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SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF SODIUM-GLUCOSE COTRANSPORTER 2 (SGLT2) INHIBITOR AND DIPEPTIDYL PEPTIDASE-4 (DPP-4) INHIBITOR BY RP-HPLC

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ABSTRACT

A rapid and precise RP-HPLC method was developed and validated for the simultaneous estimation of Dapagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, and Saxagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, in pharmaceutical formulations. The method employed a Shimadzu LC-20AD system with a photodiode array detector and an Agilent C18 column. The mobile phase consisted of methanol and sodium acetate buffer at a 55:45 ratio, with a flow rate of 1.0 ml/min, and the detection wavelength was set at 228 nm. The retention times for Dapagliflozin and Saxagliptin were 2.314 minutes and 2.904 minutes, respectively. Method validation followed ICH guidelines, demonstrating high precision with % RSD values below 2% for both drugs. The linearity of the method was confirmed with R^2 values close to 0.999 for both analytes. The LOD and LOQ were 0.439 µg/ml and 1.33 µg/ml for Dapagliflozin and 0.257 µg/ml and 0.780 µg/ml for Saxagliptin, respectively. Forced degradation studies revealed the stability of both drugs under various stress conditions, with minor degradation observed. The method was successfully applied to the analysis of the marketed formulation Qtern, achieving accurate quantification with a % assay close to the labeled claim. This method offers a reliable and efficient approach to the routine analysis of these antidiabetic agents.

KEYWORDS: RP-HPLC, Dapagliflozin, Saxagliptin, Simultaneous Estimation, Method Validation, Forced Degradation, Qtern.

INTRODUCTION

Dapagliflozin (Figure 1) is a sodium-glucose co-transporter 2 (SGLT2) inhibitor used primarily for the management of type 2 diabetes mellitus. It works by inhibiting SGLT2 in the proximal renal tubules, reducing glucose reabsorption and increasing urinary glucose excretion, which helps lower blood glucose levels. Dapagliflozin has shown benefits in weight reduction and lowering blood pressure, making it a favorable option for patients with type 2 diabetes and cardiovascular risks. Additionally, it has been indicated for use in heart failure with reduced ejection fraction (HFrEF) to reduce the risk of hospitalization and cardiovascular mortality.[1]

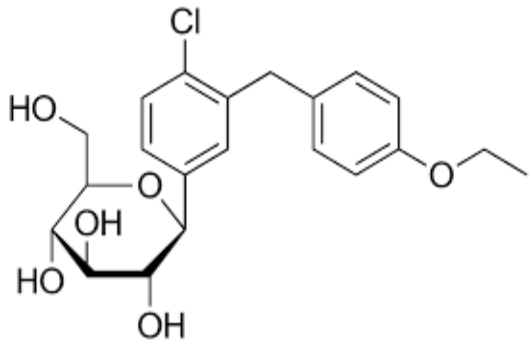

Figure 1: Chemical structure of dapagliflozin.

Saxagliptin (Figure 2) is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances glucose regulation in patients with type 2 diabetes by preventing the degradation of incretin hormones, such as GLP-1 and GIP. These hormones stimulate insulin release in response to meals and decrease glucagon secretion, improving glycemic control. Saxagliptin is typically used as an adjunct to diet and exercise and can be combined with other antidiabetic agents like metformin or SGLT2 inhibitors for enhanced efficacy. Its cardiovascular profile is neutral, but it is considered a safe and effective option for improving postprandial glucose levels in patients who require additional glycemic control.[2]

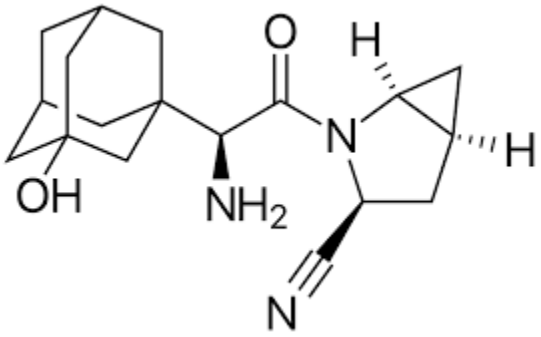

Figure 2: Chemical structure of saxagliptin.

