Analytical Methods for Simultaneous Estimation of SGLT2 Inhibitor and DPP-4 Inhibitor in their Combination for Treatment of Type 2 Diabetes Mellitus

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Abstract: The Saxagliptin Hydrochloride (SAXA) and Dapagliflozin (DAPA) Fixed-Dose Combination has recently been approved for the treatment of Mellitus type 2 diabetes. In order to simultaneously estimate SAXA and DAPA in a bulk product and its formulation, the study aimed at developing a simple, fast, sensitive, and validated UV-Spectrophotometric and reversed-phase high-performance liquid chromatography (RP-HPLC) methods. Simultaneous equation UV method was performed on Shimadzu UV-1800 Spectrophotometer based on measurement of SAXA and DAPA absorption in methanol at 210 nm and 224 nm, respectively, over 6-22 μg / mL and 12-44 μg / mL linear ranges. RP-HPLC method was designed using an HPLC system-equipped PDA detector. The method has been validated for SAXA and DAPA for a range of 8 to 22 μg / ml and from 16 to 44 μg / mL. In compliance with ICH guideline Q2(R1), the optimal approach is successfully validated. The results showed that the method was accurate (98.22–100.28 percent w / w and 99.48–100.15 percent w / w SAXA and DAPA, respectively) and precise (percentage of relative standard deviation < 2.0). Developed methods follow ICH Q2 (R1) criteria and sufficient to apply regulatory versatility for submission.

Keywords: saxagliptin hydrochloride; dapagliflozin; UV-spectrophotometry; Reverse-Phase High-Performance Liquid Chromatography.

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1. Introduction

The combination of saxagliptin (SAXA) and dapagliflozin (DAPA) may have a major glycaemic regulation advantage without the risk of weight gain and hypoglycemia, which may be linked with other medicines used to treat diabetes of type 2. SAXA is an oral hypoglycemic (anti-diabetic) dipeptidyl peptidase-4 (DPP-4) inhibitor with a IUPAC name (1S, 3S, 5S)-2[(2S)-2-(3-hydroxy-1-adamantyl)-acetyl]-2-azabicyclo[3.1.2] hexane-3-carbonitrile (Figure 1) [1]. It is used for the treatment of type II diabetes either in monotherapy or in combination with other drugs. The drug acts to suppress protein/enzyme, dipeptidyl peptidase 4 (DPP-4), competitively resulting in an increased amount of active augmentin: gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Dapagliflozin (DAPA) is a sodium-glucose co-transporter-2 inhibitor with a chemical name (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-
ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol (Figure 2). Such sodium-glucose co-transporters are responsible for reabsorbing glucose into the kidney [2-3].

![Figure 1: Structure of Saxagliptin.](image1)

![Figure 2: Structure of Dapagliflozin.](image2)

An extensive literature survey has revealed that there are few methods are reported for estimation of SAXA and DAPA individually or in combination with other drugs by UV Method [4-7], Reverse phase high performance liquid chromatographic (RP HPLC) method [8-16], tandem mass spectrometry method [17] or LC-MS [18-19]. So, we propose to develop methods that are more reliable, sensitive, and simple for simultaneous estimation of SAXA and DAPA in bulk as well as a pharmaceutical formulation. In the present work, we developed and validated the UV method and RP-HPLC method for the simultaneous estimation of saxagliptin hydrochloride and dapagliflozin in bulk drug and its formulation as per ICH guidelines Q2(R1) [20].

2. Materials and Methods

2.1. Reagents and chemicals.

CTX Lifesciences (Surat) and Zydus Healthcare (Ahmedabad) gifted the pure drug samples of Saxagliptin Hydrochloride and Dapagliflozin, respectively. HPLC grade Acetonitrile, HPLC grade methanol, and all other chemicals were obtained from LOBA. During the entire study, HPLC grade water was obtained from the Milli-Q.

2.2. Instrumentation.

Spectrophotometric experiments were run on Shimadzu UV-Visible Double Beam System UV-1800 Spectrophotometer, while Chromatography was performed with an HPLC Shimadzu LC-2010 device equipped with a quaternary pump, column oven, degasser, PDA detector, and LC solution program.

