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RESEARCH ARTICLE

Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk Drug and Pharmaceutical Dosage Forms

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Abstract: A simple, rapid, accurate, precise, and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous quantitative estimation of dapagliflozin and saxagliptin in bulk drugs and pharmaceutical tablet formulations. The ultraviolet (UV) absorption spectra of both analytes in methanol exhibited maximum absorbance (λmax) values at 225 nm for dapagliflozin and 213 nm for saxagliptin. The overlay spectra revealed a distinct isosbestic point at 220 nm, which was chosen as the optimal analytical wavelength for dual-wavelength detection. Chromatographic separation was accomplished on a C18 column (250 mm x 4.6 mm, 5 µm particle size) at a controlled column temperature of 33°C. The optimized mobile phase consisted of a mixture of methanol and 0.1% v/v aqueous acetic acid in an isocratic ratio of 70.7:29.3 v/v, delivered at a constant flow rate of 0.9 mL/min. Under these optimized chromatographic conditions, dapagliflozin and saxagliptin eluted with clean, well-resolved symmetrical peaks at retention times of approximately 4.55 min and 7.18 min, respectively, yielding a high-resolution factor of 10.25. Calibration plots showed excellent linearity over the concentration ranges of 10-50 µg/mL for dapagliflozin and 5-25 µg/mL for saxagliptin. The recovery rates obtained during accuracy studies ranged between 98.57% and 99.50%. Repeatability, intraday, and interday precision studies showed high reproducibility, with percent relative standard deviation (%RSD) values consistently below 2.0%. Sensitivity evaluations yielded limits of detection (LOD) of 0.477 µg/mL and 0.283 µg/mL for dapagliflozin and saxagliptin, respectively. The developed method was successfully validated through the assay of a commercial tablet formulation.

Keywords: Dapagliflozin; Saxagliptin; RP-HPLC; Stability-Indicating; Validation; Dual-Wavelength Detection; Quality Control.

1. Introduction

Type 2 diabetes mellitus is a progressive metabolic disorder characterized by chronic hyperglycemia, insulin resistance, and impaired insulin secretion [1]. Monotherapy often fails to sustain long-term glycemic control due to the multi-pathological nature of the disease [2]. Combining oral hypoglycemic agents with complementary mechanism pathways has become an essential clinical strategy to help patients meet individualized glycated hemoglobin (HbA1c) targets [3]. Combining a sodium-glucose cotransporter-2 (SGLT2) inhibitor with a dipeptidyl peptidase-4 (DPP-4) inhibitor offers a highly synergistic therapeutic approach without increasing the risk of hypoglycemia or body weight gain [4].

Dapagliflozin, chemically identified as (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, is a potent, selective, and reversible inhibitor of SGLT2 [5]. By targeting SGLT2 in the proximal renal tubules, it prevents glucose reabsorption into the bloodstream, facilitating the excretion of excess glucose via urine [6]. This insulin-independent action provides glucose-lowering effects alongside secondary benefits, including blood pressure reduction and weight loss [7]. Saxagliptin, chemically known as (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile, acts as a selective and competitive inhibitor of DPP-4 [8]. saxagliptin increases postprandial insulin secretion in a glucose-dependent manner and suppresses inappropriate glucagon release from pancreatic alpha cells [9] by preventing the rapid degradation of incretin hormones, such as glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).

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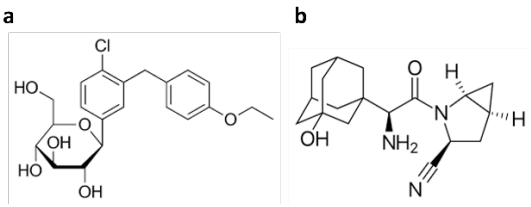

Figure 1. Structures of a. dapagliflozin and b. saxagliptin

Simultaneous prescription and co-formulation of dapagliflozin and saxagliptin (e.g., in combinations such as Tab. Dapaglyn L) necessitate the development of reliable, rapid, and economical analytical assays to monitor both active ingredients in commercial dosage forms [10]. Literature suggests that chromatographic separation of these two chemically distinct drugs can be challenging due to their widely differing polarities and ionization properties, which often lead to poor peak symmetry, peak fronting, or insufficient resolution [11].

Although independent assays and select separation methods for these drugs have been described in different matrices, many of these existing methods involve expensive solvents, long analysis runs, or complex buffer systems that increase instrumental wear [12]. The design of a simple, robust, and cost-effective isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method operating with an aqueous-organic mobile phase is highly desirable [13]. A simplified isocratic approach minimizing organic waste and utilizing common column chemistry offers significant benefits for routine quality control in testing laboratories. This investigation aims to construct, optimize, and validate a highly sensitive, specific, and stability-indicating RP-HPLC method under ICH Q2(R1) regulatory parameters to simultaneously quantify dapagliflozin and saxagliptin in bulk formulations and tablet dosage forms [14].

2. Materials and Methods

2.1. Chemicals, Reagents, and Standard Samples

High-purity active pharmaceutical ingredient (API) reference standards of Dapagliflozin and Saxagliptin were obtained as gift samples from Reliable's Shree Industrial Training Centre and Laboratory, Jalgaon, India. The commercial pharmaceutical formulation Dapaglyn L tablets (labeled to contain 10 mg of dapagliflozin and 5 mg of saxagliptin) was purchased from a local pharmacy. HPLC-grade methanol, glacial acetic acid, and purified deionized water were procured from Merck India Ltd. (Mumbai, India). All solvents, buffer components, and prepared mobile phases were filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA) and thoroughly degassed via sonication for 15 min before system introduction.

2.2. Instrumentation and Software

Chromatography was performed on an Agilent 1100 Series HPLC system equipped with a high-performance reciprocating pump (HP-1100, G1311A), a manual injector or autosampler, a column thermostat, and a diode array detector (DAD, G1314B). The system operated under Agilent ChemStation software (Revision B.04.03) for chromatogram visualization, peak integration, and quantitative data analysis. Spectrophotometric scans and absorbance measurements were cross-verified on a double-beam UV-Visible spectrophotometer.

2.3. Preparation of Stock and Working Standard Solutions

2.3.1. Primary Standard Stock Solutions

To prepare the primary standard stock solutions, accurate quantities of 10 mg of dapagliflozin and 5 mg of saxagliptin were weighed and transferred quantitatively into separate, clean, dry 10 mL volumetric flasks. Approximately 7 mL of HPLC-grade methanol was added to each flask, and the mixtures were sonicated for 10 min to ensure complete dissolution of the analytes. After achieving room temperature equilibration, the volumes were made up to the calibration mark with methanol. This process yielded primary standard stock concentrations of 1000 µg/mL for dapagliflozin and 500 µg/mL for saxagliptin. The prepared stock solutions were filtered through 0.45 µm syringe filters and stored under refrigeration at 4°C protected from light.

2.3.2. Working Standard Calibration Solutions

From the prepared primary stock solutions, appropriate aliquots were accurately transferred into a series of 10 mL volumetric flasks and diluted with the mobile phase (methanol : 0.1% aqueous acetic acid, 70.7:29.3 v/v) to yield a series of mixed working standard solutions. The final concentration range obtained for dapagliflozin was 10, 20, 30, 40, and 50 µg/mL, and the corresponding range for saxagliptin was 5, 10, 15, 20, and 25 µg/mL. These calibration solutions were prepared fresh daily.

2.4. Determination of UV Absorption and Isosbestic Point

Aliquot portions of the individual working standard solutions of dapagliflozin (10 µg/mL) and saxagliptin (10 µg/mL) in methanol were scanned in the wavelength region of 200-400 nm against a methanol blank. Absorbance profiles were recorded to locate individual absorption maxima (λmax). Spectral overlay was performed to identify the isosbestic point (common analytical wavelength) where both drugs showed balanced response profiles suitable for simultaneous HPLC quantification.

2.5. Optimization of Chromatographic Conditions

To establish stable, reproducible chromatographic peaks with low tailing factors ($T \leq 1.5$), high theoretical plate counts ($N \geq 2000$), and optimized resolution ($Rs > 2$), the mobile phase chemistry was optimized. Multiple trials with variations in organic-to-aqueous ratio, aqueous phase pH modifier, column temperature, and mobile phase flow rate were conducted. The optimized conditions were maintained throughout all validation protocols.

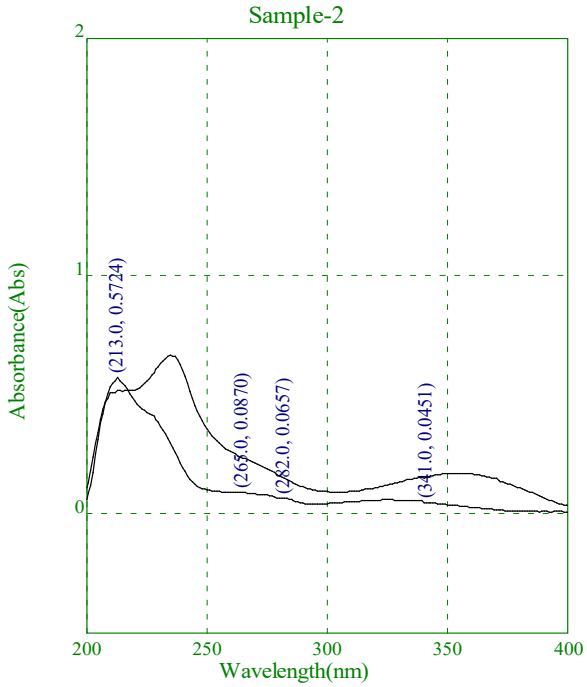

Figure 2. Isosbestic point of Dapagliflozin and Saxagliptin at 220 nm

2.6. Method Validation Protocol (ICH Q2(R1))

2.6.1. System Suitability and Specificity

System suitability was verified by running six replicate injections of a mixed standard solution containing dapagliflozin (20 µg/mL) and saxagliptin (10 µg/mL) to evaluate parameters including retention time, capacity factor, theoretical plate count, tailing factor, and resolution. Specificity was evaluated by injecting blank solutions, placebo excipient mixtures, standard drug solutions, and commercial sample preparations to ensure no interference occurred at the retention times of both analytes.

2.6.2. Linearity and Range

Linearity of the method was studied by analyzing five concentrations of mixed standard solutions in range of 10-50 µg/mL for dapagliflozin and 5-25 µg/mL for saxagliptin. Triplicate injections of each concentration level were analyzed. Calibration curves

were plotted by graphing mean peak areas (y-axis) against concentrations (x-axis), followed by linear regression analysis to calculate slope, intercept, and correlation coefficient (R^2).

2.6.3. Accuracy and Recovery

Accuracy was determined by recovery studies using the standard addition method. Pre-analyzed formulation sample solutions corresponding to 10 µg/mL of dapagliflozin and 5 µg/mL of saxagliptin were spiked with pure API standards at three different concentration levels (80%, 100%, and 120%). Specifically, pure standards corresponding to 8, 10, and 12 µg/mL of dapagliflozin and 4, 5, and 6 µg/mL of saxagliptin were added. Recoveries were evaluated in triplicate for each level, and the percentages of drug recovered and relative standard deviations were calculated.

2.6.4. Precision (System, Intraday, and Interday)

The precision of the developed method was evaluated at three levels:

- Repeatability (System Precision): Six consecutive injections of mixed standard solutions (50 µg/mL dapagliflozin and 25 µg/mL saxagliptin) were analyzed to calculate the relative standard deviation of peak areas.
- Intraday Precision: Analyzed in triplicate at three concentration levels (20, 30, and 40 µg/mL for dapagliflozin; 10, 15, and 20 µg/mL for saxagliptin) at different times within the same day.
- Interday Precision: Completed by analyzing the same three concentrations on three consecutive days.

2.6.5. Limits of Detection (LOD) and Quantification (LOQ)

Sensitivity was assessed by calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ) based on the standard deviation of the intercept response (σ) and the slope of the calibration curve (S), using the mathematical models:

$$\text{LOD} = \frac{3.3 \times \sigma}{S}$$

$$\text{LOQ} = \frac{10 \times \sigma}{S}$$

2.6.6. Robustness and Ruggedness

Robustness was evaluated by introducing small, deliberate changes in chromatographic conditions:

- Slight modification of organic composition in mobile phase (pm 1.0%).
- Deliberate variation of detection wavelength (pm 1 nm).

The impact of these deliberate parameters on chromatographic resolution, retention times, tailing factors, and quantitative recoveries was monitored. Ruggedness was verified by performing the assays under identical conditions with a second analyst in a different laboratory.

2.7. Application to Marketed Formulation

To assay the commercial formulation, twenty tablets of Dapaglyn L (labeled to contain 10 mg dapagliflozin and 5 mg saxagliptin per tablet) were weighed and finely ground in a mortar. A portion of the powder equivalent to 10 mg of dapagliflozin and 5 mg of saxagliptin was weighed and transferred to a 100 mL volumetric flask containing 70 mL of methanol. The flask was sonicated for 20 min to ensure complete extraction, followed by dilution to volume with methanol. The solution was filtered through a 0.45 µm membrane filter. From this filtered preparation, aliquots were transferred to volumetric flasks and diluted with mobile phase to yield target working concentrations of 40 µg/mL for dapagliflozin and 20 µg/mL for saxagliptin. Replicate injections of these preparations were analyzed to evaluate formulation potency.

3. Results and Discussion

3.1. UV Spectrophotometric Estimation and Wavelength Selection

To identify the optimum detection wavelength for the simultaneous quantitative determination of dapagliflozin and saxagliptin, standard solutions of each drug were prepared in methanol at a concentration of 10 µg/mL and scanned individually across the ultraviolet region of 200-400 nm. Dapagliflozin showed a distinct absorbance maximum (λmax) at approximately 225 nm. Saxagliptin exhibited its corresponding absorbance maximum at approximately 213 nm.

An overlay of the ultraviolet absorption spectra of both analytes revealed a distinct isosbestic point at 220 nm. At this wavelength of intersection, both drugs exhibited balanced and stable absorbance intensities. Consequently, 220 nm was selected as the common detection wavelength for the developed multi-wavelength diode array detection scheme. This wavelength choice ensures adequate sensitivity and response reproducibility for both analytes in their co-formulated ratios, preventing the disproportionate signal dominance of one compound over the other.

