**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 12 [11] October 2023 :184-195

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

**ORIGINAL ARTICLE** **OPEN ACCESS**

# Development of Chemometric Aided RP- HPLC Assay Approach for the Concurrent Determination of Saxagliptin Hydrochloride and Dapagliflozin Propanediol Monohydrate in Combined Dosage Form

**Nikita Shaileshbhai Patel[1*], Bhavesh Hirabhai Patel[2], Mona Kaushal[1]**
[1]Department of Pharmaceutical Quality Assurance and Chemistry, Arihant School of Pharmacy and Bio-Research Institute, Adalaj, Gandhinagar-382421, Gujarat, India.
[2]Department of Pharmaceutical Chemistry, K. B. Institute of Pharmaceutical Education and Research, Sector-23, Gandhinagar-382023, Gujarat, India.
Corresponding Author: Ms. Nikitaben Shaileshbhai Patel
Email ID: niki.pharma1990@gmail.com

**ABSTRACT**

*The peak area for multiwavelength detector responses was tested employing the PLS (partial least squares)- chemometric assessment approach. HPLC-PLS is the abbreviation for the combined use of HPLC and chemometric assessment approach. For purposes of comparison, the combined HPLC-PLS results have been validated using the HPLC technique known as the classical-HPLC method. A new chemometric approach using high- performance liquid chromatography with photodiode array detection was developed and utilized for the concurrent determination of Saxagliptin Hydrochloride and Dapagliflozin Propanediol Monohydrate in its combined dosage form. Using column C18 Kromasil, with dimensions of 250 mm*4.60 mm and particle sizes of 5 microns, and a mobile phase made up of 10 mM phosphate buffer (pH-4.5) and acetonitrile (47:53 %v/ v), a satisfactory chromatographic separation between two medicaments was carried out. Five different analytical wavelengths (220nm, 230nm, 240nm, 250nm and 260nm) were selected for the chemometric method (PLS method) for determining errors of regression at multiple wavelengths and Peak area for the concentration level was measured. The developed method resulted from Saxagliptin and Dapagliflozin eluting at 3.15 min and 5.35 min respectively. Saxagliptin and Dapagliflozin were both shown to be linear across the concentration ranges of 2–12 µg/mL and 4–24 µg/mL, respectively. The percentage recovery was discovered in between 98 to 102%. HPLC-PLS enables the elimination or reduction of calibration errors and residuals from the conventional HPLC depended on just a single wavelength. As a result, the HPLC performs better while analysing complicated mixtures.*

**Keywords:** Dapagliflozin, Saxagliptin, RP-HPLC, Chemometric, Partial Least Square, HPLC-PLS

Received 24.07.2023 Revised 21.08.2023 Accepted 27.10.2023

## INTRODUCTION

A chronic metabolic illness called diabetes mellitus causes hyperglycaemia, or raised blood sugar levels, which is normally companioned by insulin resistance. Uncontrolled production of glucose by the liver and dropped skeletal muscle glucose immersion along with dropped glycogen synthesis results in hyperglycaemia. [1]. 90 – 95 of cases of diabetes are type 2 diabetes, also referred as" adult- onset diabetes" or as" noninsulin dependent diabetes". This group includes those with relative insulin insufficiency and peripheral insulin resistance [2]. Antidiabetic medications have been evolved to control and downgrade blood sugar level in diabetics [3]. Hyperglycaemia is significantly linked to the progression of type 2 diabetes, which increases the risk of myocardial infarction, stroke, microvascular events, and mortality[4]. Because type 2 diabetes is a progressive form, patients frequently need a combination of antidiabetic medications to attain and maintain glycaemic control[5]. Keeping blood sugar levels under control seeks to prevent hyperglycaemia's acute osmotic symptoms, to avoidance of long-term blood glucose instability and delaying or preventing the onset of diabetic complications without compromising quality of life [6]. Chemically, Dapagliflozin is referred to as (1S)-1,5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl]phenyl]-D-glucitol as illustrated in Figure 1. Its molecular weight is 408.98 g/mol and its chemical formula is C24H33ClO8. The sodium glucose co-transporter 2 is specifically blocked by Dapagliflozin

Propanediol Monohydrate. It enhances glycemic control in people having type 2 diabetes by stopping kidneys from absorbing again glucose, causing the excess to be discharged in the urine [7]. Chemically, Saxagliptin is reffered to as (1S, 3S, 5S)-2[(2S)-2-amino-2-(3-hydroxy-1- adamantyl)acetyl]-2-azabicyclohexane-3-carbonitrile) as illustrated in Figure 2. Its molecular weight is 315.41 g/mol and its chemical formula is C18H25N3O2[8]. For people with type 2 diabetes mellitus (T2DM), Saxagliptin, a DPP-4 inhibitor, has been recommended as a supplement to diet and exercise to enhance glycemic control. Once daily Saxagliptin dosing before breakfast in T2DM patients reduces postprandial hyperglycemia, including after dinner, and results in prolonged suppression of plasma DPP-4 activity. This is accompanied by an elevation in plasma GLP-1 levels[9]. Chemometrics is the use of statistical and mathematical approaches to produce additional knowledge from chromatographic data. Chemometrics, in accordance to the International Chemometrics Society, is a science that uses mathematical or statistical methods to connect assessments taken from a chemical system or process to the system's condition[11]. Chemometric techniques have a wide range of uses in analytical chemistry, and recent developments in spectroscopy, chromatography, and other areas of analytical chemistry have revealed new uses for these techniques[12]. The method has been applied to pharmaceutical solid dosage forms for quality assurance and quality control [13]. Because it offers accurate, sensitive and consistent quantitative analysis of substances, HPLC is currently the method of choice for the research of various components in pharmaceutical formulations. To create linear regression functions, the traditional HPLC approach uses the peak area measured at a only one wavelength. When working with a single wavelength detector response, traditional HPLC gives us various chromatographic area discrepancies due to the injection, instrument fluctuations, and other variables. Each of these factors has an impact on the outcome of an analysis. Recently, chemometric calibration approaches were applied to assess the analytical data acquired from a variety of equipment. Combining multiwavelength HPLC technology with chemometric calibration methods removes or reduces the unfavourable condition of applying only one regression function that uses a single wavelength[14]. A review of the literature discovered that many approaches have been described for concurrent estimation of SAXA and DAPA by UV Spectrophotometric methods[15- 18], RP- HPLC methods[19- 28], RP- UPLC system[29], stability- indicating assay methods[10, 30-33], Quality by Design[34], LC-MS/MS method[35-36] and chemometric assisted UV system[37]. To our knowledge, no reports of the SAXA and for combined RP-HPLC chemometric test method have been made to yet. Our study's major goal is to use partial least square chemometric calibration on a multiwavelength collection of multivariate chromatographic data.

## MATERIAL AND METHODS

### Chemicals and reagents

Morepen Laboratories Ltd., Baddi supplied pure medicine samples of the medications SAXA and DAPA. We purchased Qtern® 5 mg/10 mg tablets (AstraZeneca AB) from the local market. These tablets consist of Dapagliflozin Propanediol Monohydrate 12.3 mg, which is equal to Dapagliflozin 10 mg, and Saxagliptin Hydrochloride 5.95 mg, which is equal to Saxagliptin 5 mg. Acetonitrile (HPLC grade), Ortho-phosphoric acid AR, and Potassium dihydrogen orthophosphate AR were provided by Merck. Water (HPLC grade) was provided by Rankem.