MATERIALS AND METHODOLOGY

Chemicals and Reagents

Pharma Life Research Lab Pharmaceuticals, Hyderabad, Telangana, provided Dapagliflozin and Saxagliptin reference standards. Qtern® tablets were obtained from a local market in Hyderabad, Telangana. Merck Ltd. provided HPLC-grade sodium acetate buffer, acetonitrile, methanol, and water. All of the other compounds in the research were classified as AR. Milli-Q water was used to prepare the mobile phase.

Instrumentation

Shimadzu® LC-20AD pumps, SPD-M20A photodiode array detector with adjustable wavelength programmability, CBM-20A system controller, and manual injector were used for chromatography. The Shimadzu® LC solution software was used to collect the data.

Preparation of Stock and Standard solutions

Separate stock solutions of Dapagliflozin and Saxagliptin were created by dissolving precisely weighed 10 mg of each medication in 100 mL of methanol to form a 100 µg ml^{-1} stock solution of each drug. These stock solutions were further diluted with the same mobile phase as needed to establish working standard solutions of 2–12 µg ml^{-1} and 1-6 µg ml^{-1} of Dapagliflozin and Saxagliptin, respectively, for linearity and other analytical procedures. The finished solution was filtered via a 0.45 µm Millipore membrane filter.

Method validation

The validated chromatographic technique was evaluated for system appropriateness, specificity, precision, accuracy, linearity, the limit of detection (LOD), the limit of quantitation (LOQ), and robustness using the protocols outlined in the ICH recommendations Q2 (R1).[3-19]

System suitability

By injecting a blank mobile phase followed by six repetitions of Dapagliflozin (5 µg ml^{-1}) and Saxagliptin (2.5 µg ml^{-1}) combination, system suitability parameters for tailing factor, repeatability, number of theoretical plates, and resolution between Dapagliflozin and Saxagliptin peaks were examined.

Linearity

To establish six standard concentrations, aliquots of drug working solutions were transferred to 10 mL volumetric flasks and diluted with the mobile phase. Linear regression analysis using the least-squares regression approach was used to assess linearity.

Precision and Accuracy

Intra-day precision was examined on the same day, while inter-day precision was examined over three days. The results were shown using a computed percent relative standard deviation. The accuracy was measured at three distinct levels: 50%, 100%, and 150%. The mean standard deviation was used to compute the accuracy percentage.

Robustness

Deliberate minute changes in chromatographic parameters such as flow rate, mobile phase ratio, wavelength, and buffer component pH have occurred. The tailing factor, percent RSD, and percentage recovery of Dapagliflozin and Saxagliptin peaks were also examined.

LOD and LOQ

The formula was used to compute the LOD and LOQ for Dapagliflozin and Saxagliptin using the linear regression equation and a standard deviation of the intercept and slope.

Forced degradation studies

Forced degradation studies for Dapagliflozin and Saxagliptin were conducted to assess their stability and method robustness under various stress conditions, including acid hydrolysis (0.5 N HCl), base hydrolysis (0.5 N NaOH), oxidation (3% H2O2), thermal, and photolytic degradation. Acid and base degradation experiments were performed by mixing 1 ml of the drug sample solution with 0.5 N HCl or 0.5 N NaOH in a 10 ml volumetric flask, then heating in a controlled temperature bath at 80°C±1°C for four hours. The samples were then neutralized and injected in duplicate under optimal chromatographic conditions. Oxidative degradation used a similar approach with 3% hydrogen peroxide to induce oxidative stress.

For thermal degradation, aliquots of the sample solutions were heated at 80°C±1°C for four hours, diluted with the mobile phase, and analyzed in triplicate. Photolytic degradation involved exposing 1 ml aliquots of the samples to visible light with a minimum of 1.2 million lux hours and 200-watt hours/square meter over a wavelength range of 320-400 nm for four hours before dilution and triplicate analysis under the same chromatographic conditions. These studies confirmed the stability of both drugs under the tested conditions, establishing method robustness.