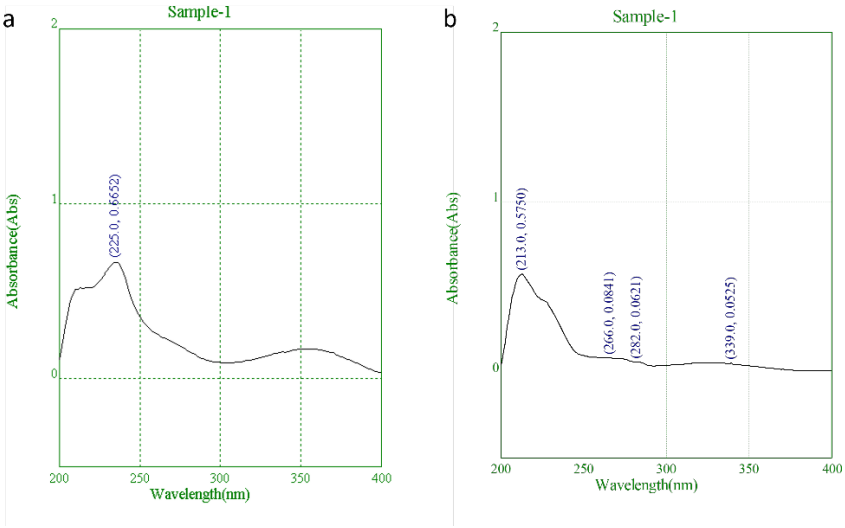

Figure 3. UV absorption spectrum of a. Dapagliflozin (10 µg/mL) in methanol b. Saxagliptin (10 µg/mL) in methanol

3.2. Chromatographic Method Development and Mobile Phase Optimization

To obtain efficient separation, optimal resolution, high theoretical plate count, and low peak asymmetry, the chromatographic system was systematically optimized through a series of twelve trials. Different mobile phase systems comprising methanol and 0.1% v/v aqueous acetic acid were evaluated under varying flow rates and columns.

3.2.1. Trial Conditions

The preliminary trials utilized higher proportions of organic modifier. In Trial 01, using a methanol to 0.1% acetic acid ratio of 85:15 v/v at a flow rate of 1.0 mL/min, two distinct peaks were resolved at retention times of 4.396 min and 6.136 min. Although separation was achieved, the peak symmetry and resolution were poor, with theoretical plate values of 891 for the first peak. Trial 02 altered the ratio to 80:20 v/v, which split the peaks and produced an interfering third peak at 5.349 min, indicating poor system selectivity under these conditions. Trials 03, 04, and 05 suffered from rapid elution, baseline noise, or single co-eluting peaks. In Trial 06, increasing the aqueous component (methanol : 0.1% acetic acid, 60:40 v/v) at a flow rate of 1.0 mL/min resulted in an excessively delayed peak at 12.044 min, which is undesirable for a rapid quality control assay. Trials 07 through 10 investigated various combinations of flow rates and compositions but consistently resulted in either split peaks, insufficient plate counts, or baseline drift. Trial 11 (methanol : 0.1% acetic acid, 71:29 v/v at 0.9 mL/min) significantly improved performance, resolving dapagliflozin at 4.463 min and saxagliptin at 5.715 min with a resolution factor of 4.79.

3.2.2. Selection of the Optimized Conditions

To further optimize resolution and peak symmetry, a final adjustment was made in Trial 12. The isocratic mobile phase composition was set to methanol and 0.1% v/v aqueous acetic acid in a ratio of 70.7:29.3 v/v at a flow rate of 0.9 mL/min. Under these optimized

conditions, dapagliflozin eluted at a retention time of 4.554 min, and saxagliptin eluted at a retention time of 7.180 min. The resolution between the two peaks was 10.25, with tailing factors of 0.97 and 0.93 for dapagliflozin and saxagliptin, respectively. The theoretical plate counts were 7553 for dapagliflozin and 9037 for saxagliptin. This chromatographic system achieved excellent separation with high baseline stability and was selected for full validation. The detailed mobile phase composition variables evaluated across the development phase are presented in Table 1, while the measured peak elution parameters for all twelve trials are shown in Table 2.

Table 1. Mobile Phase Configurations Evaluated in Development Trials

Trial Number	Mobile Phase Ratio (Methanol: 0.1% Aqueous Acetic Acid, v/v)	Flow Rate (mL/min)	Injection Volume (µL)
Trial 01	85:15	1.0	20
Trial 02	80:20	1.0	20
Trial 03	75:25	0.8	20
Trial 04	70:30	0.8	20
Trial 05	65:35	0.8	20
Trial 06	60:40	1.0	20
Trial 07	60:40	0.7	20
Trial 08	55:45	0.7	20
Trial 09	70:30	0.9	20
Trial 10	72:28	0.9	20
Trial 11	71:29	0.9	20
Trial 12	70.7:29.3	0.9	20

Table 2. System Suitability and Peak Integration Parameters from Development Trials

Trial Label	Eluted Peak	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Width (min)	Theoretical Plates (N)	Resolution (Rs)
Trial-01	Peak 1	4.396	2158.05	100.17	1.24	0.347	891	—
	Peak 2	6.136	710.43	41.43	1.15	0.220	4311	3.61
Trial-02	Peak 1	4.492	751.28	45.76	0.28	0.288	1343	—
	Peak 2	4.973	1326.25	56.74	1.27	0.426	756	0.79
	Peak 3	5.349	274.39	18.49	0.42	0.221	3245	0.68
Trial-03	Peak 1	2.922	154.71	23.93	0.49	0.088	6128	—
	Peak 2	3.172	37.00	3.47	0.24	0.192	1507	1.05
Trial-04	Peak 1	7.398	333.14	11.99	0.59	0.420	1719	—
Trial-05	Peak 1	5.080	12.49	1.90	0.83	0.117	10395	—
	Peak 2	5.201	16.92	2.14	0.44	0.147	6970	0.54
Trial-06	Peak 1	12.044	3842.60	120.48	0.40	0.460	3797	—
Trial-07	Peak 1	4.076	3018.48	76.89	1.68	0.400	575	—
	Peak 2	4.642	1308.32	55.41	0.23	0.371	867	0.86
	Peak 3	6.440	29.46	2.24	0.66	0.195	6047	3.73
	Peak 4	7.701	1432.78	38.47	1.60	0.660	755	1.73
Trial-08	Peak 1	1.767	7129.41	202.89	0.89	0.513	65	—
	Peak 2	4.581	100.28	11.39	0.55	0.164	4313	4.88
	Peak 3	4.818	220.43	10.04	0.40	0.408	772	0.49
Trial-09	Dapagliflozin	3.106	826.29	145.79	0.94	0.080	8328	—
	Saxagliptin	6.192	407.43	44.84	0.85	0.137	11305	16.70
Trial-10	Peak 1	3.142	934.91	184.75	0.96	0.074	9904	—
Trial-11	Dapagliflozin	4.463	2547.91	261.78	0.97	0.140	5644	—
	Saxagliptin	5.715	725.98	63.28	0.90	0.167	6448	4.79
Trial-12	Dapagliflozin	4.554	1907.41	233.75	0.97	0.123	7553	—
	Saxagliptin	7.180	481.93	41.04	0.93	0.178	9037	10.25

3.3. Method Validation Results (ICH Q2(R1))

3.3.1. Linearity and Range

Linearity was evaluated in triplicate across five concentration levels, ranging from 10-50 µg/mL for dapagliflozin and 5-25 µg/mL for saxagliptin. The calibration equations derived from the mean integrated peak areas were Dapagliflozin = 153.8356 C + 322.774 (R^2 = 0.99976) and Saxagliptin = 82.4144 C + 60.312 (R^2 = 0.99972)

where C represents analyte concentration in µg/mL. These calibration parameters indicate a strong linear correlation between analyte concentration and peak response. Highly reproducible peak areas were obtained at all concentration levels, with relative standard deviations (%RSD) well under 1.5%. The detailed linearity data, back-calculated concentration averages, and regression precision parameters are summarized in Tables 3, 4, and 5.