### Instrumentation

Shimadzu HPLC autosampler SIL-40/ SIL-40C equipped with LC 40D Pump and SPD-M20A PDA sensor was utilised. The injection volume for samples were 20µl. It was done by using C18 Kromasil column having dimension of 250*4.60 mm and 5 micron particle size. Lab Solution software was used to do data acquisition and integration.

### Preparation of mobile phase

A 1000 mL volumetric flask was filled with precisely weighed 1.36 g of Potassium Dihydrogen Phosphate, and 950 mL of water was then added. The mixture was sonicated and finally diluted upto the mark with water. Then pH 4.5 was adjusted with Ortho-phosphoric Acid. To eliminate contaminants, this prepared buffer (10 mM) was passed on 0.45 micron filter paper. The buffer was mixed with Acetonitrile in the ratio of 47: 53 (% v/v) into a mobile phase bottle. For 5 mins, the prepared mixture was sonicated in the ultrasonic bath for degassing and then used as the mobile phase.

### Preparation of Standard stock solutions

Dapagliflozin Propanediol Monohydrate (12.3 mg, equal to 10 mg DAPA) as well as Saxagliptin Hydrochloride (11.9 mg, equal to 10 mg SAXA) were precisely weighed and added to separate 100 mL volumetric flasks. The drug was dissolved in Acetonitrile with sonication and sufficient Acetonitrile was added to the final volume to make a 100 µg/mL stock solution.

### Preparation of solutions for the development of the calibration curve

Aliquots ranging from 0.2 mL to 1.2 mL and 0.4 mL to 2.4 mL for SAXA and DAPA, respectively were taken

in 10 mL volumetric flasks from standard stock solutions and a sufficient amount of mobile phase was added, then the volume was made up to 10 mL with mobile phase to obtain final concentrations 2, 4, 6, 8, 10, 12 µg/mL and 4, 8, 12, 16, 20, 24 µg/mL of SAXA and DAPA, respectively as the standard solutions of binary mixtures.

**Preparation of sample solution**

Twenty tablets were precisely weighed and finely pulverised. Accurately weighed powder containing 5 mg of SAXA and 10 mg of DAPA was put into a 100 mL volumetric flask, and then enough acetonitrile has been added to dissolve the medication. The final volume was adjusted with Acetonitrile after it was ultrasonically processed for 20 minutes. To get a stock sample solution, the solution was filtered with Whatman filter paper (No. 42). An aliquot of 1.6 mL from a stock sample solution was pipetted out and diluted up to 10 mL with diluent to attain a final concentration of 8 µg/mL SAXA and 16 µg/mL of DAPA. These sample solutions were assayed as per the proposed method and the % assay was calculated.

## EXPERIMENTAL

**Method development and optimization**

The mixture of solution with a concentration range of 2–12 µg/mL of SAXA and 4–24 µg/mL of DAPA was used as the concentration set. Five wavelengths (220, 230, 240, 250 and 260 nm) were used to capture the concentration set's peak area for the chemometric method (PLS method) for determining errors of regression at multiple wavelengths and at the retention time of 3.15 for SAXA and 5.35 for DAPA. As shown in Figure 3-6, the chromatogram of an optimised method for standard medications (8 µg/mL of SAXA and 16 µg/mL of DAPA) separately and in binary mixture, as well as for a sample at wavelength 240 nm. The chromatograms of the binary combination having 4 µg/mL SAXA and 8 µg/mL DAPA at different wavelengths are shown in Figures 7-11. Overlain Chromatogram of the binary mixture of 4 µg/mL of SAXA and 8 µg/mL of DAPA at different wavelengths is shown in Figure 12. Tables 1 and 2 exhibit the HPLC data set associated with the binary mixture's concentration set. The calibration curves were plotted using concentration Vs peak area in the binary mixture at different wavelengths and the regression equation derived are shown in Figures 13 and 14. The produced concentration series and corresponding measured HPLC data set were applied to the chemometric calibration procedure, PLS. The developed HPLC-PLS calibrations were used to estimate the concentrations of SAXA and DAPA within samples.

**Classical HPLC**

Plotting the chromatograms that correspond to the concentration ranging from 2–12 µg/mL SAXA and 4–24 µg/mL DAPA was done using a PDA detector that operates at five wavelengths and peak area was determined. A stainless-steel column, the $C_{18}$ Kromasil, measuring 150 mm* 4.60 mm and having 5 µm-sized particles was used to develop the chromatographic separation at ambient temperature. The mobile phase was composed of 10 mM Phosphate buffer (pH- 4.5), Acetonitrile and it was a mixture of 47: 53 v/v of each. The mobile phase's flow rate was held constant at 1.0 mL/min.

The same conditions were used for the HPLC-PLS. The peak area values for SAXA and DAPA, respectively, for the five-wavelength set of 220, 230, 240, 250, and 260 nm are shown in Tables 1 and 2 and five straight lines for both drugs were generated. As illustrated in Figures 13 and 14, the calibration was done by plotting the analyte peak area versus the SAXA and DAPA concentration. Regression equations were discovered to have correlation coefficients higher than 0.99. For the SAXA and DAPA analysis process, two equations with highest coefficients of regression at 240 nm were selected from the generated calibration equations. SAXA and DAPA had good separation and resolution under optimised conditions, and their usual retention times were approximately 3.15 min and 5.35 min, respectively. The calibration formula provides good linearity and reliable outcomes for SAXA and DAPA at the wavelength point under test. As illustrated in Figures 3-6, chromatogram of a method that has been optimised for standard medicines in binary combination and as individual compounds.

## RESULTS

**Method Validation**

Based on how well they performed in providing precise analytical data, the efficacy of both methods were assessed. To achieve this, an accuracy study was performed by adding a known amount of SAXA ranging from 2-12 µg/mL and DAPA ranging from 4-24 µg/mL to a known concentration of tablet formulation having 8 µg/mL of SAXA and 16 µg/mL of DAPA and the recovery of added amount of drug was determined by developed calibration method. The mean recovery and %RSD of our suggested approaches were calculated and shown in Table 3. For the evaluation of both medicines, these approaches showed consistent accuracy and increased precision. Interference and systemic mistakes were not seen during the analysis process.

**Statistical Calculations for the calibration techniques**

Based on the correlation between the actual and predicted levels during the calibration and prediction stages, respectively, the suggested HPLC-multivariate calibrations were evaluated for their effectiveness on the variables defined by the standard errors of calibration and prediction. Table 4 summarises the statistical findings after using least-squares linear regression modelling to the actual and anticipated concentrations. As shown in Table 4, significant correlation coefficients among the actual and anticipated concentrations were found during the HPLC-PLS calibration application, showing good precision as well as accuracy.