RESULTS AND DISCUSSIONS

Method development

The typical chromatogram of Dapagliflozin and Saxagliptin displays two distinct peaks, with Dapagliflozin eluting at a retention time of approximately 2.273 minutes and Saxagliptin at 2.783 minutes. The chromatogram shows well-separated, sharp peaks, indicating effective separation of the two compounds under the specified chromatographic conditions. The peak shape and baseline resolution suggest a stable and reproducible method suitable for the simultaneous estimation of Dapagliflozin and Saxagliptin in combined formulations. The detection was carried out using UV absorption, with both analytes showing clear and identifiable peaks, making this method viable for routine analysis.

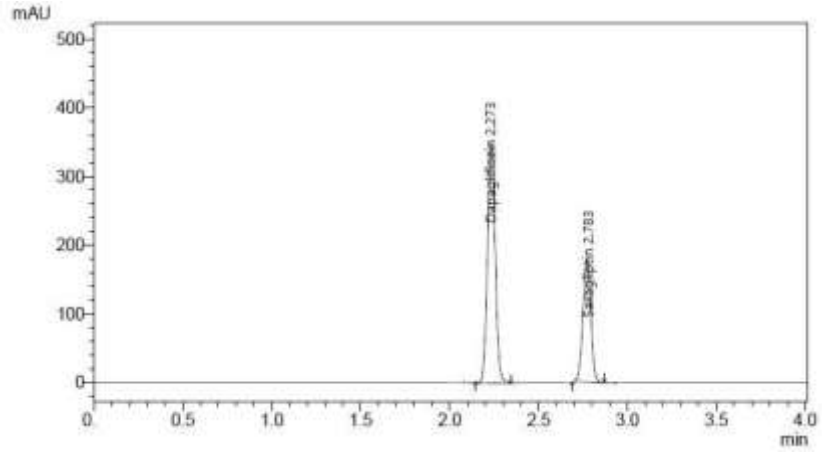

Figure 3: Typical Chromatogram of Dapagliflozin and Saxagliptin.

System suitability

The chromatographic analysis of Dapagliflozin and Saxagliptin resulted in USP tailing factors of 1.34 and 1.43, respectively, indicating symmetrical peak shapes for both compounds. The USP plate count was found to be 5832 for Dapagliflozin and 4392 for

Saxagliptin, suggesting efficient column performance and good resolution of the analytes, making the method suitable for accurate quantification.

Accuracy

The recovery study for Dapagliflozin and Saxagliptin at different spiking levels (50%, 100%, and 150%) demonstrated consistent and reliable results. For Dapagliflozin, the % recovery was 100.33%, 100.37%, and 99.87% at 50%, 100%, and 150% spiking levels, respectively, with % RSD values ranging from 0.60 to 1.70, indicating high precision. Similarly, Saxagliptin showed % recoveries of 100.67%, 100.83%, and 100.37% across the same levels with % RSD values between 1.80 and 1.92, reflecting good accuracy and repeatability. The overall % recovery for both drugs remained close to 100%, demonstrating the robustness and reliability of the analytical method for quantifying these drugs.

Table 1: Accuracy Data of Dapagliflozin and Saxagliptin.

Name of the drug	% Level spiking	% RSD	% Recovery
Dapagliflozin	50%	1.70	100.33
	100%	0.89	100.37
	150%	0.60	99.87
Saxagliptin	50%	1.92	100.67
	100%	1.858	100.83
	150%	1.80	100.37

System precision

The precision analysis of the method showed that the mean peak areas for Dapagliflozin and Saxagliptin were 71,250.33 and 55,507.50, respectively, with standard deviations (SD) of 820.04 and 520.3. The % RSD values for the peak areas were 0.98% for Dapagliflozin and 1.16% for Saxagliptin, indicating good system precision. For the % assay, the mean values were 100.2% for Dapagliflozin and 102.35% for Saxagliptin, with SDs of 1.08 and 1.52. The % RSD for the % assay was 1.18% for Dapagliflozin and 1.02% for Saxagliptin, demonstrating that the method is precise and reproducible, meeting the acceptance criteria of a % RSD below 2%.

Table 2: System Precision Results of DAPA and SEXA.