Table 3. Chromatography Properties across the Selected Linearity Range

Concentration (µg/mL) (Dapa + Saxa)	Injection Number	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
10 + 5	Injection 01	Dapa	4.554	1907.41	233.75	0.97	7553	10.25
		Saxa	7.180	481.93	41.04	0.93	9037	
	Injection 02	Dapa	4.537	1908.11	231.76	0.96	7297	10.04
		Saxa	7.143	489.94	41.29	0.92	8723	
20 + 10	Injection 01	Dapa	4.552	3367.91	415.63	0.96	7756	10.12
		Saxa	7.113	871.70	75.29	0.91	9095	
	Injection 02	Dapa	4.529	3352.84	395.86	0.97	7081	10.05
		Saxa	7.113	871.93	75.02	0.91	9095	
30 + 15	Injection 01	Dapa	4.524	4903.09	602.64	0.97	7661	10.31
		Saxa	7.132	1282.70	110.51	0.93	9143	
	Injection 02	Dapa	4.540	4931.72	601.03	0.95	7428	9.98
		Saxa	7.103	1296.42	110.84	0.92	8843	
40 + 20	Injection 01	Dapa	4.523	6479.97	790.84	0.97	7452	9.93
		Saxa	7.067	1710.74	144.61	0.92	8755	
	Injection 02	Dapa	4.527	6473.10	756.68	0.97	6893	9.99
		Saxa	7.110	1701.78	145.93	0.92	9087	
50 + 25	Injection 01	Dapa	4.533	7988.43	943.86	0.97	7096	10.05
		Saxa	7.119	2119.51	180.84	0.92	9109	
	Injection 02	Dapa	4.541	8094.50	979.04	0.96	7310	10.08
		Saxa	7.119	2138.62	183.61	0.93	9110	

Table 4. Calibration Regression Dynamics of Dapagliflozin

Concentration (µg/mL)	Replicate Peak Area	Mean Peak Area	Standard Deviation (SD)	relative Standard Deviation (%RSD)
10	1907.41 / 1908.11	1907.76	0.50	0.03%
20	3367.91 / 3352.84	3360.37	10.65	0.32%
30	4903.09 / 4931.72	4917.41	20.24	0.41%
40	6479.97 / 6473.10	6476.53	4.86	0.07%
50	7988.43 / 8094.50	8041.46	75.00	0.93%

Table 5. Calibration Regression Dynamics of Saxagliptin

Concentration (µg/mL)	Replicate Peak Area	Mean Peak Area	Standard Deviation (SD)	relative Standard Deviation (%RSD)
5	481.93 / 489.94	485.93	5.67	1.17%
10	871.70 / 871.93	871.82	0.16	0.02%
15	1282.70 / 1296.42	1289.56	9.70	0.75%
20	1710.74 / 1701.78	1706.26	6.34	0.37%
25	2119.51 / 2138.62	2129.07	13.51	0.63%

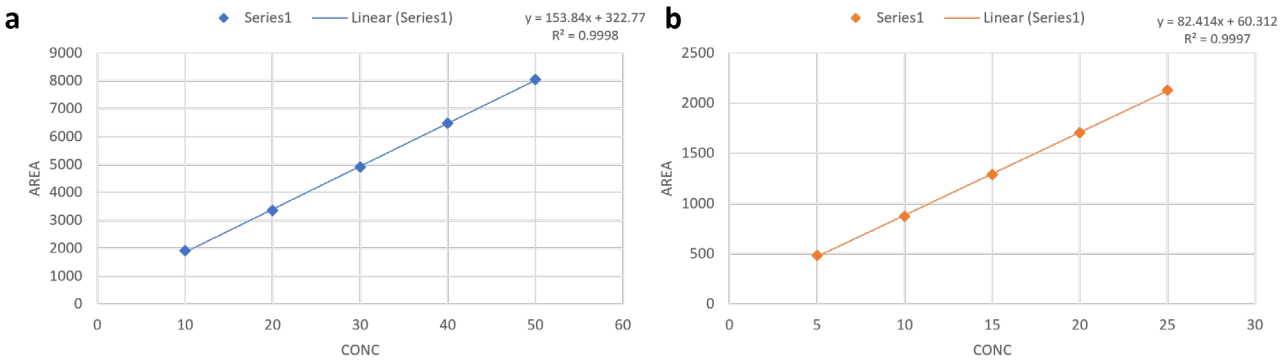

Figure 4. Calibration curve of a. Dapagliflozin and b. Saxagliptin

3.3.2. Accuracy and Recovery Studies

Accuracy was verified by spiking known quantities of pure API reference standard into formulation pre-analyzed samples at levels of 80%, 100%, and 120%. Mean percentage recovery rates were in the range of 98.57%-99.22% for dapagliflozin and 98.57%-99.50% for saxagliptin. These recovery values are well within the acceptable regulatory limits (98.0%-102.0%). No co-eluting peaks or active interferences from excipient matrix complexes were detected in any accuracy run. System response parameters for these recovery trials are listed in Table 6, while final recovery statistical assessments are reported in Table 7 and Table 8.

Table 6. Peak Resolution During Recovery

Recovery Spike Level	Replicate Run	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
80% Spike	Injection 01	Dapa	4.544	3059.21	380.03	0.96	7726	10.32
		Saxa	7.115	795.27	69.73	0.92	9579	
	Injection 02	Dapa	4.541	3062.89	376.09	0.96	7716	10.33
		Saxa	7.116	794.94	69.54	0.92	9580	
100% Spike	Injection 01	Dapa	4.540	3386.68	419.91	0.96	7715	10.25
		Saxa	7.113	882.35	75.58	0.92	9330	
	Injection 02	Dapa	4.539	3383.89	412.13	0.96	7503	10.26
		Saxa	7.110	879.71	76.84	0.91	9564	
120% Spike	Injection 01	Dapa	4.540	3687.95	454.54	0.96	7508	10.21
		Saxa	7.100	960.68	83.95	0.92	9537	
	Injection 02	Dapa	4.541	3660.57	450.82	0.96	7717	10.19
		Saxa	7.117	954.67	81.76	0.92	9106	

Table 7. Results of Recovery for Dapagliflozin

Accuracy Level	Standard API Spiked (µg/mL)	Experimental Area	Quantity Recovered (µg/mL)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
80%	8.0	3062.89	7.82	98.78%	0.01	0.01%
	8.0	3059.21	7.79	98.77%		
100%	10.0	3386.68	9.92	99.22%	0.13	0.13%
	10.0	3383.89	9.90	99.04%		
120%	12.0	3687.95	11.88	99.01	0.31	0.31%
	12.0	3660.57	11.70	98.57%		

Table 8. Results of Recovery for Saxagliptin

Accuracy Level	Standard API Spiked (µg/mL)	Experimental Area	Quantity Recovered (µg/mL)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
80%	4.0	794.94	3.91	98.89%	0.07	0.07%
	4.0	795.27	3.92	98.99%		
100%	5.0	882.35	4.98	99.50%	0.45	0.46%
	5.0	879.71	4.94	98.86%		
120%	6.0	960.68	5.93	98.76%	0.13	0.13%
	6.0	954.67	5.85	98.57%		