**Assay of marketed sample**

SAXA and DAPA in tablet were quantitatively analysed using HPLC-PLS and traditional HPLC methods. The two drugs combined dosage form Qtern® 5 mg/10 mg tablets (AstraZeneca AB) is purchased from the local pharmacy. Three replicates were performed for the analysis. Each medication's average peak area was computed, and the amount of drug within the sample was measured. The outcomes are presented in Table 5. Both methods produced results that were extremely close to the stated value of a commercial pharmaceutical dose forms. Both used approaches showed good agreement.

**Results Evaluation by Statistical Tests**

T-test was used for the significant differences between the two methods and results are displayed in Table 6. The calculated statistical values did not exceed theoretical statistical values, proving that there was no discernible difference between two procedures. The results of all statistical tests' numerical findings showed that the methodologies under investigation are appropriate for identifying both substances in pharmaceutical dosage forms. Both techniques can be effectively applied to commercial formulations because the determined statistically P-value did not surpass the theoretical P-value, indicating that there is no discernible distinction between the two approaches.

## DISCUSSION

The introduction of multiwavelength PDA sensors to the HPLC systems makes achievable concurrent chromatographic finding of samples at multiwavelength. The obtained multiwavelength detections yield different peak area information. These HPLC results can now be analysed quantitatively with concurrent data gathering at many wavelengths because of the addition of multivariate calibration processes. the concurrent quantitative measurement of SAXA and DAPA in samples, a novel idea is the use of multivariate method PLS to the acquired chromatogram exhibits better absorption and meeds good peak separation in chromatograms. The identical data processing is required for HPLC-chemometric calibrations as it is for HPLC calculations using a single wavelength. The traditional HPLC approach that utilises one wavelength of detection response was utilised as well for the testing of the combinations of both drug samples in order to compare these HPLC-PLS. The experimental findings from the HPLC-PLS approach were compared with the results from the classical- HPLC method. Both techniques were utilized for the concurrent quantitative analysis of SAXA and DAPA in tablets.

**Table 1: Linearity data for Saxagliptin at different wavelength (n=5)**

| Saxagliptin Conc. (µg/mL) | Wavelength | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 220 nm | | 230 nm | | 240 nm | | 250 nm | | 260 nm | |
| | Area ± SD | % RSD | Area ± SD | % RSD | Area ± SD | % RSD | Area ± SD | % RSD | Area ± SD | % RSD |
| 2 | 102283± 1319.45 | 1.29 | 115832 ± 903.49 | 0.78 | 122465 ± 1016.46 | 0.83 | 101135 ± 1203.51 | 1.19 | 112582± 1103.30 | 0.98 |
| 4 | 228901± 2701.03 | 1.18 | 238439± 2527.45 | 1.06 | 233990 ± 2714.28 | 1.16 | 213384 ± 1536.36 | 0.72 | 210924 3206.04 | 1.52 |
| 6 | 317289 ± 3157.35 | 0.96 | 326739± 4018.89 | 1.23 | 347291 ± 5313.55 | 1.53 | 328891 ± 2927.13 | 0.89 | 312289 4309.59 | 1.38 |
| 8 | 422847± 6004.43 | 1.42 | 409274± 4624.80 | 1.13 | 455902 ± 5789.96 | 1.27 | 412259 ± 5606.72 | 1.36 | 423368± 7239.59 | 1.71 |
| 10 | 519029± 8667.78 | 1.67 | 528847± 7721.17 | 1.46 | 563927 ± 6315.98 | 1.12 | 511390 ± 7261.74 | 1.42 | 510923 6795.28 | 1.33 |
| 12 | 622309± 9583.56 | 1.54 | 612902± 8458.05 | 1.38 | 688820 ± 9574.60 | 1.39 | 643902 ± 7791.21 | 1.21 | 634902 11618.71 | 1.83 |

**Table 2: Linearity data for Dapagliflozin at different wavelength (n=5)**

| Dapagliflozin Conc. (µg/mL) | Wavelength | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 220 nm | | 230 nm | | 240 nm | | 250 nm | | 260 nm | |
| | Area ± SD | % RSD | Area ± SD | % RSD | Area ± SD | % RSD | Area ± SD | % RSD | Area ± SD | % RSD |
| 4 | 312692± 2001.23 | 0.64 | 322190 ± 2351.99 | 0.73 | 366654 ± 2163.26 | 0.59 | 334902 ± 2444.78 | 0.73 | 322230 ± 1482.26 | 0.46 |
| 8 | 611289± 7152.08 | 1.17 | 634092 ± 9701.61 | 1.53 | 645807 ± 10397.49 | 1.61 | 639392 ± 8184.22 | 1.28 | 611293 ± 6968.74 | 1.14 |
| 12 | 954733± 12316.06 | 1.29 | 982371 ± 16503.83 | 1.68 | 991234 ± 12291.30 | 1.24 | 922837 ± 10704.91 | 1.16 | 1050342± 14074.58 | 1.34 |
| 16 | 1289342± 13667.03 | 1.06 | 1209386± 15963.90 | 1.32 | 1294408± 14109.05 | 1.09 | 1209381± 16568.92 | 1.37 | 1200283± 20044.73 | 1.67 |
| 20 | 1633809± 23200.09 | 1.42 | 1666278± 21161.73 | 1.27 | 1588823 ± 17953.70 | 1.13 | 1539808 24021.00 | 1.56 | 1522891± 19493.00 | 1.28 |
| 24 | 1822732± 30439.62 | 1.67 | 1902883± 29684.97 | 1.56 | 1920394± 27269.59 | 1.42 | 1902281± 28343.99 | 1.49 | 1822818± 21693.53 | 1.19 |

**Table 3: Recovery data obtained by proposed methods**

| Amount Added Conc. (µg/mL) | | Amount Recovered Conc. (µg/mL) | | | | % Recovery | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Classical HPLC | | HPLC-PLS | | Classical HPLC | | HPLC-PLS | |
| SAXA | DAPA | SAXA | DAPA | SAXA | DAPA | SAXA | DAPA | SAXA | DAPA |
| 2 | 16 | 1.96 | 15.83 | 1.97 | 15.9 | 98.11 | 98.93 | 98.50 | 99.38 |
| 4 | 16 | 3.96 | 15.69 | 3.97 | 15.87 | 98.95 | 98.04 | 99.25 | 99.19 |
| 6 | 16 | 5.94 | 15.84 | 5.93 | 15.92 | 99.02 | 99.03 | 98.83 | 99.50 |
| 8 | 16 | 8.01 | 15.90 | 7.92 | 15.97 | 100.18 | 99.38 | 99.00 | 99.81 |
| 10 | 16 | 9.92 | 15.80 | 9.87 | 15.86 | 99.25 | 98.73 | 98.70 | 99.13 |
| 12 | 16 | 11.89 | 15.93 | 11.9 | 16.04 | 99.12 | 99.58 | 99.17 | 100.25 |
| 8 | 4 | 7.86 | 4.01 | 7.9 | 3.97 | 98.24 | 100.13 | 98.75 | 99.25 |
| 8 | 8 | 7.88 | 7.94 | 8.07 | 8.14 | 98.47 | 99.29 | 100.88 | 101.75 |
| 8 | 12 | 7.84 | 12.00 | 8.1 | 11.93 | 98.04 | 100.04 | 101.25 | 99.42 |
| 8 | 16 | 7.94 | 15.86 | 7.97 | 16.06 | 99.26 | 99.14 | 99.63 | 100.38 |
| 8 | 20 | 7.97 | 19.90 | 7.93 | 19.66 | 99.57 | 99.49 | 99.13 | 98.30 |
| 8 | 24 | 7.86 | 24.09 | 7.89 | 23.67 | 98.26 | 100.39 | 98.63 | 98.63 |
| Average | | | | | | 98.87 | 99.35 | 99.31 | 99.58 |
| SD | | | | | | 0.660 | 0.651 | 0.879 | 0.901 |
| %RSD | | | | | | 0.667 | 0.656 | 0.885 | 0.905 |