2.3. UV Spectroscopic method (Method A).

2.3.1. Selection of solvent for SAXA and DAPA.

Methanol was ideal for the spectrophotometric analysis of SAXA and DAPA, according to the solubility testing. SAXA solutions of 6-22 μg / mL and DAPA solutions of 12-44 μg / mL were prepared in methanol, and the spectrum was recorded between 200-400 nm.
2.3.2. Preparation of internal standards.

2.3.2.1. SAXA standard stock solution (500 µg/mL).

Weighed 5 mg of SAXA to a volumetric flask of 10 mL. The flask has been shaken and methanol is the amount to have a 500 µg / mL SAXA solution.

2.3.2.2. DAPA standard stock solution (1000 µg/mL).

Weighed and transferred 10 mg of DAPA to a 10 ml volumetric flask. The flask has been shaken up, and methanol was used to produce a 1000 µg / mL DAPA solution.

2.3.2.3. SAXA standard working solution (50 µg/mL).

A 10ml SAXA was taken and transferred from the above stock solution into a 100ml volumetric flask. The flask has been shaken, and methanol has been added to the mark for a solution containing SAXA 50 µg/mL.

2.3.2.4. DAPA standard working solution (100 µg/mL).

10 ml of DAPA from the above stock solution was taken and transferred 100 mL volumetric flask. The flask was shaken, and volume was made up to the mark with methanol to give a solution containing 100 µg/mL of DAPA.

2.3.3. Selection of analytical wavelength for SAXA and DAPA.

6-22 µg / mL solutions were prepared in diluents for SAXA, and between 200-400 nm of the spectrum was recorded. Similarly, DAPA solutions of 12-44 µg / mL were prepared in diluents, and spectrums between 200-400 nm were recorded. The wavelength at which maximum absorbance was measured was called the drug's λmax.

2.3.4. Establishment of the calibration curve.

Individually a calibration curve was plotted for SAXA over a concentration range of 6-22 µg / mL and for DAPA 12-44 µg / mL. Accurately calibrated SAXA and DAPA work-stock solution was transferred to volumetric flasks of 10mL and diluted with methanol to the limit. The absorbance was measured at selected wavelengths 210 and 224 nm for each solution. For SAXA and DAPA, the calibration curves were constructed by plotting absorbance versus concentration, and the coefficient of regression was stated.

2.3.5. Simultaneous equation method [21,22].

A simultaneous equation approach was used for estimating SAXA and DAPA using spectrophotometry. This approach involves two wavelengths. Select one wavelength at which SAXA displays maximum absorbance, thus choosing the second wavelength at which DAPA displays maximum absorbance. At chosen wavelengths, i.e., 210 nm and 224 nm, the absorptivity of both the drugs determined.

From the above data of absorptivity, the generated equation for both the drugs is as under simultaneous equation

\[ A1 = ax1 cx + ay1 cy \]  
\[ A2 = ax2 cx + ay2 cy \]
where, 
ax1 and ax2 are absorptivities of SAXA at 210 and 224 nm, respectively 
ay1 and ay2 are absorptivities of DAPA at 210 and 224 nm, respectively 
A1 and A2 are absorbances of the diluted test sample at 210 and 224 nm, respectively 

Using Cramer’s rule and matrices, the equation (1) and (2) can be written as,

\[ C_x = \frac{(A2 \cdot ay1 - A1 \cdot ay2)}{(ax2 \cdot ay1 - ax1 \cdot ay2)} \]
\[ C_y = \frac{(A1 \cdot ax2 - A2 \cdot ax1)}{(ax2 \cdot ay1 - ax1 \cdot ay2)} \]

By putting the values of absorptivity

where 1) A1 and A2 are absorbances of the sample at 210nm and 224nm, respectively 
2) Cx is the concentration of SAXA in µg/mL 
3) Cy is the concentration of DAPA in µg/mL

2.3.6. Method validation.

A detailed and full validation of the system was carried out according to the Q2(R1) guidelines of the ICH. The approach has been checked for linearity, precision, and accuracy, analyte recovery, roughness, and reproducibility [23].