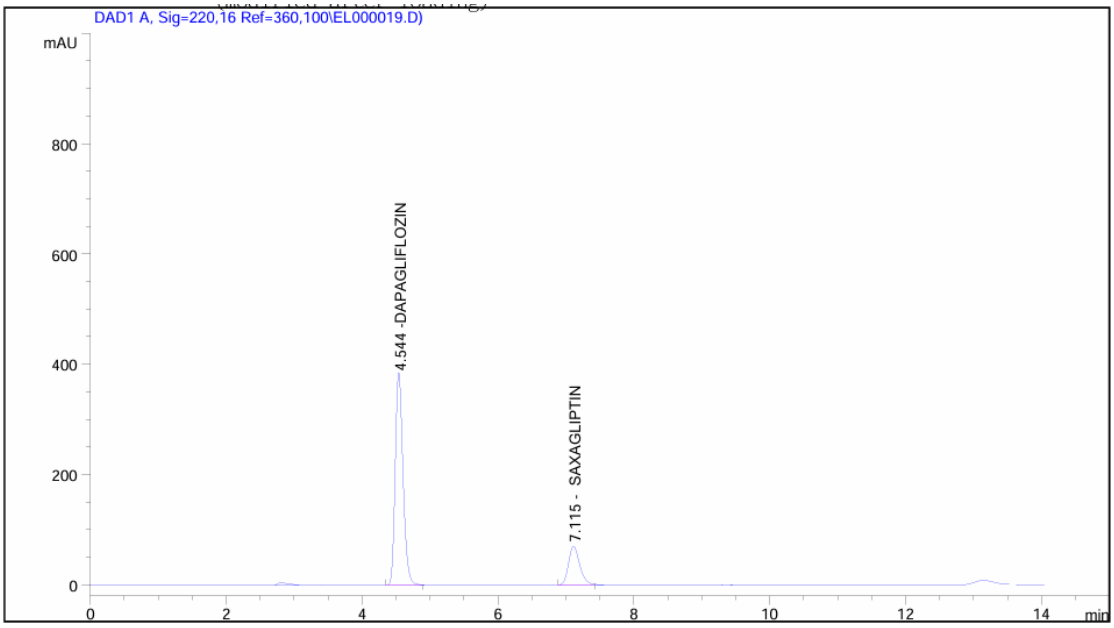

Figure 5. Chromatograms of the recovery spikes for accuracy at 80%

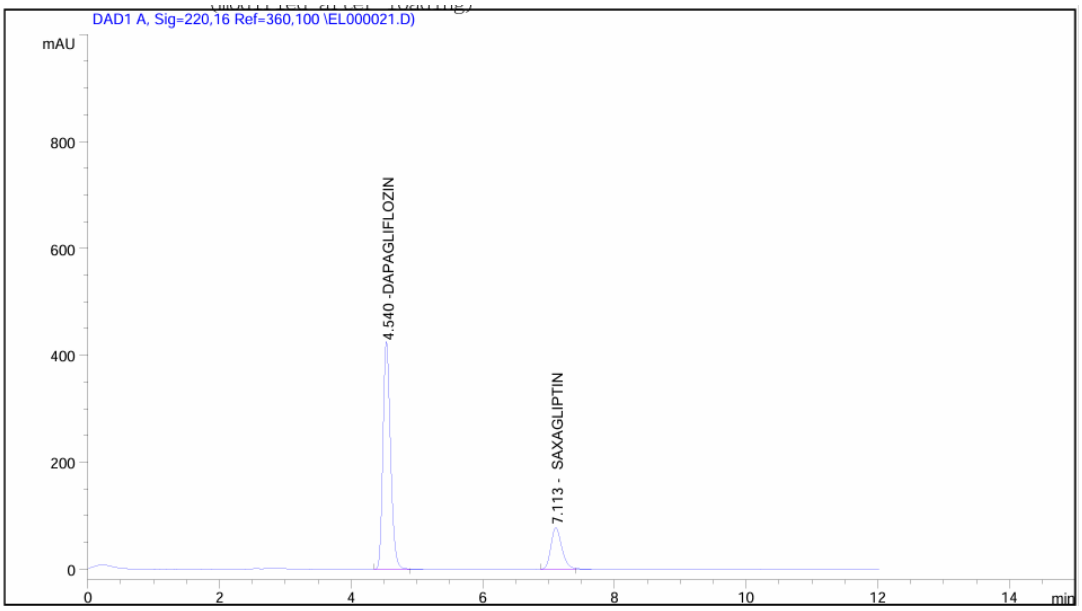

Figure 6. Chromatograms of the recovery spikes for accuracy at 100% levels

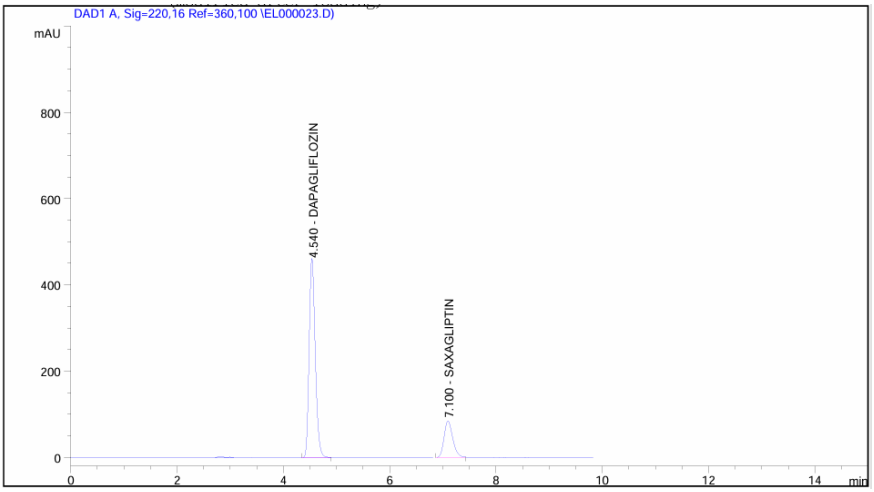

Figure 7. Chromatograms of the recovery spikes for accuracy at 120% levels

3.3.3. System Precision

Intraday Precision: Intraday precision was evaluated by analyzing three concentration levels on the same day. For dapagliflozin, the mean recoveries at concentrations of 20, 30, and 40 µg/mL were 99.59%, 100.16%, and 100.53%, with calculated % RSD values of 0.13%, 0.01%, and 0.03%, respectively. For saxagliptin, the corresponding mean recoveries at concentrations of 10, 15, and 20 µg/mL were 99.17%, 99.36%, and 100.08%, with corresponding % RSD values of 0.04%, 0.13%, and 0.48%, respectively. These values show high method reproducibility within the same day. The peak parameters for the intraday injections are summarized in Table 9, and the precision statistical evaluations are reported in Table 10 and Table 11.

Table 9. Peak Parameters Observed during Intraday Precision Evaluation

Selected Concentration (Dapa + Saxa, µg/mL)	Replicate Run	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
20 + 10	Injection 01	Dapa	4.546	3389.22	419.00	0.97	7735	10.37
		Saxa	7.131	877.78	76.65	0.93	9621	
	Injection 02	Dapa	4.542	3383.03	416.80	0.96	7721	10.30
		Saxa	7.127	877.29	75.95	0.92	9367	
30 + 15	Injection 01	Dapa	4.548	4943.59	606.48	0.97	7532	10.19
		Saxa	7.141	1287.41	111.91	0.92	9166	
	Injection 02	Dapa	4.545	4944.52	606.00	0.96	7525	10.13
		Saxa	7.123	1289.71	110.56	0.92	9120	
40 + 20	Injection 01	Dapa	4.546	6508.34	800.64	0.97	7733	10.29
		Saxa	7.130	1704.10	149.49	0.93	9375	
	Injection 02	Dapa	4.539	6505.84	784.88	0.95	7304	10.12
		Saxa	7.126	1715.62	147.85	0.92	9128	

Table 10. Intraday Precision Statistics for Dapagliflozin

Nominal Concentration (µg/mL)	Replicate Area	Measured Quantity (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
20	3389.22 / 3383.03	19.92	99.59%	99.59%	0.13	0.13%
30	4943.59 / 4944.52	30.05	100.16%	100.16%	0.01	0.01%
40	6508.34 / 6505.84	40.21	100.53%	100.53%	0.03	0.03%

