level</th>
<th>Amount Spiked (μg/mL)</th>
<th>Amount recovered (μg/mL)</th>
<th>% Recovery</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50%</td>
<td>50</td>
<td>49.53313</td>
<td>99.07</td>
<td>Mean = 99.13, SD = 0.13, %RSD = 0.13</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>50</td>
<td>49.64345</td>
<td>99.29</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>50</td>
<td>49.52552</td>
<td>99.05</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100%</td>
<td>100</td>
<td>99.84408</td>
<td>99.84</td>
<td>Mean = 99.78, SD = 0.0675, %RSD = 0.07</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>100</td>
<td>99.70917</td>
<td>99.71</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>100</td>
<td>99.77721</td>
<td>99.78</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>150%</td>
<td>150</td>
<td>149.555</td>
<td>99.70</td>
<td>Mean = 99.66, SD = 0.2726, %RSD = 0.27</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>150</td>
<td>149.8553</td>
<td>99.07</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>150</td>
<td>149.0463</td>
<td>99.29</td>
<td></td>
</tr>
</tbody>
</table>

Linearity:
The linearity of the method was established by determining the absorbance of different concentrations of Ertugliflozin and Sitagliptin over a range of 3.75-22.5μg/ml and 25-150μg/ml respectively. The results are given in Tables 8 and Fig. 5,6.

Table 8: Linearity table for Ertugliflozin and Sitagliptin.

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Ertugliflozin</th>
<th>Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc (μg/mL)</td>
<td>Peak area</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3.75</td>
<td>106567</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>197094</td>
</tr>
<tr>
<td>4</td>
<td>11.25</td>
<td>295390</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>376237</td>
</tr>
<tr>
<td>6</td>
<td>18.75</td>
<td>484397</td>
</tr>
<tr>
<td>7</td>
<td>22.5</td>
<td>573322</td>
</tr>
</tbody>
</table>
Limit of Detection (LOD) and Limit of Quantitation (LOQ):
The limit of detection and limit of quantification were evaluated by serial dilutions of Ertugliflozin and Sitagliptin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Ertugliflozin and Sitagliptin was found to be 0.43 μg/mL and 0.74 μg/mL respectively and the LOQ value 1.30 μg/mL and 2.24 μg/mL respectively.

Assay:
Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out (Table 9).

Table 9: Assay data of Ertugliflozin and Sitagliptin combination marketed formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labelled claim(mg)</th>
<th>Drug found(mg)</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertugliflozin</td>
<td>15</td>
<td>14.88</td>
<td>99.18%</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>100</td>
<td>99.13</td>
<td>99.13%</td>
</tr>
</tbody>
</table>
Table 10: Forced Degradation studies of Ertugliflozin and Sitagliptin

<table>
<thead>
<tr>
<th>Degradation Condition</th>
<th>Ertugliflozin (%)</th>
<th>Sitagliptin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>4.80</td>
<td>4.44</td>
</tr>
<tr>
<td>Alkali</td>
<td>3.74</td>
<td>3.98</td>
</tr>
<tr>
<td>Oxidation</td>
<td>2.10</td>
<td>2.78</td>
</tr>
<tr>
<td>Thermal</td>
<td>1.83</td>
<td>2.41</td>
</tr>
<tr>
<td>UV</td>
<td>1.54</td>
<td>1.30</td>
</tr>
<tr>
<td>Water</td>
<td>0.75</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Forced degradation studies:
The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out for acid hydrolysis (2N HCl heated for 30 min at 60°C), alkali hydrolysis (2 N NaOH heated for 30 min at 60°C), oxidative degradation (20%H₂O₂ heated at 60°C for 30 min) and thermal degradation (samples placed in an oven at 105°C for 6 h). For photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 7 days. Results are shown in Tables 10.

DISCUSSION:
In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Ertugliflozin and Sitagliptin in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Buffer (Potassium dihydrogen Ortho Phosphate): Acetonitrile (70:30 V/V) with 1.0 ml/min flow rate was quite robust.

The optimum wavelength for detection was 240 nm at which better detector response for drug was obtained. The average retention time for Ertugliflozin and Sitagliptin were found to be 3.203 and 2.106 min. The calibration was linear in concentration range of 3.75-22.5mcg/ml for Ertugliflozin and 25-150mcg/ml for Sitagliptin. The low values of % RSD indicate the method is precise and accurate. Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % RSD. reported was found to be less than 2 %.The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION:
The proposed method for the assay of the popular anti-diabetic drugs Ertugliflozin and Sitagliptin in the commercially available tablet formulation is simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

ACKNOWLEDGEMENT:
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REFERENCES:


