Development and Validation of Stability Indicating HPTLC Method for Simultaneous Estimation of Dapagliflozin and Linagliptin

Anchal Shukla
Indukaka Ipcowala College of Pharmacy, A constituent college of The CVM University

Usmangani K. Chhalotiya
usmangani.chhalotiya@cvmu.edu.in

Dimal Shah
Indukaka Ipcowala College of Pharmacy, A constituent college of The CVM University

Jinal Tandel
Indukaka Ipcowala College of Pharmacy, A constituent college of The CVM University

Heta Kachhiya
Indukaka Ipcowala College of Pharmacy, A constituent college of The CVM University

Mital Parmar
Indukaka Ipcowala College of Pharmacy, A constituent college of The CVM University

Research Article

Keywords: Dapagliflozin, Linagliptin, HPTLC, Validation, Forces Degradation Studie, Diabetes mellitus type II

Posted Date: March 28th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4147349/v1

License: ☕️ TECTED This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.
Abstract

For the treatment of Type 2 Diabetes Mellitus, Dapagliflozin selectively inhibits the sodium-glucose co-transporter-2, while Linagliptin competitively and reversibly inhibits dipeptidyl peptidase-4 in a fixed dose combination (1:1). Dapagliflozin and Linagliptin estimation in bulk and tablet formulation was accomplished by the development and validation of a precise and accurate HPTLC method. The procedure used Toluene: Chloroform: Methanol: Triethylamine (7:2:1:0.2 v/v/v) as the mobile phase and HPTLC aluminum plates pre-coated with silica gel 60 F254 as the stationary phase used. Dapagliflozin and Linagliptin were determined to have $R_f$ values of 0.23 and 0.40 correspondingly. At 224 nm, densitometric analysis was performed in the absorbance mode. For Dapagliflozin and Linagliptin 200–1200 ng/band were the linear ranges in which the procedure produced results. Forced degradation studies were performed on both Dapagliflozin and Linagliptin active pharmaceutical ingredients like acid hydrolysis, base hydrolysis, chemical oxidation, dry heat and photodegradation studies. Dapagliflozin and Linagliptin are susceptible to acid hydrolysis, base hydrolysis, chemical oxidation while both the chemical substances are stable to dry heat and photolytic studies.

Introduction

Dapagliflozin: (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(methyl-4-ethoxyphenyl)phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol is Dapagliflozin chemical name. Its chemical formula is $C_{12}H_{25}ClO_6$, and its weight is 408.9 g/mol (Fig. 1(a)). It has a pKa value of 12.6 and a log P value of 2.7. By blocking sodium glucose co-transporter 2 (SGLT2), which is found at the proximal tubule site of the kidney, it works as an inhibitor. It falls under the category of anti-diabetics since it reduces blood sugar by preventing the kidneys from reabsorbing glucose.

Linagliptin is known by the IUPAC 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione has the chemical formula $C_{25}H_{28}N_8O_2$ and a molecular weight of 472.5 g/mol (Fig. 1(b)). Its pKa value is 8.6 and its log P value is. It has an anti-glycemic effect by suppressing the release of glucagon and increasing insulin production by inhibiting dipeptidyl peptidase 4. Because the two medications work in tandem to complement one another, using them together to treat Type 2 Diabetes is advantageous.

After a thorough literature review, it was determined that there are a number of ways to estimate the dosage and bulk of Dapagliflozin, including UV Spectroscopy, the RP-HPLC method, and the use of analytical techniques like RP-HPLC, HPTLC, UPLC, etc. in combination with other medications like metformin and saxagliptin. Linagliptin can also be estimated in bulk and dosage form using a variety of techniques, including UV Spectroscopy, the RP-HPLC method, and the use of analytical techniques like HPTLC and RP-HPLC in combination with other medications like metformin and empagliflozin.

We learned from the thorough literature review that this combination does not have an HPTLV technique. As a result, the RP-HPLC method for the 1:1 fixed dose combination of Dapagliflozin and Linagliptin in
bulk and pharmaceutical dosage form was developed. It is accurate, sensitive and exact.