2.3.6.1. Linearity.

Appropriate aliquots from SAXA (1.2 – 4.4 mL) and DAPA (1.2 – 4.4 mL) standard working solution were transferred to different volumetric flasks of 10 ml capacity separately. The volume was made up to the mark with methanol to obtain a concentration of 6–22 µg/mL of SAXA and 12-44 µg/mL of DAPA. The solutions were analyzed at 210 nm and 224 nm, and the calibration curve was constructed by plotting average Absorbance vs. Conc., and the regression equation was computed. The mean absorptivity values were also calculated for SAXA and DAPA.

2.3.6.2. Precision.

**Intraday Precision.** Lower, middle, and higher concentrations of the calibration curve were selected for intraday precision for SAXA and DAPA. Appropriate aliquots of SAXA (1.2, 2.8, and 4.4 mL) and DAPA (1.2, 2.8, and 4.4 mL) were transferred to different volumetric flasks of 10 ml individually. The volume was made up to the mark with methanol to obtain concentrations of 6, 14, and 22 µg/mL of SAXA and 12, 28, and 44 µg/mL of DAPA, respectively. The solutions were analyzed at 210 nm and 224 nm three times on the same day, and absorbance was recorded.

**Interday Precision.** Lower, middle, and higher concentrations of the calibration curve were selected for intraday precision for SAXA and DAPA. Appropriate aliquots of SAXA (1.2, 2.8, and 4.4 mL) and DAPA (1.2, 2.8, and 4.4 mL) were transferred to different volumetric flasks of 10 ml individually. The volume was made up to the mark with methanol to obtain concentrations of 6, 14, and 22 µg/mL of SAXA and 12, 28, and 44 µg/mL of DAPA, respectively. The solutions were analyzed at 210 nm and 224 nm three times on three consecutive days, and absorbance was recorded.

2.3.6.3. Repeatability.

Aliquots of 2.8 mL of SAXA and 2.8 mL of DAPA were pipette out and transferred to volumetric flasks of 10 mL individually. The volume was made up to the mark with methanol
to obtain concentrations of 14 µg/mL of SAXA and 28 µg/mL of DAPA, respectively. Repeatability was established by performing the experiment six times consecutively, and absorbances were recorded.

2.3.6.4. Accuracy.

The validity and reliability of the proposed method were evaluated using the standard method of addition via a recovery study. To check the accuracy of the proposed method recovery study, 3 different levels of standard addition (80 percent, 100 percent, and 120 percent) were performed from pre-analyzed samples.

2.3.6.5. Limit of Detection (LOD).

An individual analytical procedure's detection limit is the lowest quantity of the analyte in a sample that can be detected but not generally quantified as an exact value. The calibration curve was repeated six times, measuring the standard deviation of the intercepts and the mean of the slopes. Then, mathematical terms were used to calculate the LOD.

\[
LOD = 3.3 \frac{\sigma}{S}
\]

where, \( \sigma \) = the standard deviation of the response
\( S \) = the slope of the calibration curve

2.3.6.6. Limit of Quantification (LOQ).

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The calibration curve was repeated six times, and the standard deviations of the intercepts and means of slopes were calculated. Then LOD was measured by using the mathematical expression.

\[
LOQ = 10 \frac{\sigma}{S}
\]

where, \( \sigma \) = the standard deviation of the response
\( S \) = the slope of the calibration curve

2.3.6.7. Robustness.

The robustness of an analytical procedure is a measurement of its capacity to remain unaffected by small, but deliberate variations in method parameters. The robustness of the developed method was tested by varying detection wavelength (±2 nm) of optimized conditions.

2.3.6.8. Assay of the developed formulation containing SAXA and DAPA.

A quantity of equivalent to 5 mg of SAXA and 10 mg of DAPA was taken and dissolved in 10 mL of methanol. The solution was filtered through Whatman filter paper no. 41. From this 1 mL was pipette out in a 100 mL volumetric flask, and volume was made up to mark to obtain 50 µg/mL of SAXA and 100 µg/mL of DAPA. The final solution having a concentration of 14 µg/mL of SAXA and 28 µg/mL of DAPA was prepared and analyzed.
2.4. RP-HPLC Method.

2.4.1. Preparation of standard stock solution.

5mg of SAXA and 10 mg DAPA were weighed separately and transferred to 10 mL volumetric flasks. Both of them were dissolved in methanol, and volume was made up to mark with methanol giving final solution containing 500 µg/mL and 1000 µg/mL, respectively.