Table 11. Intraday Precision for Saxagliptin

Nominal Concentration (µg/mL)	Replicate Area	Measured Quantity (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
10	877.78 / 877.29	9.92	99.17%	99.17%	0.04	0.04%
15	1287.41 / 1289.71	14.90	99.36%	99.36%	0.13	0.13%
20	1704.10 / 1715.62	20.02	100.08%	100.08%	0.48	0.48%

Interday Precision: Interday precision was evaluated by analyzing the same concentrations on three consecutive days. For dapagliflozin, the mean recoveries at concentrations of 20, 30, and 40 µg/mL were 100.36%, 100.18%, and 100.12%, with calculated % RSD values of 0.03%, 0.02%, and 0.04%, respectively. For saxagliptin, the corresponding mean recoveries at concentrations of 10, 15, and 20 µg/mL were 100.53%, 100.18%, and 101.22%, with calculated % RSD values of 0.06%, 0.16%, and 0.29%, respectively. These low % RSD values confirm the ruggedness and long-term reproducibility of the developed method. The system peak properties observed across multiple days are detailed in Table 12, with the precision statistics reported in Table 13 and Table 14.

Table 12. Peak Properties Observed during Interday Precision Studies

Concentration Level (Dapa + Saxa, µg/mL)	Replicate Run	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
20 + 10	Injection 01	Dapa	4.525	3410.44	428.76	0.98	7707	10.12
		Saxa	7.021	888.35	79.12	0.96	9638	
	Injection 02	Dapa	4.526	3409.15	428.76	0.98	7708	10.12
		Saxa	7.023	889.12	79.12	0.96	9638	
30 + 15	Injection 01	Dapa	4.522	4945.67	623.04	0.97	7866	10.19
		Saxa	7.037	1297.17	115.31	0.93	9492	
	Injection 02	Dapa	4.520	4944.41	623.04	0.97	7865	10.19
		Saxa	7.034	1300.16	115.31	0.93	9492	
40 + 20	Injection 01	Dapa	4.510	6483.87	818.67	0.96	7825	10.20
		Saxa	7.017	1725.06	152.08	0.94	9564	
	Injection 02	Dapa	4.515	6480.24	818.67	0.96	7821	10.20
		Saxa	7.015	1732.15	152.08	0.94	9566	

Table 13. Interday Precision Statistics for Dapagliflozin

Concentration (µg/mL)	Replicate Area	Measured Quantity (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
20	3410.44 / 3409.15	20.07	100.36%	100.36%	0.03	0.03%
30	4945.67 / 4944.41	30.05	100.18%	100.18%	0.02	0.02%
40	6483.87 / 6480.24	40.05	100.12%	100.12%	0.04	0.04%

Table 14. Interday Precision for Saxagliptin

Nominal Concentration (µg/mL)	Replicate Area	Measured Quantity (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
10	888.35 / 889.12	10.05	100.53%	100.53%	0.06	0.06%
15	1297.17 / 1300.16	15.03	100.18%	100.18%	0.16	0.16%
20	1725.06 / 1732.15	20.24	101.22%	101.22%	0.29	0.29%

3.3.4. Repeatability (System Suitability)

To verify repeatability, standard solutions corresponding to 50 µg/mL of dapagliflozin and 25 µg/mL of saxagliptin were prepared and analyzed in replicate. The peak response was highly reproducible, with Calculated % RSD values of 0.01% and 0.41% for dapagliflozin and saxagliptin, respectively. This shows the consistency of the column packing and mobile phase flow dynamics. Chromatographic measurements and statistics are summarized in Table 15 and Table 16.

Table 15 Repeatability Peak Measurements

Sample Concentration (Dapa + Saxa, µg/mL)	Injection Number	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
50 + 25	Injection 01	Dapa	4.516	8116.81	1010.46	0.98	7633	10.07
		Saxa	7.026	2172.60	191.72	0.95	9341	
	Injection 02	Dapa	4.509	8118.44	1024.80	0.96	7821	10.00
		Saxa	6.986	2185.28	193.14	0.95	9235	

Table 16. Repeatability Results

Analyte	Concentration (µg/mL)	Experimental Area	Recovered Quantity (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
Dapagliflozin	50.0	8116.81 / 8118.44	50.68	101.36%	101.36%	0.01	0.01%
Saxagliptin	25.0	2172.59 / 2185.28	25.71	102.83%	102.83%	0.41	0.41%

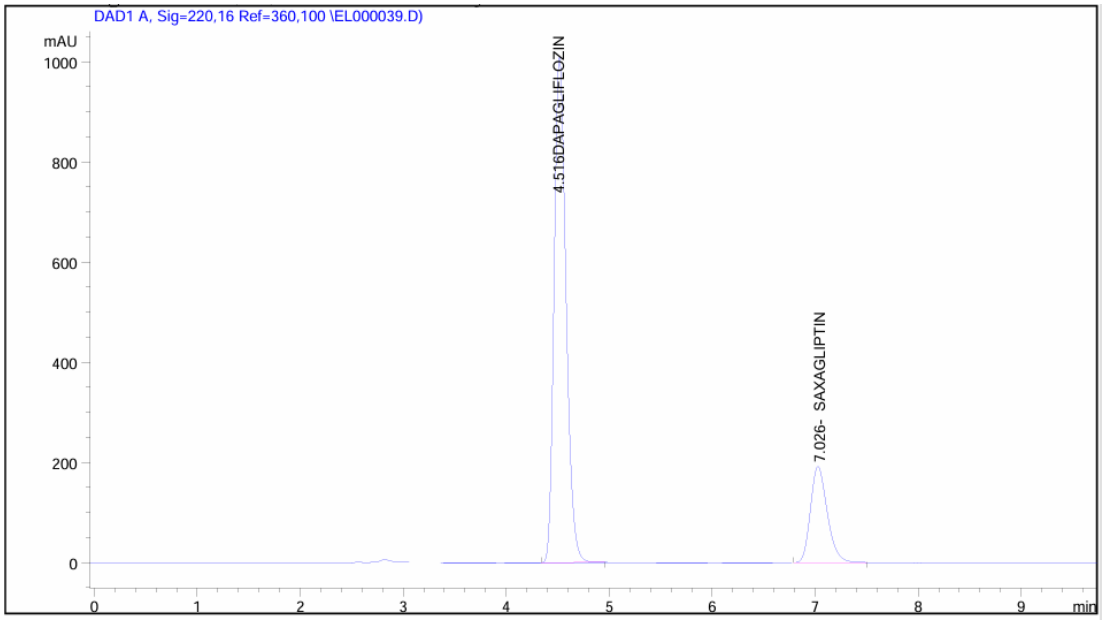

Figure 8. Chromatogram of Repeatability at 50 + 25 µg/mL

3.3.5. Limits of Detection (LOD) and Limit of Quantification (LOQ)

Sensitivity limits were calculated based on the standard deviation of the intercept response (σ) and the slope (S) of the calibration curve. For dapagliflozin, the standard deviation was 22.25, yielding an LOD of 0.0031 µg/mL and an LOQ of 0.0094 µg/mL. For saxagliptin, the standard deviation of the response was 7.08, yielding an LOD of 0.2833 µg/mL and an LOQ of 0.8585 µg/mL.

These low values show the sensitivity of the developed method, making it suitable for trace impurity monitoring, dissolution studies, and degradation product evaluations.