**Experimental**

**Standard API, Chemicals and Materials**

Linagliptin API and Dapagliflozin API were supplied by a reputable pharmaceutical company with purity 99.75 and 99.40, respectively. The 5 mg tablets of Linagliptin (marketed as Linares) and Dapagliflozin (marketed as Dapagold5). The Acetonitrile, Methanol, Toluene, Chloroform, Triethylamine were supplied by SRL Chemicals Pvt. Ltd., Mumbai, India.

**Wavelength Selection**

Dapagliflozin and Linagliptin are freely soluble in acetonitrile and individual solutions at a concentration of 10µg/ml are prepared. Using a UV-Visible double beam spectrometer, both solutions were scanned from 400 nm to 200 nm in wavelength. The iso-absorptive point was discovered to be 224 nm after scanning.

**HPTLC System**

Using a Camag Linomat 5 sample applicator (Switzerland) and a Camag 100 µl sample syringe (Hamilton, Switzerland), the samples were applied in the form of 6 mm wide bands on pre-coated HPTLC silica gel aluminum plate 60 F$_{254}$ (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany). Densitometric scanning of the generated chromatogram with a slit dimension of 4 x 0.45 mm was performed using the Camag TLC scanner 4.

**Standard solution preparation**

10 mg each of Linagliptin and Dapagliflozin were weighed, transported to separate 10-milliliter volumetric flasks, and swirl to dissolved with a little amount of acetonitrile. To bring the volume up to 10 milliliters, acetonitrile is added. The prepared solution has a 1000µg/ml concentration. A workable solution containing 100µg/ml was prepared from this stock solution by appropriately diluting it.

**Calibration curve determination**

From the working solution of 100µg/ml the aliquots of 2µl, 4µl, 6µl, 8µl, 10µl, 12µl were applied on the HPTLC silica gel aluminum plate 60 F$_{254}$ using the Camag Linomat 5 sample applicator using 100 µl Hamilton sample syringe.

**Validation**

The HPTLC method was validated in accordance with the Q2 (R2) guideline of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).

**Linearity**
Six calibrations were conducted in the concentration range of 200ng/ml to 1200ng/ml to determine the linearity of Dapagliflozin and Linagliptin in a fixed dose ratio (1:1). A straight-line equation was used to plot Peak Area v/s Concentration on the graph in order to determine the calibration cure.

**Precision**

The degree to which values or measurements from a homogenous sample are similar is known as precision. Six injections of the linearity range's middle concentration (600ng/band) were made to carry out the repeatability research.

Two investigations, intraday and interday, were carried out to achieve intermediate precision. While interday was done on different days, intraday was done on the same day. To find the intermediate precision, three concentrations—the lowest at 200ng/band, the middle at 600ng/band, and the highest at 1200ng/band were selected. The computed Mean of Area and %RSD was derived from the identified peak areas.

**Accuracy**

The degree to which the observed value and the true value agree is known as accuracy. The accuracy studies were conducted using the usual spiking approach. Using this technique, the standard solution was spiked at 80%, 100%, and 120% concentrations in the Dapagliflozin and Linagliptin sample solution, with a concentration of 600ng/band. There were three injections of each spiked sample. Calculations were made for Mean Area and Recovery Percentage.

**Limit of Quantification and Detection**

The lowest concentration at which the method can consistently detect the analyte inside the matrix (instead of measuring it) is called “limit of detection”. The term "limit of quantification" describes the smallest amount or lowest concentration of a material that can be determined using a certain analytical method while still adhering to the predetermined degrees of precision, accuracy, and uncertainty. The formula below can be used to calculate both.

\[ \text{LOD} = 3.3 \times \frac{\sigma}{s} \text{ and } \text{LOQ} = 10 \times \frac{\sigma}{s} \]

Where \( \sigma \) is the standard deviation of y-intercepts of regression lines and S is the average slope of the calibration curves.