2.4.2. Preparation of binary mixture.

10ml of SAXA and DAPA standard stock solution was added to 100 mL volumetric flask, and volume was made up to mark with methanol to obtain a mixture containing 50 µg/mL and 100 µg/mL, respectively.

2.4.3. Preparation of mobile phase.

The mobile phase acetonitrile was filtered through a 0.45micron pore size membrane filter using vacuum filtration assembly. Phosphate buffer was prepared, and pH was adjusted with glacial acetic acid (pH 4).

2.4.4. Validation of developed RP-HPLC method.

Validation was done as per ICH guideline Q2 (R1). The developed RP-HPLC methods were validated concerning parameters such as linearity, precision, accuracy, specificity, robustness.

2.4.4.1. Linearity.

Linearity was studied by preparing a standard solution at 6 different concentrations. Each concentration was repeated 6 times. The preparation of solutions for the calibration curve for the linearity study was done by the following procedure. Aliquot equivalent to 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4 mL of solution was transferred into 10 ml volumetric flasks and diluted up to the mark by using methanol. From each solution, 20µL volume was injected into the HPLC system and analyzed. The calibration curve was obtained by plotting the respective peak area against concentration in µg/mL, and the regression equation was computed.

The linearity of SAXA and DAPA was found 8-22 µg/mL and 16-44 µg/mL, respectively. Linearity was assessed in terms of slope, intercept, and correlation coefficient.

2.4.4.2. Precision.

Intraday Precision. Intraday precision was determined by analyzing the sample solution three times in one day for three different concentrations. (Combined standard samples of concentrations 10, 16, 22 µg/mL for SAXA and 20, 32, 44 µg/mL for DAPA).

Interday Precision. Interday precision was determined by analyzing the sample solution on three different days for three different concentrations. (Combined standard samples of concentrations 10, 16, 22 µg/mL for SAXA and 20, 32, 44 µg/mL for DAPA).

2.4.4.3. Repeatability.

To demonstrate precision, repeated measurement of the developed method was checked. Six sets of both (16 µg/mL of SAXA and 32 µg/mL of DAPA) were prepared.
Duplicate injections of each set of assay preparation into liquid chromatography were injected and chromatograms were recorded. The repeatability was expressed in terms of relative standard deviation (RSD).

2.4.4.4. Accuracy.

The accuracy of the method was carried out at three levels in the range of 80, 100, and 120% of the working concentration of the sample. The calculated amount of SAXA and DAPA working standard solutions were added in the sample solution-containing volumetric flask to prepare 80%, 100%, and 120% level of the working concentration.

For 80% level of recovery, 2 mL of the sample solution and 1.6 mL of standard solution was pipette out in a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a final concentration of 18 µg/mL of SAXA and 36 µg/ml of DAPA. The solution was analyzed three times, and areas were recorded.

For a 100% level of recovery, 2 mL of the sample solution and 2 mL of standard solution was pipette out in a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a final concentration of 20 µg/mL of SAXA and 40 µg/mL of DAPA. The solution was analyzed three times, and areas were recorded.

For a 120% level of recovery, 2 mL of the sample solution and 2.4 mL of standard solution was pipette out in a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a final concentration of 22 µg/mL of SAXA and 44 µg/mL of DAPA. The solution was analyzed three times, and areas were recorded.

2.4.4.5. LOD and LOQ.

The calibration curve was repeated three times, and the standard deviation (SD) of the intercepts was calculated. The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e., 3.3 for LOD and 10 for LOQ) ratio using the following equations

\[ \text{LOD} = 3.3 \frac{\sigma}{S} \quad \text{and} \quad \text{LOQ} = 10 \frac{\sigma}{S} \]

where, \( \sigma \) = Standard deviation of the response

\( S \) = Slope of the calibration curve

2.4.4.6. Robustness.

The robustness of the developed method was tested by varying detection wavelength (±2nm), flow rate (±1mL/min) of optimized conditions. There were changed the following parameters, one by one, and observed their effect on system suitability test and assay.