3.3.6. Specificity

Method specificity was evaluated in the presence of common tablet excipients. Chromatograms of the tablet formulations showed clean, well-resolved peaks for both active ingredients, with no interfering peaks at their respective retention times (4.563 min for dapagliflozin and 7.156 min for saxagliptin). Assay values of 99.45% (% RSD = 0.024%) for dapagliflozin and 98.83% (% RSD = 0.416%) for saxagliptin were obtained, confirming that the method is highly selective and unaffected by the excipient matrix. System response and peak recovery profiles from the specificity tests are shown in Table 17 and Table 18.

Table 17. Specificity Run System Response

Specified Concentration (Dapa + Saxa, µg/mL)	Replicate Run	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
20 + 10	Injection 01	Dapa	4.563	3381.12	415.64	0.95	7763	10.12
		Saxa	7.156	877.15	75.29	0.93	9562	
	Injection 02	Dapa	4.563	3382.15	395.86	0.97	7056	10.05
		Saxa	7.156	872.36	75.02	0.93	9063	

Table 18. Specificity Performance

Analyte	Nominal Concentration (µg/mL)	Experimental Area	Quantity Recovered (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
Dapagliflozin	20.0	3381.12 / 3382.15	19.89	99.43%	99.45%	0.02	0.024%
Saxagliptin	10.0	877.15 / 872.37	9.91 / 9.85	99.12% / 98.54%	98.83%	0.41	0.416%

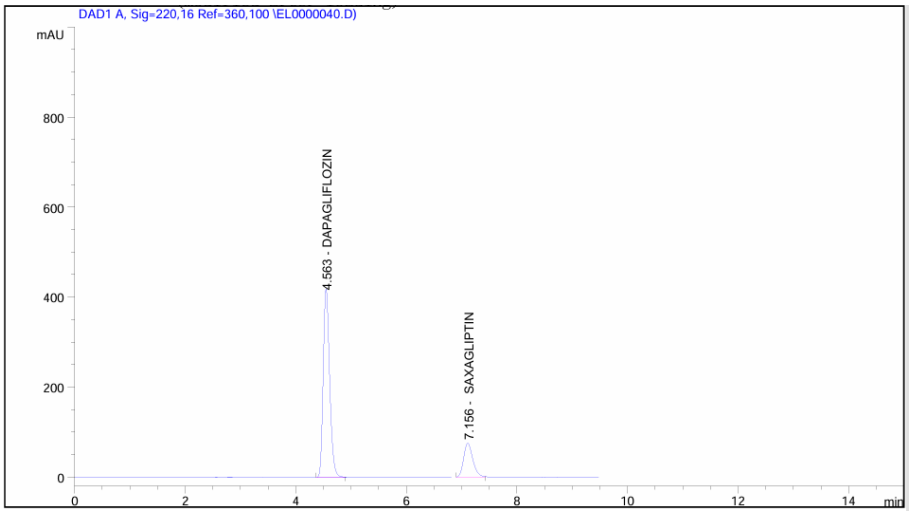

Figure 9. Chromatogram of specificity at 20+ 10 µg/mL

3.3.7. Ruggedness

Method ruggedness was verified by performing the assay under slightly different operating conditions (including multiple analysts and laboratories). The calculated recoveries remained near 100%, with low % RSD values of 0.16% for dapagliflozin and 0.37% for saxagliptin. These results show that the method is highly rugged and suitable for routine analytical applications. Chromatographic responses under different operating environments are detailed in Table 19 and Table 20.

Table 19. Peak Properties in Ruggedness Trials

Test Level (Dapa + Saxa, µg/mL)	Replicate Run	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
20 + 10	Injection 01	Dapa	4.523	3367.54	415.63	0.95	7723	10.12
		Saxa	7.153	875.64	75.29	0.93	9063	
	Injection 02	Dapa	4.563	3360.64	395.86	0.96	7056	10.05
		Saxa	7.162	879.90	75.02	0.92	9123	

Table 20. Ruggedness

Analyte	Concentration (µg/mL)	Experimental Area	Recovered Quantity (µg/mL)	Recovery Result (%)	Mean Recovery (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
Dapagliflozin	20.0	3367.54 / 3360.64	19.80 / 19.75	98.99% / 98.76%	98.87%	0.16	0.161%
Saxagliptin	10.0	875.64 / 879.90	9.89 / 9.95	98.94% / 99.45%	99.19%	0.37	0.368%

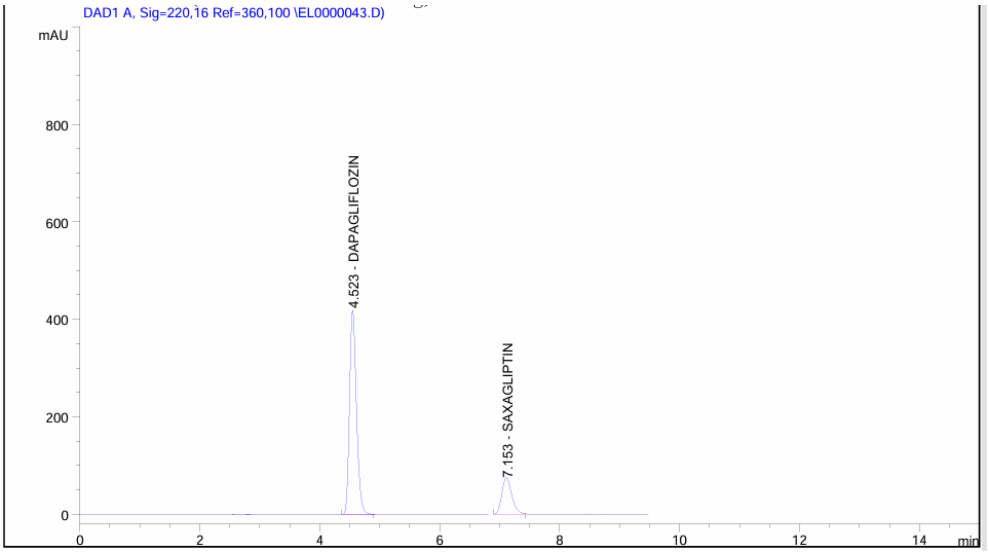

Figure 10. Chromatogram of Ruggedness at 20+ 10 µg/mL

3.3.8. Robustness

Robustness was evaluated by introducing small, deliberate modifications in mobile phase composition (organic ratio shifted to 69.7:30.3 v/v and 71.7:28.3 v/v) and detection wavelength (pm 1 nm around the 220 nm setpoint, evaluating limits at 219 nm and 221 nm). These small deliberate changes did not cause significant variations in peak area, retention times, tailing factors, or resolution. The low % RSD values obtained confirm that the method is highly robust and suitable for routine quality control laboratories. Chromatographic measurements under these modified conditions are summarized in Table 21 and Table 22.