**Robustness**

Whether or not the results remain consistent even in significantly altered settings is how one evaluates the robustness of an analytical procedure. It is a method's ability to function even if small changes are made. A few modest adjustments were made to the flow rate, analytical wavelength, and mobile phase composition to assess the method's robustness. 600ng/band was chosen as the middle concentration for the robustness analysis. The areas acquired after the injection were then used to calculate the mean area and percentage RSD.
Assay of Synthetic Mixture

The Dapagold 5 tablet of Dapagliozin and the Linares tablet of Linagliptin were precisely weighed to 5 mg in a 10-milliliter flask, dissolved in a tiny amount of acetonitrile, and the volume was then increased to 10 ml to yield a 500µg/ml solution. Withdraw 2 ml in each separate flask and top it off to 10 ml to yield solutions containing 100µg/ml. Apply 6µl on the silica plate using Camag Linomat 5 sample applicator and Camag 100 µl sample syringe by enabling over spotting; this will yield a sample of 600ng/band of Dapagliozin and Linagliptin (1:1) solution. This sample was analysed using the recently established HPTLC technology. The regression equation was then used to determine how much Dapagliozin and Linagliptin were present in the solution.

Forced Degradation Study:

The purpose of the forced deterioration study was to determine the intrinsic stability of both medications.

Heat Induced Acid Hydrolysis

A 1ml aliquot of the Dapagliozin stock solution (1000 ng/ml) was placed in a 10 ml volumetric flask. 1 of 0.01N HCl were added to the same flask. For two hours, the solution was heated to 70ºC. After allowing the sample to cool, it was neutralized with 0.01 N NaOH and brought up to the required amount with Acetonitrile. 6µl of the aforementioned solution were spotted on a TLC plate. In a similar manner, samples were made with a stock solution of Linagliptin (1000µg/ml) and investigated. Additionally, a mixture of 1ml each of Linagliptin and Dapagliozin (1000 µg/ml) was used in the investigation, both in the same 10 ml volumetric flask. 1ml of 0.01N HCl were added to the same flask.

Heat Induced Alkali Hydrolysis

In a 10 ml volumetric flask, an aliquot of 1 ml of the Dapagliozin stock solution (1000µg/ml) was taken. 1ml of 0.01N NaOH were added to the same flask. For four hours, the solution was heated to 70ºC. After allowing the sample to cool, it was neutralized with 1 ml of 0.01 N HCl and brought up to the required amount with Acetonitrile. 6µl of the aforementioned solution was spotted on a TLC plate. In a similar manner, samples were made with a stock solution of Linagliptin (1000 µg/ml) and investigated. Using an aliquot of 1ml each of Dapagliozin and Linagliptin (1000 µg/ml) in the same 10 ml volumetric flask, the investigation was also conducted in combination by adding 1 ml of 0.01N NaOH, cooling and neutraling with 1 ml of 0.01 N HCl, 6 µl of the aforementioned solution was spotted on a TLC plate.

Heat Induced Chemical Oxidation

Aliquot of (1 ml) of stock solution (1000µg/ml) of Dapagliozin was taken in 10 ml volumetric flask. To the same flask, 1 ml of 3% H2O2 was added. The sample was heated at 70ºC for 3 hours and allowed to cool and was made up to the mark with acetonitrile, from the above solution 6µl was spotted on TLC plate. Similarly, samples were prepared using Linagliptin (1000µg/ml) stock solution and study was carried out. The study was also carried out in mixture using aliquot of (1 ml) of Dapagliozin and Linagliptin (1000 µg/ml) in same 10 ml volumetric flask. To the same flask, 1ml of 3% H2O2 was added.
The sample was heated at 70°C for 3 hours and allowed to cool and was made up to the mark with acetonitrile, from above solution, 6 µl was spotted on TLC plate.