Table 4: Statistical Calculations for the proposed methods

| Parameter | Classical HPLC | | HPLC-PLS | |
|---|---|---|---|---|
| | SAXA | DAPA | SAXA | DAPA |
| SEP (Standard Error of Prediction) | 0.7746 | 0.5239 | 0.4433 | 0.2013 |
| SEC (Standard Error of Calibration) | 0.4382 | 0.3892 | 0.2281 | 0.1672 |
| Intercept | 9046.1 | 44461 | 8378.5 | 53332 |
| Slope | 56146 | 77864 | 60258 | 92367 |
| Regression Coefficient | 0.9996 | 0.9994 | 0.9999 | 0.9999 |

**Table 5: Assay of Tablet (n=6)**

| Qtern® 5 mg/10 mg tablets (SAXA: 5 mg and DAPA: 10 mg) | | |
|---|---|---|
| Drug | % ASSAY (mean ± %RSD) | |
| | Classical HPLC | HPLC-PLS |
| SAXA | 98.12 ± 0.698 | 99.25 ± 0.834 |
| DAPA | 98.45 ± 0.594 | 99.56 ± 0.926 |

**Table 6: Comparison of Proposed method's result by t-test**

| Parameter | Saxagliptin | | Dapagliflozin | |
|---|---|---|---|---|
| | Classical HPLC | HPLC-PLS | Classical HPLC | HPLC-PLS |
| **Mean** | 98.87 | 99.31 | 99.35 | 99.58 |
| **Std Deviation** | 0.66 | 0.879 | 0.651 | 0.901 |
| **P-Value (Calculated)** | 0.1217 | | 0.2528 | |
| **P-Value (Theoretical)** | 1.796 | | | |