Precision Type	Peak Areas of Dapagliflozin	Peak Areas of Saxagliptin	% Assay of Dapagliflozin	% Assay of Saxagliptin
Mean	71250.33	55507.50	100.2	102.35
SD (±)	820.04	520.3	1.08	1.52
RSD (%)	0.98	1.16	1.18	1.02

Intermediate precision

The precision analysis at different concentrations of Dapagliflozin and Saxagliptin demonstrated consistent and reproducible results. For Dapagliflozin at 4.0 µg/ml and Saxagliptin at 2.0 µg/ml, the mean peak areas were 61,329.67 and 44,477.67, with % RSD values of 1.42% and 0.90%, respectively. At concentrations of Dapagliflozin 5.0 µg/ml and Saxagliptin 2.5 µg/ml, the mean areas were 75,713.33 for Dapagliflozin and 55,899 for Saxagliptin, showing % RSDs of 0.93% and 1.80%. For Dapagliflozin 6.0 µg/ml and Saxagliptin 3.0 µg/ml, the mean areas were 90,981.33 and 68,851.33, with % RSD values of 1.29% for Dapagliflozin and 0.62% for Saxagliptin. All % RSD values were within acceptable limits (less than 2%), indicating that the method provides precise results across the tested concentration ranges.

Interday precision

The % RSD results across different concentrations of Dapagliflozin and Saxagliptin indicate good precision. For Dapagliflozin at 4.0 µg/ml and Saxagliptin at 2.0 µg/ml, the % RSDs were 0.58% and 0.53%, respectively. At 5.0 µg/ml of Dapagliflozin and 2.5 µg/ml of Saxagliptin, the % RSDs were 0.42% and 1.10%. For 6.0 µg/ml Dapagliflozin and 3.0 µg/ml Saxagliptin, the % RSDs were 0.28% and 1.78%. All values are within the acceptable range (below 2%), indicating the method's reliability and repeatability.

Linearity

The correlation coefficient R2 (0.9999) shows that DAPA and SAXA's analytical calibration curves were linear in the relevant ranges. The linear regression equations for Dapagliflozin and Saxagliptin are 15108x + 1385.7 (R2 = 0.9998) and 17071x + 550.47 (R2 = 0.9997).

Table 3: Linearity results of Dapagliflozin and Saxagliptin.

Dapagliflozin		Saxagliptin	
Standard Concentration (µg/ml)	**Peak area**	**Standard Concentration (µg/ml)**	**Peak area**
2	32183	1	17543
4	61382	2	34557
6	91483	3	52457
8	123034	4	68648
10	151382	5	85134
12	183384	6	103456

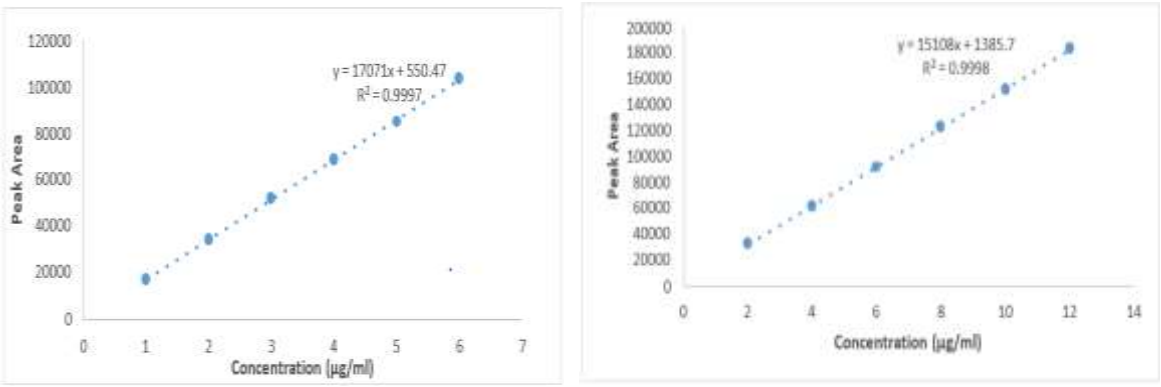

Figure 4: Calibration curves of Dapagliflozin and Saxagliptin.