*Changed wavelength by ±2nm.* Appropriate aliquot, 2.8 mL was taken from a working standard solution into a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a concentration of 14 µg/mL of SAXA and 28 µg/mL of DAPA. This solution was analyzed at an optimized HPLC condition changed flow rate (±2nm) to check robustness.

*Changed flow rate by ±2mL/min.* Appropriate aliquot, 2.8 mL was taken from a working standard solution into a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a concentration of 14 µg/mL of SAXA and 28 µg/mL of DAPA. This solution was analyzed at an optimized HPLC condition changed flow rate (±1mL/min) to check robustness.
2.4.4.7. Assay of the marketed formulation by RP-HPLC method.

Weigh 20 tablets and determine average weight. Accurately weigh and transfer the quantity of tablet contents equivalent to about 5 mg of SAXA and 10 mg of DAPA transfer into a 10 mL amber-colored volumetric flask and add methanol and sonicated for about 20 min. Dilute it up to mark with methanol and mix to get a solution containing 500 µg/mL of SAXA and 1000 µg/mL of DAPA. Take 10 mL aliquot in a separate 100 mL amber-colored volumetric flask. Dilute it up to mark with methanol to get a solution containing 50 µg/mL of SAXA and 100 µg/mL of DAPA. Take 3.2 mL of an aliquot in a separate 10 mL amber colored volumetric flask. Dilute it up to mark with methanol to get a solution containing 16 µg/mL of SAXA and 32 µg/mL of DAPA. The analysis procedure was repeated 5 times.

3. Results and Discussion

3.1. Method Validation (UV Method).

3.1.1. Linearity.

Linearity was found between 6 to 22 µg/mL for SAXA (Figure 3) and 12 to 44 µg/mL DAPA (Figure 4), respectively. Linear regression data for the calibration curves (n=6) shows a good linear relationship over the concentration range of 6 to 22 µg/mL for SAXA (Figure 5,6) and 12 to 44 µg/mL DAPA (Figure 7,8) respectively. The solutions were analyzed at 210nm and 224nm, and the calibration curve was constructed by plotting average Absorbance vs. Conc., and the regression equation was computed.

Figure 3. Overlay spectra of SAXA (6 to 22 µg/mL) in methanol at 210 nm.

Figure 4. Overlay spectra of DAPA (12 to 44 µg/mL) in methanol at 224 nm.
Figure 5. Calibration curve for SAXA at 210 nm.

Figure 6. Calibration curve for SAXA at 224 nm.

Figure 7. Calibration curve for DAPA at 224 nm.
3.1.2. Precision.

3.1.2.1. Intraday precision.

Intraday precision was performed, and %RSD values were within the acceptance criteria.

3.1.2.2. Interday precision.

Interday precision was performed and %RSD values were well within the acceptance criteria.

3.1.3. Repeatability.

It was performed, and %RSD values were found to be less than 2%.

3.1.4. Accuracy.

Accuracy was performed, and % Recovery was found to be within 98-102% at all three levels. This indicates that SAXA and DAPA can be recovered successfully in the presence of excipients. (As shown in Table 1 and 2).

Table 1. Accuracy (% Recovery) data for SAXA at 210 nm (n=3).

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Sample Conc. (µg/mL)</th>
<th>Conc. of Std added (µg/mL)</th>
<th>Total Conc. (µg/mL)</th>
<th>Abs</th>
<th>Mean Abs¹</th>
<th>Amt Recovered (µg/mL)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>0.387</td>
<td>0.384</td>
<td>18.01</td>
<td>100.08</td>
<td>0.09</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.385</td>
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<td></td>
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<td></td>
<td></td>
<td>0.380</td>
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<tr>
<td>100%</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>0.430</td>
<td>0.429</td>
<td>20.33</td>
<td>101.67</td>
<td>0.07</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.433</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.425</td>
<td></td>
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</tr>
<tr>
<td>120%</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>0.461</td>
<td>0.460</td>
<td>21.93</td>
<td>99.69</td>
<td>0.05</td>
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<td></td>
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<td>0.465</td>
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<td></td>
<td></td>
<td>0.455</td>
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</tr>
</tbody>
</table>

¹Mean absorbance of n=3
Table 2. Accuracy (% Recovery) data for DAPA at 224 nm (n=3).