Table 21. Robustness Peak Parameters

Parameter Varied	Mobile/Wavelength Setting	Replicate Run	Analyte	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
Mobile Phase Composition	69.7:30.3	Injection 01	Dapa	4.406	4947.94	631.93	0.96	7637	9.50
			Saxa	6.698	1301.41	118.41	0.95	9130	
		Injection 02	Dapa	4.405	4952.18	631.93	0.96	7635	9.50
			Saxa	6.696	1303.75	118.41	0.95	9132	
	71.7:28.3	Injection 01	Dapa	4.612	4964.83	584.11	0.97	7156	10.48
			Saxa	7.401	1296.06	106.63	0.94	8921	
		Injection 02	Dapa	4.615	4957.46	584.11	0.97	7155	10.48
			Saxa	7.400	1303.08	106.63	0.94	8922	
Detection Wavelength	219 nm	Injection 01	Dapa	4.546	4790.66	587.69	0.97	7528	10.13
			Saxa	7.141	1400.51	119.31	0.93	8939	
		Injection 02	Dapa	4.549	4805.66	587.69	0.97	7529	10.13
			Saxa	7.140	1405.55	119.31	0.93	8940	
	221 nm	Injection 01	Dapa	4.541	5086.96	617.94	0.97	7510	10.28
			Saxa	7.137	1097.79	89.40	0.91	9391	
		Injection 02	Dapa	4.543	5045.57	617.94	0.97	7510	10.28
			Saxa	7.139	1104.96	89.40	0.91	9391	

Table 22. Robustness Results

Target Parameter	Analyte	Modified Condition	Conc (µg/mL)	Experimental Area	Mean Area	Standard Deviation (SD)	relative Standard Deviation (%RSD)
Mobile Phase Ratio	Dapagliflozin	69.7:30.3	30.0	4947.94 / 4952.18	4950.10	3.00	0.06%
		71.7:28.3	30.0	4964.83 / 4957.46	4961.15	5.21	0.11%
	Saxagliptin	69.7:30.3	15.0	1301.41 / 1303.75	1302.60	1.65	0.13%
		71.7:28.3	15.0	1296.06 / 1303.08	1299.57	4.97	0.38%
Wavelength	Dapagliflozin	219 nm	30.0	4790.66 / 4805.66	4798.20	10.60	0.22%
		221 nm	30.0	5086.96 / 5045.57	5066.27	29.27	0.58%
	Saxagliptin	219 nm	15.0	1400.51 / 1405.55	1403.00	3.56	0.25%
		221 nm	15.0	1097.79 / 1104.96	1101.37	5.07	0.46%

3.4. Quantitative Evaluation of Commercial Formulation (Tab. Dapaglyn L)

The validated RP-HPLC method was applied for the quantitative determination of dapagliflozin and saxagliptin in Dapaglyn L tablets. The integrated peak areas of the sample solutions were compared against the standard calibration curves.

For dapagliflozin, the average recovery was 100.71% of the label claim (% RSD = 0.305%). For saxagliptin, the average recovery was 100.55% of the label claim (% RSD = 0.057%). These values indicate excellent recovery within the tablet formulation.

System peak parameters for the commercial assay are summarized in Table 23, and the formulation assay results are reported in Table 24.

Table 23. System Peak Integration Parameters for Commercial Formulations

Sample Target	Replicate Run	Eluting Peak	Retention Time (min)	Peak Area (mAU.s)	Peak Height (mAU)	Tailing Factor (T)	Plates (N)	Resolution (Rs)
Tab. Dapaglyn L	Injection 01	Dapa	4.535	6531.53	784.12	0.95	7292	10.09
		Saxa	7.098	1718.22	148.18	0.91	9290	
	Injection 02	Dapa	4.539	6504.80	784.45	0.95	7306	10.14
		Saxa	7.114	1716.88	149.24	0.91	9332	

Table 24. Assay of Formulation

Active Pharmaceutical Ingredient (API)	Nominal Quantity (µg/mL)	Experimental Area	Quantity Recovered (µg/mL)	Recovery Result (%)	Mean Formulation Potency (%)	Standard Deviation (SD)	relative Standard Deviation (%RSD)
Dapagliflozin	40.0	6531.53 / 6504.80	40.37 / 40.20	100.92% / 100.49%	100.71%	0.31	0.305%
Saxagliptin	20.0	1718.22 / 1716.88	20.12 / 20.10	100.59% / 100.51%	100.55%	0.06	0.057%

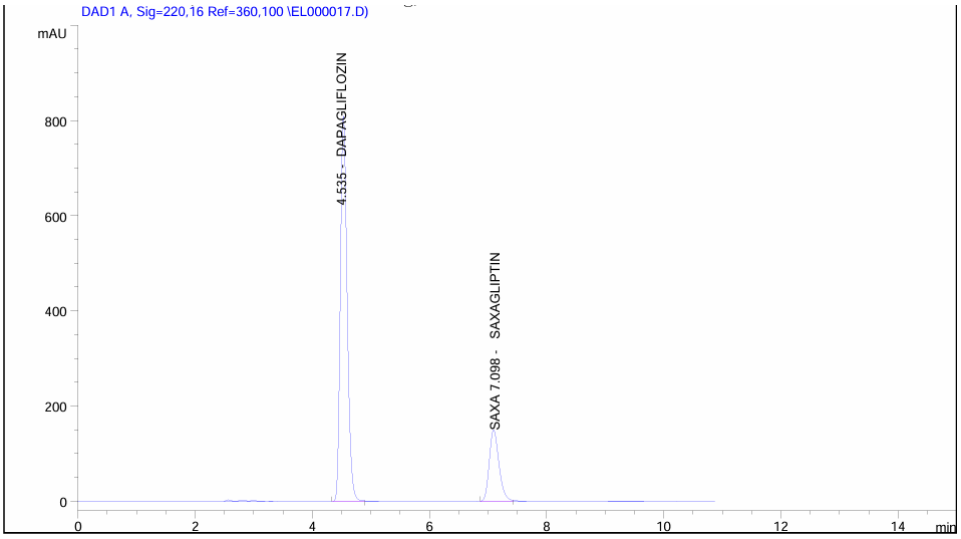

Figure 11. Chromatogram of assay study at 40 + 20 µg/mL

4. Conclusion

An efficient, accurate, precise, and stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of dapagliflozin and saxagliptin in bulk drug forms and commercial pharmaceutical formulations. The UV profiles in methanol showed absorption maxima at 225 nm for dapagliflozin and 213 nm for saxagliptin, with a well-resolved isosbestic point at 220 nm. Consequently, 220 nm was selected as the common detection wavelength. The chromatographic system was optimized across twelve trials. The optimal separation was achieved in Trial 12 using a C18 column (250 mm x 4.6 mm, 5 µm particle size) with an isocratic mobile phase consisting of methanol and 0.1% v/v aqueous acetic acid in a ratio of 70.7:29.3 v/v at a flow rate of 0.9 mL/min and a column temperature of 33°C. This setup resolved dapagliflozin and saxagliptin with sharp, symmetrical peaks ($T \leq 0.97$) at retention times of 4.554 min and 7.180 min, respectively, yielding a resolution of 10.25 and theoretical plate counts exceeding 7500. Validation was performed in accordance with ICH Q2(R1) guidelines. The method showed excellent linearity across the ranges of 10-50 µg/mL for dapagliflozin ($R^2 = 0.9998$) and 5-25 µg/mL for saxagliptin ($R^2 = 0.9997$). The recovery rates obtained during accuracy studies (98.57%-99.50%) were within regulatory limits. Precision evaluations (including repeatability, intraday, and interday runs) yielded % RSD values below 2.0%, showing high method reproducibility. The low LOD values (0.0031 µg/mL for dapagliflozin and 0.2833 µg/mL for saxagliptin) confirm the sensitivity of the method. Robustness and ruggedness

testing showed minimal variation in response under small, deliberate variations in organic ratio, wavelength, or analyst. The developed method was successfully applied to analyze a commercial tablet formulation (Tab. Dapaglyn L). The recoveries were near 100% of the label claims (100.71% for dapagliflozin and 100.55% for saxagliptin) with low standard deviations, and no interference from the excipient matrix was detected. These results confirm that this simple, robust, and cost-effective isocratic method is suitable for routine quality control and stability testing of these co-formulated drugs in pharmaceutical laboratories.

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