**Thermal Degradation**

After 4 hours at 70° to 80°C in dry heat, 10 mg each of Dapagliflozin and Linagliptin powder was transferred to two separate volumetric flasks (10 ml each). To dissolve the powder, a few ml of acetonitrile was added to each of the flask, then more acetonitrile was added until the desired amount was reached, yielding 1000 µg/ml of Dapagliflozin and Linagliptin. 1ml of the Dapagliflozin solution (1000 µg/ml) were pipetted out in 10ml volumetric flask and made volume with acetonitrile. 6 µl of the aforementioned solution was spotted on the TLC plate. Same process was repeated for Linagliptin. Pipette out 1ml each of the Dapagliflozin (1000 µg/ml) and Linagliptin (1000 µg/ml) solutions, then transfer them to the same flask. The contents were then diluted with acetonitrile to produce a standard solution of a combination of medications (100 µg/ml for Dapagliflozin and 100 µg/ml for Linagliptin). On the TLC plate, 6 µl of the aforementioned solution was observed.

**Photolytic (UV Light) Degradation**

Accurately weight 10mg of both the active pharmaceutical ingredients individually and transfer to two separate petri dishes. After being exposed to UV light for 24 hours, add few ml of acetonitrile to in both the petri dish to dissolve the powders of Dapagliflozin and Linagliptin. The solutions were transferred into two separate 10 ml volumetric flasks, then more acetonitrile was added until the desired volume was reached, yielding 1000 µg/ml of Dapagliflozin and Linagliptin. Pipette out 1ml of aliquots from both 1000 µg/ml of Dapagliflozin and Linagliptin and makeup volume with acetonitrile to produce solution of 100 µg/ml of Dapagliflozin and 100 µg/ml of Linagliptin. 6 µl of the Dapagliflozin solution (100 µg/ml) and Linagliptin (100 µg/ml) were seen on the TLC plate. Pipette out 1ml each of the Dapagliflozin (1000 µg/ml) and Linagliptin (1000 µg/ml) solutions, then transfer them to the same flask. The contents were then diluted with acetonitrile to produce a standard solution of a combination of medications (100 µg/ml for Dapagliflozin and 100 µg/ml for Linagliptin). On the TLC plate, 6 µl of the aforementioned solution was observed.

The last concentration of the considerable number of solutions 100 ng/band (6µl) was applied on the pre-coated HPTLC plates and densitogram were recorded.

**Results and Discussion**

**Wavelength Selection**

Acetonitrile was used as a solvent to create separate solutions of Dapagliflozin and Linagliptin at a concentration of 10µg/ml. using a UV-Visible double beam spectrometer, both solutions were scanned from 400 nm to 200 nm in wavelength. The iso-absorptive point was discovered to be 224 nm after scanning as shown in Fig. 2.
Mobile Phase optimization

Toluene: Chloroform: Methanol: Triethylamine (7:2:1:0.2) was the mobile phase that generated a symmetrical and selective peak for Dapagliflozin at \( R_f 0.23 \) and Linagliptin at \( R_f 0.40 \) as shown in Fig. 3.

Validation

The HPTLC method was validated in accordance with the Q2 (R2) guideline of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The results are shown as follows.

Linearity

The developed HTPLC method revealed that the correlation coefficients of 0.9978 and 0.9973 for Dapagliflozin and Linagliptin were linear within the specified concentration range of 200–1200ng/band. Figure 4 shows the Overlay of the Densitogram of calibration range. The value of regression analysis is shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dapagliflozin</th>
<th>Linagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (ng/band)</td>
<td>200–1200</td>
<td>200–1200</td>
</tr>
<tr>
<td>Regression Coefficient ((R^2))</td>
<td>0.997</td>
<td>0.997</td>
</tr>
<tr>
<td>Slope of regression equation</td>
<td>1.1499</td>
<td>2.9126</td>
</tr>
<tr>
<td>Standard deviation of slope</td>
<td>0.0075</td>
<td>0.0271</td>
</tr>
<tr>
<td>Intercept of regression</td>
<td>29.48</td>
<td>248.57</td>
</tr>
<tr>
<td>Standard deviation of intercept</td>
<td>9.0445</td>
<td>12.2410</td>
</tr>
</tbody>
</table>