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Sample Conc. (µg/mL)</th>
<th>Conc. Of Std added (µg/mL)</th>
<th>Total Conc. (µg/mL)</th>
<th>Abs</th>
<th>Mean Abs¹</th>
<th>Amt Recovered (µg/mL)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>20</td>
<td>16</td>
<td>36</td>
<td>1.469</td>
<td>1.483</td>
<td>36.3</td>
<td>100.83</td>
<td>0.22</td>
</tr>
<tr>
<td>100%</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>1.651</td>
<td>1.662</td>
<td>40.74</td>
<td>101.85</td>
<td>0.87</td>
</tr>
<tr>
<td>120%</td>
<td>20</td>
<td>24</td>
<td>44</td>
<td>1.745</td>
<td>1.761</td>
<td>43.19</td>
<td>98.15</td>
<td>0.30</td>
</tr>
</tbody>
</table>

¹Mean absorbance of n=3

3.1.5. LOD and LOQ.

LOD and LOQ values were calculated using the mathematical equation.

3.1.6. Robustness.

The robustness of the developed method was checked by varying the λmax. The results show that % RSD values are less than 2% at varied maxima indicating that the developed method is robust in terms of change in λmax by ±2 nm.

3.1.7. Analysis of the developed formulation.

The results of the assay were found to be within of label claim for formulations containing SAXA and DAPA as shown in Table 3.

Table 3. Assay data for SAXA and DAPA (n=3).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (mg)</th>
<th>Sample conc. (µg/mL)</th>
<th>Abs at 210 nm</th>
<th>Abs at 224 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAXA</td>
<td>5</td>
<td>14</td>
<td>0.305</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.310</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.308</td>
<td>0.174</td>
</tr>
<tr>
<td></td>
<td>% Assay ± SD</td>
<td>100.33 ± 0.002</td>
<td>99.87 ± 0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.81</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>DAPA</td>
<td>10</td>
<td>28</td>
<td>1.95</td>
<td>1.165</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.180</td>
<td>1.149</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.182</td>
<td>1.142</td>
</tr>
<tr>
<td></td>
<td>% Assay ± SD</td>
<td>100.19 ± 0.008</td>
<td>100.41 ± 0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.68</td>
<td>1.02</td>
<td></td>
</tr>
</tbody>
</table>

Figure 9. Developed chromatogram of SAXA (16μg/mL) and DAPA (32μg/mL) by HPLC.
3.2. **HPLC method.**

Various trials for varying ratios of the mobile phase, gradient system, diluents, wavelength, flow, column, and column temperature were performed. The final chromatographic conditions are mentioned below based on trials, as mentioned in Table 4, while the optimized chromatography is shown in Figure 9.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Shimadzu HPLC system equipped quaternary pump and PDA detector</td>
</tr>
<tr>
<td>Column</td>
<td>C18 Inertsil ODS (150×4.6) mm, 5μ</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Phosphate buffer: Acetonitrile (55:45), pH 4.0 adjusted by glacial acetic acid</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 μL/min</td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>220 nm</td>
</tr>
<tr>
<td>Run time</td>
<td>5 min</td>
</tr>
<tr>
<td>Diluent</td>
<td>Methanol</td>
</tr>
<tr>
<td>Oven Temperature</td>
<td>25°C</td>
</tr>
</tbody>
</table>

3.2.1. **RP-HPLC method validation.**

The developed HPLC method was validated as per ICH Q2 (R1) guidelines.

3.2.1.1. **System suitability.**

System suitability was assessed using a sample as six homogenous injections. (Table 5).

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Tailing Factor</th>
<th>Theoretical Plate</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAXA</td>
<td>2.144</td>
<td>406272</td>
<td>1.272</td>
<td>3188.124</td>
<td>--</td>
</tr>
<tr>
<td>DAPA</td>
<td>3.156</td>
<td>1656776</td>
<td>0.925</td>
<td>3702.009</td>
<td>4.865</td>
</tr>
</tbody>
</table>

3.2.1.2. **Linearity.**

Overlay chromatogram of mixture showing linearity of SAXA (8–22 μg/mL) and DAPA (22–44 μg/mL) at 220 nm (Figure 10). The regression data for the calibration curves (n=6) shows a good linearity relationship over the concentration range of 8-22 μg/mL for SAXA (Figure 11) and 16-44 μg/mL for DAPA (Figure 12) in mixture with respect to the peak area. Linearity data of SAXA and DAPA shows %RSD less than 2. (Table 6).