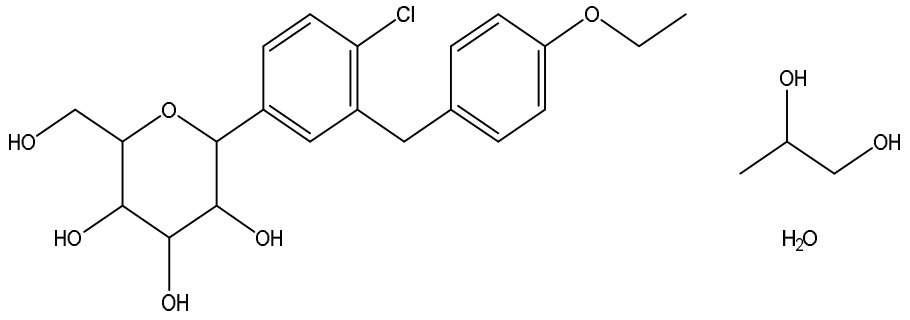


Figure 1: Dapagliflozin Propanediol Monohydrate

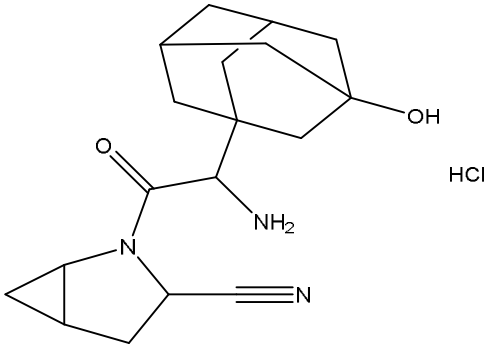


Figure 2: Saxagliptin Hydrochloride

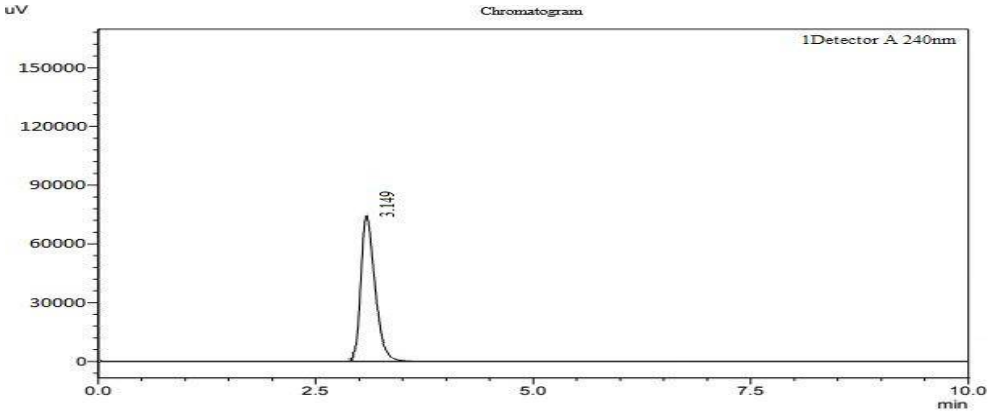


**Figure 3: Chromatogram of Saxagliptin at 240 nm (SAXA-8 µg/mL)**

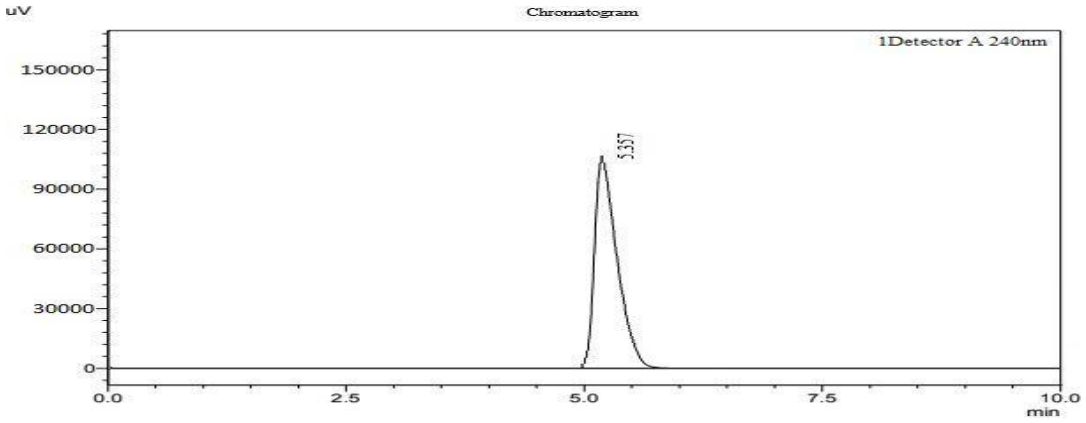


**Figure 4: Chromatogram of Dapagliflozin at 240 nm (DAPA-16 µg/mL)**

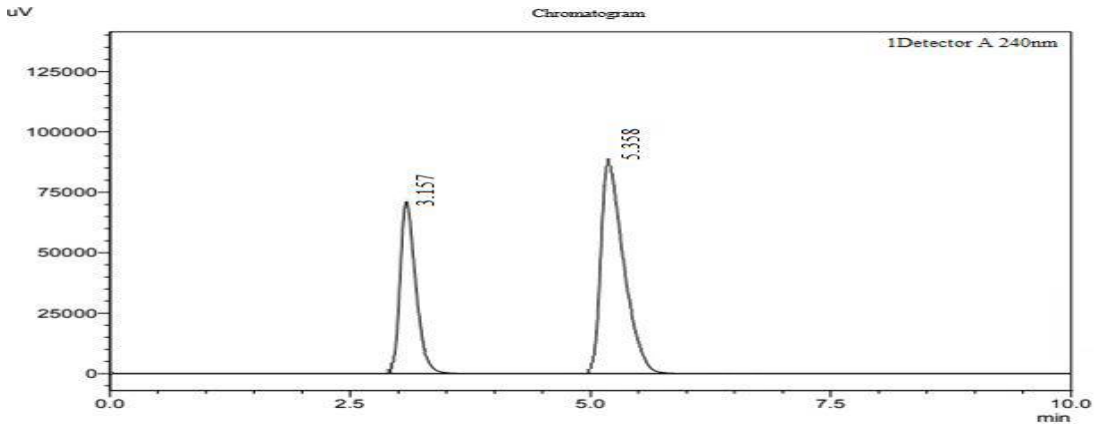


**Figure 5: Chromatogram of Sample at 240 nm (SAXA-8 µg/mL and DAPA-16 µg/mL)**

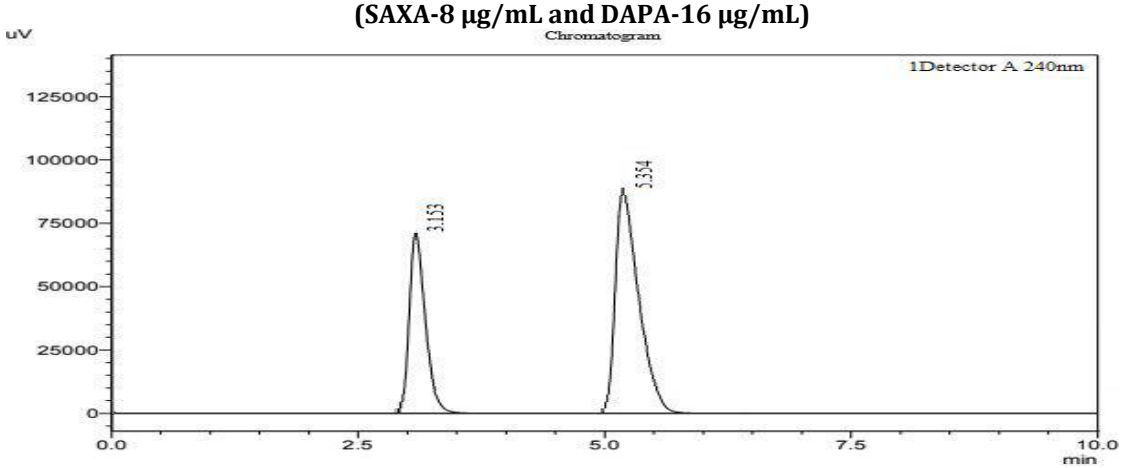


Figure 6: Chromatogram of binary mixture at 240 nm (SAXA-8 µg/mL and DAPA-16 µg/mL)