Precision

Dapagliflozin and Linagliptin have percentage RSD value ranges for Interday precision of 0.59–1.88 and 0.39–1.48%, respectively, and Intraday precision of 0.35–0.62% and 0.51–1.81%, for Dapagliflozin and Linagliptin, respectively. When intraday and interday measurements are made, the precision result demonstrates that the values are near to one another. Table 2 shows the summary of validation parameters of proposed HPTLC method for Dapagliflozin and Linagliptin.
Table 2

Result of validation parameters of RP-HPLC method for Dapaglirozin and Linagliptin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dapaglirozin</th>
<th>Linagliptin</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (ng/band)</td>
<td>200–1200</td>
<td>200–1200</td>
<td>-</td>
</tr>
<tr>
<td>Retention Factor (Rf)</td>
<td>0.23</td>
<td>0.40</td>
<td>-</td>
</tr>
<tr>
<td>Detection Limit (ng/band)</td>
<td>25.80</td>
<td>13.09</td>
<td>-</td>
</tr>
<tr>
<td>Quantitation Limit (ng/band)</td>
<td>72.22</td>
<td>42.14</td>
<td>-</td>
</tr>
<tr>
<td>Accuracy (% Recovery)</td>
<td>98.82–101.44</td>
<td>98.35–101.01</td>
<td>&gt;98% &lt;102%</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
<td>-</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
<td>Robust</td>
<td>% RSD value&lt; 2%</td>
</tr>
</tbody>
</table>

Precision (%RSD)

<table>
<thead>
<tr>
<th></th>
<th>Interday Precision (n = 3)</th>
<th>Intraday Precision (n = 3)</th>
<th>Repeatability Study (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.59–1.88</td>
<td>0.39–1.48</td>
<td>%RSD value&lt; 2%</td>
</tr>
<tr>
<td></td>
<td>0.35–0.62</td>
<td>0.51–1.81</td>
<td>%RSD value&lt; 2%</td>
</tr>
</tbody>
</table>

Repeatability (n = 6) 1.88 1.65 %RSD value< 2%

Assay (% Recovery) 100.7 99.8 >98% - <102%

RSD is Relative Standard Deviation and “n” is number of determinations.

Accuracy

The accuracy studies were conducted using the usual spiking approach. The regression equation was used to compute the percentage recovery of Linagliptin and Dapaglirozin. The percentage recovery of Dapaglirozin and Linagliptin was found to be 98.82–101.44% and 98.35–101.01%, respectively. The results fall between the range of 98 to 102% which indicates that the procedure is deemed accurate.

Limit of Detection and Limit of Quantification

The results indicate that the approach is very sensitive for both detection and quantification. Specifically, the LOD and LOQ for Dapaglirozin were determined to be 25.80ng/band and 72.22ng/band, while the corresponding values for Linagliptin were 13.09 ng/band and 42.14ng/band respectively.