![Overlay chromatogram of mixture showing linearity of SAXA (8–22 μg/mL) and DAPA (22–44 μg/mL) at 220 nm.](image)
Figure 11. Calibration Curve for SAXA.

![Figure 11](image1.png)

Figure 12. Calibration Curve for DAPA.

![Figure 12](image2.png)

Table 6. Linearity data of SAXA and DAPA (n=6).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. (µg/mL)</th>
<th>Peak area Mean±SD</th>
<th>% RSD</th>
<th>Conc. (µg/mL)</th>
<th>Peak area Mean±SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>8</td>
<td>202156±4410</td>
<td>1.18</td>
<td>16</td>
<td>834613±3958</td>
<td>0.47</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>252329±1830</td>
<td>0.72</td>
<td>20</td>
<td>1095381±638</td>
<td>0.05</td>
</tr>
<tr>
<td>3.</td>
<td>12</td>
<td>297284±5386</td>
<td>1.81</td>
<td>24</td>
<td>1273055±9887</td>
<td>0.77</td>
</tr>
<tr>
<td>4.</td>
<td>14</td>
<td>344760±1398</td>
<td>0.40</td>
<td>28</td>
<td>1463528±2191</td>
<td>0.14</td>
</tr>
<tr>
<td>5.</td>
<td>16</td>
<td>405429±2821</td>
<td>0.69</td>
<td>32</td>
<td>1691868±30634</td>
<td>1.81</td>
</tr>
<tr>
<td>6.</td>
<td>18</td>
<td>442463±1163</td>
<td>1.62</td>
<td>36</td>
<td>1843934±3596</td>
<td>0.19</td>
</tr>
<tr>
<td>7.</td>
<td>20</td>
<td>500817±1233</td>
<td>0.24</td>
<td>40</td>
<td>2122818±7747</td>
<td>0.36</td>
</tr>
<tr>
<td>8.</td>
<td>22</td>
<td>556461±4073</td>
<td>0.73</td>
<td>44</td>
<td>2305784±8416</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Linearity Equation

SAXA:

\[ y = 25112x - 1464.6 \]

\[ R^2 = 0.9984 \]

DAPA:

\[ y = 51715x + 27412 \]

\[ R^2 = 0.9975 \]

3.2.1.3. Precision.

The %RSD value of each concentration for intraday and interday precision for SAXA and DAPA was found NMT 2%. This value shows that the developed method was precise.
3.2.1.4. Repeatability.

The six-replicate injection for both the drugs was injected, and the chromatogram is recorded for the same concentration. The relative standard deviation was found to be NMT 2%, indicating that the method is repeatable.

3.2.1.5. Accuracy.

Accuracy was performed, and % Recovery was found to be within 98-102% at all three levels. This indicates that the SAXA (Table 7) and DAPA (Table 8) can be recovered successfully in presence of excipients.

Table 7. Accuracy data of SAXA (n=3).

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Sample Conc. (µg/mL)</th>
<th>Conc. of Std added (µg/mL)</th>
<th>Total Conc. (µg/mL)</th>
<th>Peak Area</th>
<th>Mean Peak Area¹</th>
<th>Amt. Recovered (µg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>443546</td>
<td>445178</td>
<td>17.78</td>
<td>98.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>445864</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>446126</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>484208</td>
<td>491868</td>
<td>19.64</td>
<td>98.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>499315</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>492083</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>556748</td>
<td>556454</td>
<td>22.21</td>
<td>100.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>556740</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>555876</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Mean area of n=3.

Table 8. Accuracy data of DAPA (n=3).