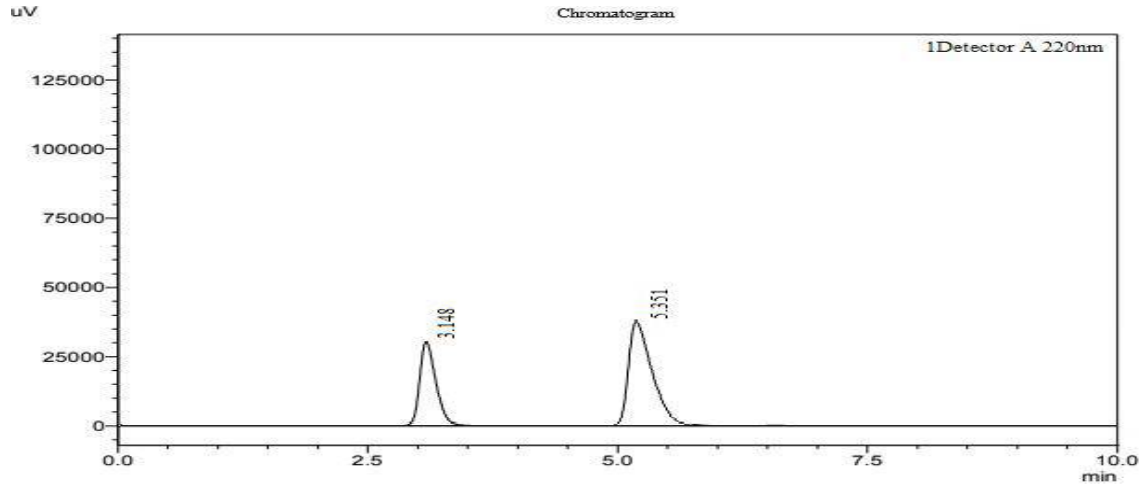


**Figure 7: Chromatogram of binary mixture at 220 nm (SAXA-4 µg/mL and DAPA-8 µg/mL)**

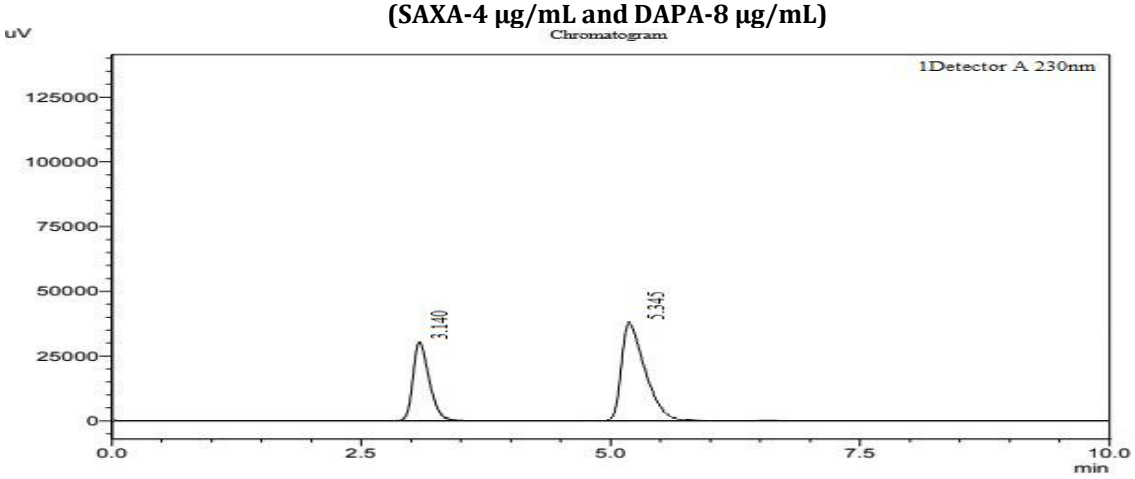


**Figure 8: Chromatogram of binary mixture at 230 nm (SAXA-4 µg/mL and DAPA-8 µg/mL)**

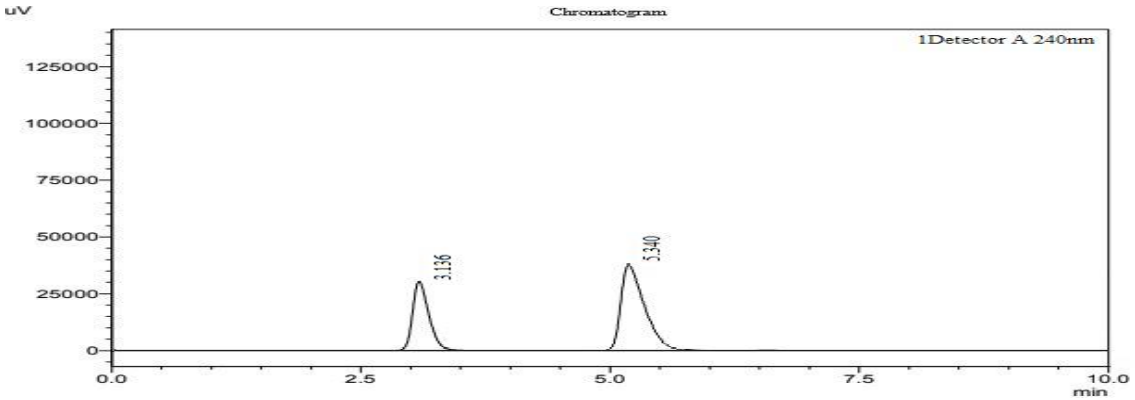


**Figure 9: Chromatogram of binary mixture at 240 nm (SAXA-4 µg/mL and DAPA-8 µg/mL)**

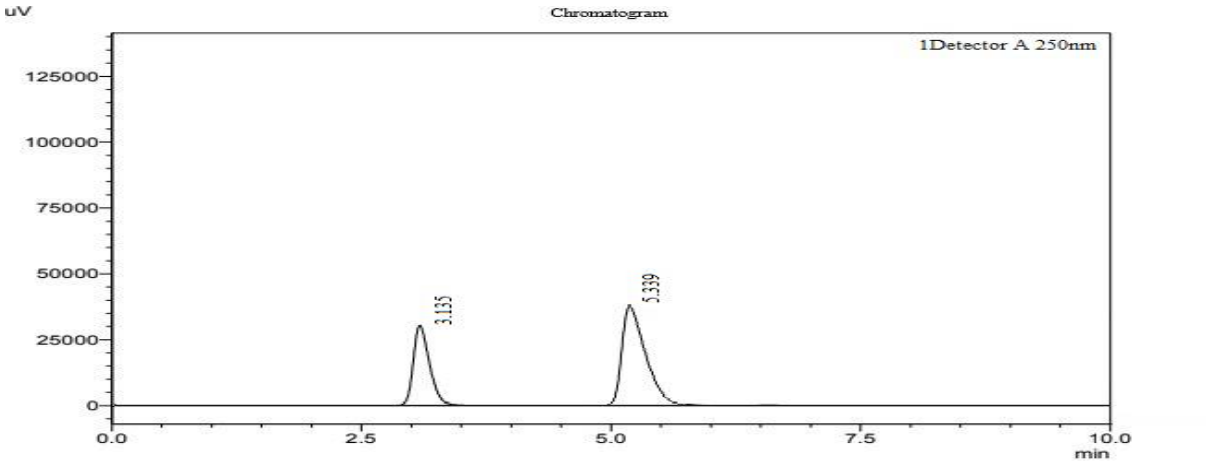


**Figure 10: Chromatogram of binary mixture at 250 nm (SAXA-4 µg/mL and DAPA-8 µg/mL)**

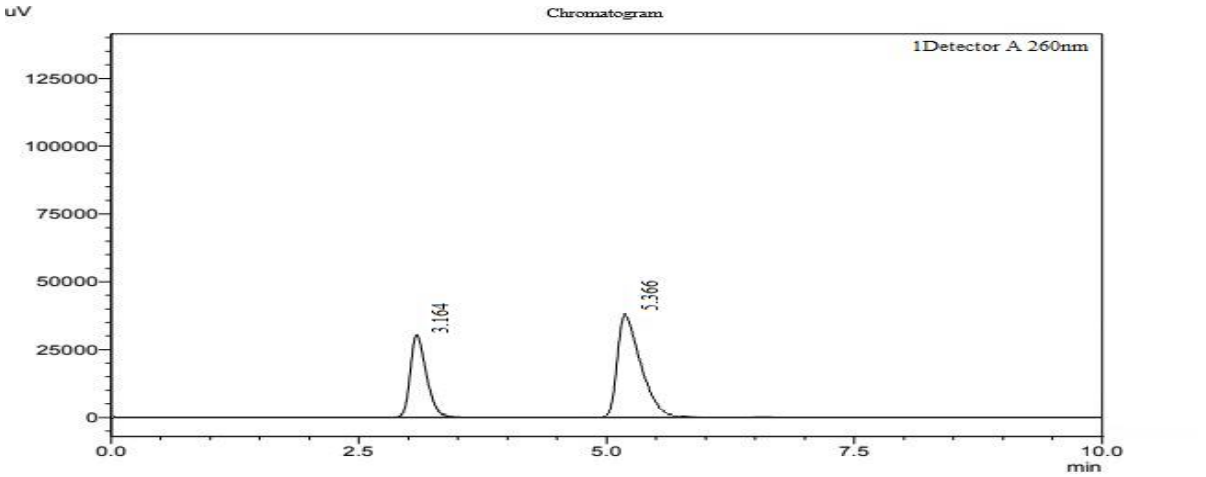


**Figure 11: Chromatogram of binary mixture at 260 nm (SAXA-4 µg/mL and DAPA-8 µg/mL)**

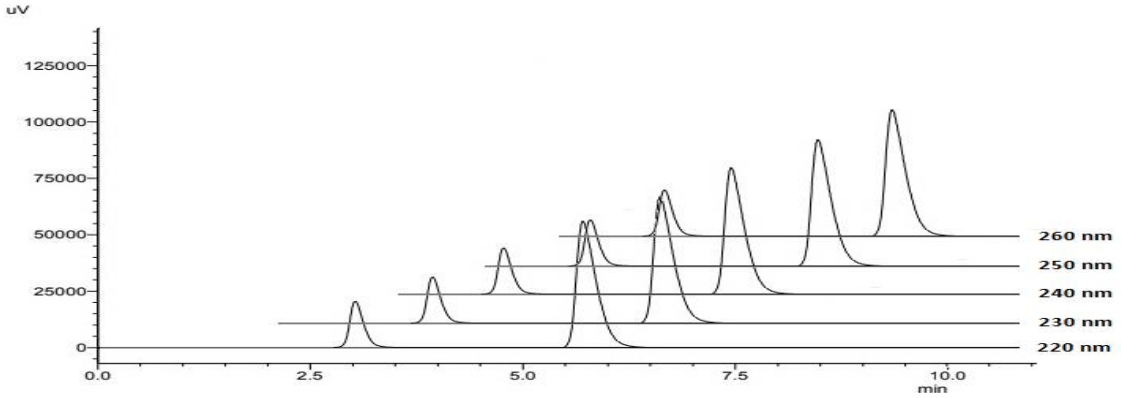


Figure 12: Overlain Chromatogram of binary mixture at different wavelengths (SAXA-4 µg/mL and DAPA-8 µg/mL)