Robustness

Some deliberate modifications were made to the test parameters to carry out the robustness investigation. The modifications included a minor shift in the analytical wavelength from 224 nm to 223 nm and 225 nm, a change in saturation time from 40 min to 35 min and 45 min, and a change in the
composition of the mobile phase from toluene: chloroform: methanol: Triethylamine (7:2:1:0.2) to 7.2:2:0.8:0.2 and 6.8:2:1.2:0.2. The percentage RSD was found to be less than 2% which demonstrates that Dapagliflozin and Linagliptin may be assessed using this method with minor adjustments to the optimized chromatographic conditions are shown in Table 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimized Condition</th>
<th>Change in Condition</th>
<th>Rf</th>
<th>Mean area ± SD (n = 3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Wavelength</td>
<td>224 nm</td>
<td>223 nm</td>
<td>0.20</td>
<td>747.5667 ± 6.07</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>225 nm</td>
<td>0.20</td>
<td>808.0667 ± 7.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Change in Saturation time</td>
<td>40 min</td>
<td>35 min</td>
<td>0.18</td>
<td>7.031595 ± 1.90</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 min</td>
<td>0.21</td>
<td>1075.3 ± 7.35</td>
<td>0.68</td>
</tr>
<tr>
<td>Change in Mobile Phase Composition</td>
<td>7:2:1:0.2 v/v/v/v</td>
<td>7.2:2:0.8:0.2 v/v/v/v</td>
<td>0.25</td>
<td>938.7 ± 3.54</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8:2:1.2:0.2 v/v/v/v</td>
<td>0.23</td>
<td>1131.4 ± 20.58</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Table 3
Robustness study for Linagliptin (600ng/band)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimized Condition</th>
<th>Change in Condition</th>
<th>Rf</th>
<th>Mean area ± SD (n = 3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Wavelength</td>
<td>224 nm</td>
<td>223 nm</td>
<td>0.38</td>
<td>1749.767 ± 17.89</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>225 nm</td>
<td>0.38</td>
<td>1770.733 ± 27.22</td>
<td>1.53</td>
</tr>
<tr>
<td>Change in Saturation time</td>
<td>40 min</td>
<td>35 min</td>
<td>0.35</td>
<td>1967.667 ± 20.57</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 min</td>
<td>0.35</td>
<td>2553.867 ± 16.76</td>
<td>0.65</td>
</tr>
<tr>
<td>Change in Mobile Phase Composition</td>
<td>7:2:1:0.2 v/v/v/v</td>
<td>7.2:2:0.8:0.2 v/v/v/v</td>
<td>0.45</td>
<td>2674.733 ± 10.36</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8:2:1.2:0.2 v/v/v/v</td>
<td>0.43</td>
<td>2048.667 ± 18.50</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Assay of Synthetic Mixture

The recommended methodology was utilized to examine the commercially available tablet formulations of Dapagliflozin and Linagliptin and the percentage amount of drug was found to be 100.7% and 99.8%.
Figure 5 displays the overlay densitogram, selectivity, and specificity of the Linagliptin standard and formulation along with the Dapagliflozin standard.

Peak purity spectra of Dapagliflozin and Linagliptin in calibration curve is shown in Fig. 6.

**Forced Degradation:**

Dapagliflozin and Linagliptin were degraded in acid hydrolysis with 0.01 N HCl at 70°C for 2 hours. Figure 7 represents the densitogram of combined drug under the acid degradation study.

Dapagliflozin and Linagliptin were significantly degraded in alkali degradation using 0.01 N NaOH at 70°C for 4 hours. Figure 8 displays the densitogram of combined drugs in alkali degradation study.

Dapagliflozin and Linagliptin were somewhat vulnerable to oxidative stress conditions. Figure 9 displays the densitogram of during chemical oxidative breakdown.

Dapagliflozin and Linagliptin were more prone to thermal degradation conditions. Figure 10 displays the mixture's densitogram during thermal degradation.

Dapagliflozin and Linagliptin were found to be slightly vulnerable to photo degradation conditions. Figure 11 displays the mixture's densitogram in UV photolytic degradation.

With no interference, all of the degradant peaks could be clearly separated from the drug peak, and a detector was used to measure the peak purity. For the analysis of stability samples, the suggested approach is applicable which represent the Dapagliflozin and Linagliptin are susceptible to acid, alkali, chemical oxidative, dry heat and photolytic stress conditions. Table 4 represent the results of forced degradation studies.
### Table 4
Result of Force Degradation Studies

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time(hr)</th>
<th>%Amount of Dapagliozin Degradant</th>
<th>%Amount of Linagliptin Degradant</th>
<th>Rf value of degradants of Dapagliozin</th>
<th>Rf value of degradants of Linagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid 0.01 N HCl</td>
<td>2</td>
<td>18.91%</td>
<td>19.21%</td>
<td>0.03</td>
<td>0.46, 0.77</td>
</tr>
<tr>
<td>Base 0.01N NaOH</td>
<td>4</td>
<td>16.96%</td>
<td>19.66%</td>
<td>0.22</td>
<td>0.30</td>
</tr>
<tr>
<td>Oxidation 3% H₂O₂</td>
<td>3</td>
<td>15.54%</td>
<td>7.05%</td>
<td>0.05, 0.25</td>
<td>0.42</td>
</tr>
<tr>
<td>Dry Heat*</td>
<td>4</td>
<td>20%</td>
<td>17.58%</td>
<td>0.28</td>
<td>0.52</td>
</tr>
<tr>
<td>Photo Degradation#</td>
<td>24</td>
<td>12.45%</td>
<td>6.61%</td>
<td>0.03</td>
<td>0.59</td>
</tr>
</tbody>
</table>