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Sample Conc. (µg/mL)</th>
<th>Conc. of Std added (µg/mL)</th>
<th>Total Conc. (µg/mL)</th>
<th>Peak Area</th>
<th>Mean Peak Area¹</th>
<th>Amt. Recovered (µg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>20</td>
<td>16</td>
<td>36</td>
<td>1854992</td>
<td>1830516</td>
<td>35.86</td>
<td>99.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1822824</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1813732</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>2097835</td>
<td>2085420</td>
<td>39.79</td>
<td>99.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2072219</td>
<td></td>
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<td></td>
<td></td>
<td>2086205</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>20</td>
<td>22</td>
<td>44</td>
<td>2243201</td>
<td>2254911</td>
<td>44.07</td>
<td>100.15</td>
</tr>
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<td>2280277</td>
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<td></td>
<td></td>
<td>2241254</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Mean area of n=3.

3.2.1.6. LOD and LOQ.

The LOD and LOQ of the drugs were calculated using the following equation.
LOD = 3.3 σ/S
LOQ = 10 σ/S
where, σ = standard deviation of the response, S = slope of the calibration curve.

3.2.1.7. Robustness.

The robustness of the developed method was checked by varying flow rates (±0.1mL/min) and wavelength (±2 nm). The results show that % RSD values are less than 2%

3.2.1.8. Analysis of developed formulation.

The results of the assay were found to be within of label claim for formulations containing SAXA and DAPA, as shown in Table 9.
Table 9. Assay data for SAXA and DAPA (n=3).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (mg)</th>
<th>Sample Conc. (µg/mL)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>400980</td>
</tr>
<tr>
<td>SAXA</td>
<td>5</td>
<td>16</td>
<td>405403</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>406978</td>
</tr>
<tr>
<td>% Assay ± SD</td>
<td></td>
<td>101.02± 3109</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>DAPA</td>
<td>10</td>
<td>32</td>
<td>1705610</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1701686</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1711731</td>
</tr>
<tr>
<td>% Assay ± SD</td>
<td></td>
<td>101.45± 5062</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Discussion.

In the development of the RP-HPLC method for estimating SAXA and DAPA in tablet formulation, it was found that the mobile phase composition of phosphate buffer and acetonitrile (55:45) was more appropriate for the simultaneous estimation of both the drugs, and the volume of acetonitrile resulted in a major change in retention time. These optimal conditions were achieved baseline separation of both drugs with a reasonable resolution and run time of less than 5 min. The HPLC process has been approved according to Q2(R1) of the ICH Guidelines. The percentage of assay results for combination drugs is found to be within approval requirements (98-102 percent), result summary shown in Table 9.

UV method was based on the measurement of absorbance of Saxagliptin Hydrochloride and Dapagliflozin in methanol at 210 nm and 224 nm, respectively. The linearity range was selected 6-22 µg/mL and 12-44 µg/mL for Saxagliptin Hydrochloride and Dapagliflozin, respectively. The validation parameters were performed according to the ICH guidelines Q2(R1). The precision results, expressed by intra-day and inter-day for both the drugs, were % RSD < 2 %.

4. Conclusions

This research involves systematically developing a simple, fast, accurate, and cost-effective UV spectrophotometric method for the simultaneous estimation of SAXA and DAPA. Better percentage recoveries and proper validation showed the appropriateness and efficiency of the proposed analytical procedures. Consequently, this approach can be implemented successfully for the routine consists of dosage form analysis comprising SAXA and DAPA. The RP-HPLC method optimized the resolution as a response in a relatively short time (5 min) between SAXA and DAPA. All system suitability parameters are observed in optimal condition, and it was found with the range for SAXA and DAPA estimation. In addition, the validation analysis helped identify the best conditions by verifying that the approach was selective, specific, accurate, linear, precise, and robust. The percentage assay values for formulations research were found to be between 98-102%. The methods developed have been validated in accordance with ICH Q2 (R1) guidelines. In conclusion, simple, selective, sensitive, and accurate UV Spectroscopic and RP-HPLC methods have been developed and validated in agreement with ICH guidelines Q2 (R1) for the routine quality of the dosage form analyzes containing SAXA and DAPA. This method developed satisfies the guidelines of ICH Q2 (R1) and is appropriate for regulatory submission under regulatory flexibility.
Funding

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Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

References

12. Maher, H.M.; Abdelrahman, A.E.; Alzoman, N.Z.; Aljohar, H.I. Stability-indicating capillary electrophoresis method for the simultaneous determination of metformin hydrochloride, saxagliptin hydrochloride, and