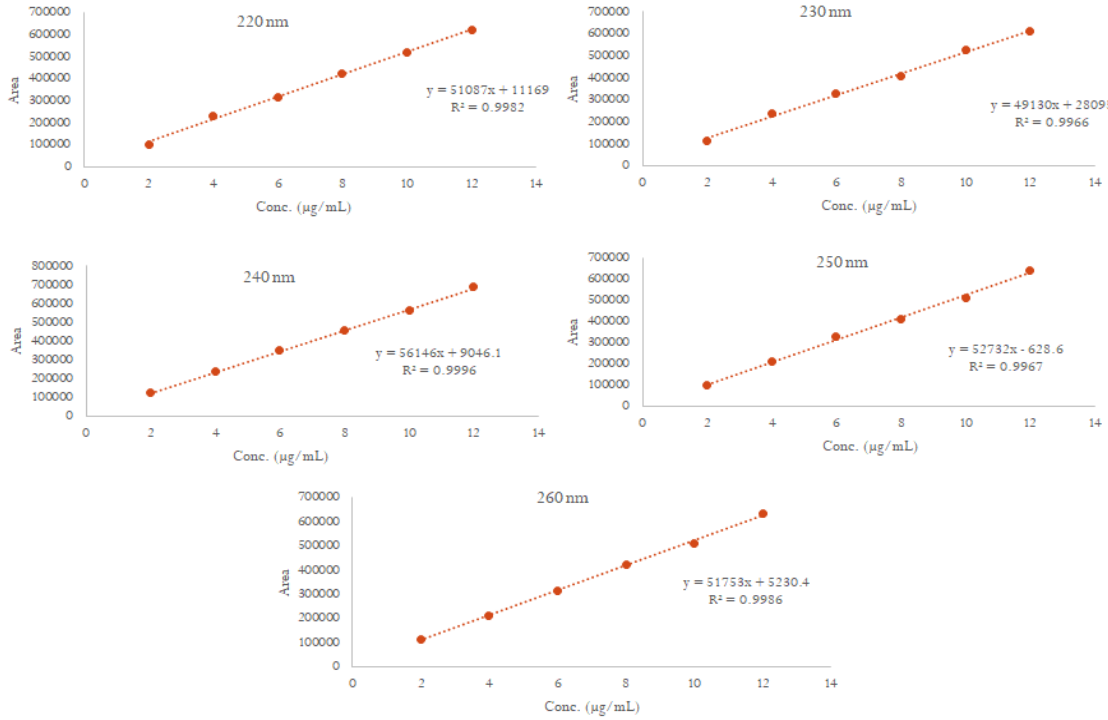


**Figure 13: Calibration Curve of Saxagliptin (2-12 µg/mL) at different wavelengths**

## CONCLUSION

Till today there is no chemometric assay method (HPLC-PLS) for the concurrent estimation of SAXA and DAPA in combined dosage form. For the concurrent determination of SAXA and DAPA in combination dose form, a straightforward, affordable, and cost-effective HPLC-PLS was developed with outstanding sensitivity. A unique approach for the concurrent quantitative determination of SAXA and DAPA in samples is the application of the multivariate method-PLS to the obtained chromatographic information. With HPLC-PLS, calibration inaccuracies and residuals from conventional HPLC that only uses one wavelength can be removed or reduced. As a result, the HPLC performs better while analysing complicated mixtures.

## LIST OF ABBREVIATIONS

SAXA: Saxagliptin Hydrochloride; DAPA: Dapagliflozin Propanediol Monohydrate; PLS: Partial Least Square; HPLC: High-performance liquid chromatography

## ACKNOWLEDGEMENTS

Thanks to Morepen Laboratories Ltd., Baddi for giving free samples of pure medications, the writers are grateful to them.

**CONFLICTS OF INTEREST:** None

## REFERENCES

1. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Flower, R. J. Rang and Dale's Pharmacology. Elsevier. 2007.
2. American Diabetes Association. (2018). Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. Diabetes care, 41(Supplement_1), S13-S27.
3. Introduction of diabetes mellitus. https://www.diabetes.co.uk/diabetes-medication/antidiabetic-drugs.html [Last accessed on 17 Aug 2020].
4. Fonseca, V. A. (2009). Defining and characterizing the progression of type 2 diabetes. Diabetes care, 32(Suppl 2), S151.
5. Rosenstock, J., Hansen, L., Zee, P., Li, Y., Cook, W., Hirshberg, B., & Iqbal, N. (2015). Dual add-on therapy in type 2 diabetes poorly controlled with Metformin monotherapy: a randomized double-blind trial of Saxagliptin plus dapagliflozin addition versus single addition of Saxagliptin or Dapagliflozin to Metformin. Diabetes care, 38(3), 376-383.
6. Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., Diamant, M., Ferrannini, E., Nauck, M., ... & Matthews, D. R. (2012).

American Diabetes Association (ADA); European Association for the Study of Diabetes (EASD). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes care, 35(6), 1364-1379.