*Drugs heat for 4 hrs at 70–80 °C in Hot air oven, # Exposed to UV light for 24 hrs

### Conclusion

Presenting HPTLC method in pharmaceutical investigation represent a major advance in quality assurance. The validation parameters low percentage RSD value suggests that the high-performance thin layer chromatography method is suitable for routine analysis and the quantitative simultaneous determination of Dapagliozin and Linagliptin in bulk and combination. The method is proven to be reproducible, selective, accurate, resilient, and exact by statistical analysis of the data. Dapagliozin and Linagliptin are susceptible to acid and base hydrolysis, chemical oxidation, dry heat and photolytic degradation stress condition. In which, degradant peaks of Dapagliozin and Linagliptin could be clearly separated from the parent drug peaks, and a detector was used to measure the peak purity. For the analysis of stability samples, the suggested approach is therefore applicable.

### Declarations

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. All authors associated with this research work declared that there is no conflict of interest for publication of work. This research did not receive any specific grant from any funding agencies in the public, commercial, or not-for-profit sectors.

### Author Contribution

All authors associated with this research work declared that there is no conflict of interest for publication of work. All authors have read and approved the manuscript. The contribution of each authors are
Anchal Shukla: She is a postgraduate student and above work has been carried out by her as dissertation work. Usmangani Chhalotiya: He is a mentor of Ms. Anchal Shukla and under his noble guidance proposed method has been developed and validated as per ICH guideline. He is also giving training for ease of operation of sophisticated reverse phase liquid chromatography instrument and involved in interpretation of data. Dimal Shah: Through his good relationship with pharmaceutical industry we have received all active pharmaceutical ingredients and he is having sound technical knowledge Waters HPLC software system. Jinal Tandel: under her noble guidance student can understand the concept of mobile phase selection and how to optimize chromatographic conditions. Heta Kachhiya: under her noble guidance student can understand the concept of mobile phase selection and how to optimize chromatographic conditions. She is also involved in interpretation of data. Mital Parmar: She is a co-mentor of Anchal Patel helping hand throughout cited research work.

**Acknowledgements**

The authors are extremely appreciative Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, SICART and The Charutar Vidya Mandal University, Vallabh Vidyanagar for providing necessary facilities to carry out research work.

**References**


Figures
Figure 1

(a): Chemical structure of Dapagliflozin

(b): Chemical Structure of Linagliptin
Figure 2

Overlay UV-Visible Spectra of Dapagliflozin and Linagliptin (10 µg/ml)

Figure 3

Densitogram of Dapagliflozin and Linagliptin (600 ng/band)
Figure 4
3D Overlay densitogram of Dapagliflozin and Linagliptin (200–1200 ng/band)

Figure 5
Overlay Densitogram of standard and synthetic mixture of Dapagliflozin and Linagliptin (600 ng/band)
Figure 6

Peak purity spectra of Dapagliflozin and Linagliptin in calibration curve

Figure 7

Combined densitogram Dapagliflozin (600ng/band) and Linagliptin (600ng/band) in 0.01 N HCl reflux for 2 hours at 70°C
Figure 8

Combined densitogram Dapagliflozin (600ng/band) and Linagliptin (600ng/band) in 0.01 N NaOH reflux for 4 hours at 70ºC

Figure 9

Combined densitogram of Dapagliflozin (600ng/band) and Linagliptin (600ng/band) in 3% H₂O₂ reflux for 3 hours at 70-80 ºC
Figure 10

Combined densitogram of Dapagliflozin (600ng/band) and Linagliptin (600ng/band) in dry heat for 4 hours at 70-80ºC.

Figure 11

Combined Densitogram of Dapagliflozin (600ng/band) and Linagliptin (600ng/band) in UV exposure for 24 hrs.