Robustness

The % RSD results for Dapagliflozin and Saxagliptin under different conditions indicate consistent and reliable performance of the method. For changes in flow rate, the % RSD values for retention time, tailing factor, and % recovery ranged from 0.33% to 1.46%, demonstrating minimal variability. When varying pH, the % RSD values for both drugs were below 2%, ranging from 1.11% to 1.92%, indicating good stability across pH changes. Adjustments in wavelength resulted in % RSD values from 0.78% to 1.47%, showing precise detection. For variations in the organic ratio of the mobile phase, the % RSD values remained within 0.45% to 1.93%, highlighting the method's robustness across different mobile phase compositions. All % RSD values were below the acceptance threshold of 2%, confirming the method's reliability under varied analytical conditions.

LOD and LOQ

The sensitivity of the analytical method for detecting and quantifying Dapagliflozin and Saxagliptin was evaluated by determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD is the lowest drug concentration that can be detected but not necessarily quantified with acceptable precision. At the same time, the LOQ is the minimum concentration that can be quantitatively measured with suitable precision and accuracy. The LOD was determined to be 0.439 µg/ml for Dapagliflozin, indicating the method's ability to detect deficient drug concentrations in a sample. The LOQ for Dapagliflozin was 1.33 µg/ml, representing the lowest concentration reliably quantified. For Saxagliptin, the LOD was 0.257 µg/ml, showing the method's higher sensitivity in detecting this drug at even lower levels. The LOQ for Saxagliptin was 0.780 µg/ml, allowing for accurate and precise quantification at this concentration. These results reflect the method's high sensitivity and suitability for trace analysis of both Dapagliflozin and Saxagliptin in various sample matrices.

Assay

The marketed formulation Qtern, containing 10 mg of Dapagliflozin and 5 mg of Saxagliptin, was analyzed to determine each component's retention time, peak area, and % assay. Dapagliflozin exhibited a retention time of 2.314 minutes with a peak area of 763,628 and an % assay of 99.23%, indicating that the measured concentration was very close to the labeled amount. Saxagliptin showed a retention time of 2.904 minutes, with a peak area of 542,920 and an % assay of 100.23%, confirming accurate quantification per the formulation's label claim. The results demonstrate the method's effectiveness for simultaneously estimating both active ingredients in the Qtern tablet.

Forced degradation studies

The degradation studies of Dapagliflozin and Saxagliptin under various stress conditions revealed their stability profiles and susceptibility to different degradation mechanisms. For Dapagliflozin, the retention time (Rt) they were varied slightly across conditions, with acid hydrolysis showing an Rt of 2.26 minutes and a % drug degradation of 9.22%, while base hydrolysis resulted in 6.33% degradation at an Rt of 2.45 minutes. Oxidative stress caused 7.22% degradation, with a purity angle of 0.454 and a purity threshold of 0.594, indicating that the drug remained within acceptable purity limits. Thermal and photolytic conditions resulted in lower degradation, at 1.56% and 0.92%, respectively. For Saxagliptin, acid conditions led to the highest degradation at 12.21% with an Rt of 2.77 minutes, while base hydrolysis resulted in 9.45% degradation at an Rt of 3.02 minutes. Oxidative conditions caused 5.67% degradation, and thermal and photolytic conditions resulted in minimal degradation at 1.65% and 1.13%, respectively. The purity angle and threshold values across all conditions for both drugs suggest that the method can effectively separate degraded products from the parent compound, confirming the stability-indicating nature of the analytical method.

CONCLUSION

The developed RP-HPLC method for simultaneous estimating Dapagliflozin and Saxagliptin in pharmaceutical formulations has proven accurate, precise, and robust. The method validation, conducted according to ICH guidelines, confirmed that the method exhibits good linearity, sensitivity, and repeatability. The LOD and LOQ values demonstrate the method's ability to detect and quantify trace levels of both drugs effectively. Forced degradation studies indicated that the method could distinguish between the active drugs and their

degradation products, ensuring its suitability for stability testing. Additionally, applying this method to the analysis of the marketed formulation, Qtern validated its practical utility, achieving results that align closely with the product's labeled content. Overall, this method is an efficient and reliable option for the routine quality control of Dapagliflozin and Saxagliptin, contributing to accurately assessing these widely used antidiabetic agents.

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