7. Patel, N. S., & Patel, B. H. (2020). Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Dapagliflozin Propanediol and Metformin Hydrochloride in Tablet Dosage Form. Int. j. pharm. sci. drug. res., 12(6), 660-667.
8. Aswini, R., Eswarudu, M. M., & Babu, P. S. (2018). A review on analytical methods for estimation of Dapagliflozin and Saxagliptin in bulk and in pharmaceutical dosage forms. International Journal of Research in Pharmacy and Chemistry, 8(3), 460-468.
9. The Merck Index, RSC publishing, 15th edition
10. Deepan, T., & Dhanaraju, M. D. (2018). Stability indicating HPLC method for the simultaneous determination of Dapagliflozin and Saxagliptin in bulk and tablet dosage form. Curr. Issues Pharm. Medical Sci., 31(1), 39-43.
11. B Bansal, A., Chhabra, V., Rawal, R. K., & Sharma, S. (2014). Chemometrics: A new scenario in herbal drug standardization. J Pharm Anal., 4(4), 223-233.
12. Wiberg, K. Multivariate Spectroscopic Methods for the Analysis of Solutions, Ph. D. Dissertation, Stockholm University SE-106 91 Stockholm, Sweden, 2004.
13. Singh, I., Juneja, P., Kaur, B., & Kumar, P. (2013). Pharmaceutical applications of chemometric techniques. int. sch. res. notices, 1-13.
14. Özdemir, A. (2005). A new application of chemometric techniques to HPLC data for the simultaneous analysis of a two-component mixture, J. Liq. Chromatogr. Relat. Technol., 28 (14), 2179-94.
15. Bhadauria, R. S., & Agarwal, V. (2019). Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in marketed formulation. J. drug deliv. ther., 9(4-s), 1160-1164.
16. Suthar, A. M., Prajapati, L. M., Joshi, A. K., Patel, J. R., & Kharodiya, M. L. (2018). Estimation of Saxagliptin Hydrochloride and Dapagliflozin Propendiol Monohydrate in combined dosage form. J. innov. appl. pharm. sci., 01-07.
17. Lotfy, H. M., Mohamed, D., & Elshahed, M. S. (2019). Novel univariate spectrophotometric determination of the recently released solid dosage form comprising Dapagliflozin and Saxagliptin via factorized response spectra: assessment of the average content and dosage unit uniformity of tablets. Spectrochim. Acta - A: Mol. Biomol., 5, 2-13.
18. Abdel-Gawad, S. A., Arab, H. H., & Hassan, S. A. (2021). Signal processing techniques for the spectrophotometric quantitation of binary mixture of Dapagliflozin and Saxagliptin: A comparative study. Trop. J. Pharm. Res., 20(7), 1489-1496.
19. Rao, B. R., Rao, V. V., & Venkateswarlu, B. S. (2019). RP-HPLC method for simultaneous estimation of Dapagliflozin and Saxagliptin in bulk samples. J. Pharm. Sci. & Res., 11(1), 254-257.
20. Godge, R. K., Shinde, G. S., & Joshi, S. (2019). Simultaneous estimation and validation of Dapagliflozin and Saxagliptin in bulk drug and dosage form by RP-HPLC. Research journal of science and technology, 11(1), 59-63.
21. More, S. S., Sonawane, S. S., Chhajed, S. S., & Kshirsagar, S. J. (2018). Development and validation of RP-HPLC method for simultaneous estimation of Saxagliptin and Dapagliflozin in tablets. AJPTech, 8(3), 145-148.
22. Donepudi, S., & Achanta, S. (2019). Simultaneous estimation of Saxagliptin and Dapagliflozin in human plasma by validated high performance liquid chromatography-ultraviolet method. Turk J Pharm Sci, 16(2), 227.
23. Padmaja, B. R., Sivagami, B., Chandrasekar, R., & Babu, M. N. (2018). A highly validated RP-HPLC method development for the simultaneous estimation of Dapagliflozin and Saxagliptin in tablet dosage forms. Int J Pharm Sci Drug Res, 10(5), 372-378.
24. Kommineni, V., Chowdary, K. P. R., & Prasad, S. V. U. M. (2018). Development and validation of a new HPLC method for the simultaneous estimation of Saxagliptin e and Dapagliflozin and its application in pharmacokinetic studies. Int Res J Pharm Med Sci, 1, 16-24.
25. Phanindra, A. (2022). Sensitive RP HPLC method for the simultaneous estimation of Dapagliflozin and Saxagliptin in plasma. Int. J. Early Child. Spec. Educ., 14(3), 2928-33.
26. Urooj, A., Sundar, P. S., Vasanthi, R., Raja, M. A., Dutt, K. R., Rao, K. N. V., & Ramana, H. (2017). Development and validation of RP-HPLC method for simultaneous estimation of Dapagliflozin and Metformin in bulk and in synthetic mixture. World J Pharm Pharm Sci, 6(7), 2139-2150.
27. Suthar, N., Marvaniya, V., & Patni, P. (2018). Stability indicating method development and validation for the simultaneous estimation of Dapagliflozin and Saxagliptin in synthetic mixture by HPLC. Pharma Science Monitor, 9(2), 112-28.
28. Manasa, M., & Aanandhi, V. M. (2021). Stability indicating simultaneous method development and validation of Dapagliflozin and Saxagliptin by RP-HPLC. Res J Pharm Technol, 14(2), 1045-1049.
29. Madhavi, S., & Rani, A. P. (2017). Development and validation of a method for simultaneous determination of Dapagliflozin and Saxagliptin in a formulation by RP-UPLC. World J Pharma Res, 6(12), 904-16.
30. Usman, M. R. M., Ahmad, S., Deore, C., Shaikh, T., Jain, B., & Imran, M. (2020). Stability indicating RP-HPLC method for determination of Saxagliptin and Dapagliflozin in bulk and tablet dosage forms. J. Pharm. Sci. Res., 12(4), 499-506.
31. Singh, N., Bansal, P., Maithani, M., & Chauhan, Y. (2018). Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of Dapagliflozin and Saxagliptin in fixed-dose combination. New J.

Chem., 42(4), 2459-2466.
32. Chaudhary, A., & Singh, B. K. (2021). Stability-indicating RP-HPLC method for simultaneous determination of antidiabetic drugs, Dapagliflozin and Saxagliptin. J. Adv. Sci. Res., 12(03 Suppl 1), 67-75.
33. Kommineni, V., Chowdary, K. P. R., & Prasad, S. V. U. M. (2017). Development of a new stability indicating RP-HPLC method for simultaneous estimation of Saxagliptine and Dapagliflozin and its validation as per ICH guidelines. Indo Am. j. pharm., 4(09), 2920-2932.
34. Gundala, A., Prasad, K. V. S. R. G., & Koganti, B. (2019). Application of quality by design approach in RP-HPLC method development for simultaneous estimation of Saxagliptin and Dapagliflozin in tablet dosage form. Braz. J. Pharm. Sci., 55, 1-10.
35. Surendran, S., Paul, D., Pokharkar, S., Deshpande, A., Giri, S., & Satheeshkumar, N. (2019). A LC-MS/MS method for simultaneous estimation of a novel anti-diabetic combination of Saxagliptin and Dapagliflozin using a polarity switch approach: application to in vivo rat pharmacokinetic study. Anal. Methods., 11(2), 219-226.
36. Goday, S., Shaik, A. R., & Avula, P. (2018). Development and validation of a LC-ESI-MS/MS based bioanalytical method for Dapagliflozin and Saxagliptin in human plasma. Indian J. Pharm. Educ. Res., 52(4), S277-86.
37. Elhassan, M. M., Mahmoud, A. M., Hegazy, M. A., & Mowaka, S. (2023). Univariate and Multivariate Determination of Dapagliflozin and Saxagliptin in Bulk and Dosage Form. J. AOAC Int., 106(3), 794-803.

**CITATION OF THIS ARTICLE**

Nikita Shaileshbhai Patel, Bhavesh Hirabhai Patel, Mona Kaushal. Development of Chemometric Aided RP- HPLC Assay Approach for the Concurrent Determination of Saxagliptin Hydrochloride and Dapagliflozin Propanediol Monohydrate in Combined Dosage Form fish. Bull. Env.Pharmacol. Life Sci., Vol 12 [11] October 2023: 184-195