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Estimation of Alogliptin and Dapagliflozin in Synthetic Mixture by RP-HPLC Method

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Abstract

The primary objective of the proposed research was to develop and validate analytical methods for the simultaneous quantification of Alogliptin and Dapagliflozin in a synthetic mixture. Alogliptin and Dapagliflozin are medications used for managing diabetes, with Alogliptin inhibiting the enzyme Dipeptidyl peptidase-4 and Dapagliflozin belonging to the class of sodium-glucose cotransporter 2 inhibitors. High-performance liquid chromatography method development was conducted using a C18 column (250 mmX4.6 mm, 5 µm particle size). Chromatographic separation was achieved by isocratic mode using a mobile phase consisting of methanol and 0.1% ortho-phosphoric acid (pH=3.5 ± 0.01) in the ratio of 95:05 % v/v. The flow rate was maintained at 1 ml/min, and the selected wavelength for estimation was 210 nm. The retention time was determined to be 2.193 min for Alogliptin and 3.901 min for Dapagliflozin. The drugs' responses showed linear behaviour with correlation coefficient values of nearly 0.999 for both Alogliptin and Dapagliflozin over a linearity range of 10–60 µg/ml. Recoveries from studies were found to be 99.89% for Alogliptin and 100.06% for Dapagliflozin, indicating high accuracy. The method demonstrated sensitivity with low limit of detection and limit of quantification values. The method was validated according to the International Council for Harmonisation guideline Q2(R1) and was found to be linear, precise, accurate, sensitive, and robust. One significant benefit of the present study was the optimization of the mobile phase, which did not require a buffer solution. The proposed research has the potential for quantitative and qualitative analysis of both drugs in synthetic mixtures and will be further utilized for stability studies and the development of bioanalytical methods in biological fluids.

Keywords: Alogliptin, Dapagliflozin, high-performance liquid chromatography, validation, diabetes

INTRODUCTION

Diabetes is a metabolic condition characterized by abnormalities in protein, carbohydrate, and fat metabolism, stemming from either insufficient insulin production or tissue insensitivity to insulin. Common symptoms among diabetic patients include fatigue, excessive thirst, frequent urination, and weight loss. Type-2 Diabetes Mellitus (T2DM) is prevalent among the population and is marked by inadequate insulin secretion due to insulin resistance. It is a progressive disease, with its prevalence and incidence increasing with age. T2DM constitutes 80%–90% of all diabetes cases, with intra-abdominal obesity commonly observed among patients, indicating a link to insulin resistance. This type of diabetes is strongly associated with factors such as family history, advanced age, obesity, and sedentary lifestyle.

Various drugs are available for the treatment of T2DM, including alpha-glucosidase inhibitors, dipeptidyl peptidase-4 (DPP-4), and sodium-glucose cotransporter 2 (SGLT2) inhibitors. The market offers a plethora of options for diabetes

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management, emphasizing the necessity for quality medications to effectively control blood glucose levels and provide relief to patients.

Numerous methods exist for estimating Alogliptin (ALO) and Dapagliflozin (DAPA) individually, utilizing Ultraviolet (UV) spectroscopy [1–5], Reverse phase high-performance liquid chromatography (RP-HPLC) [6–22], and liquid chromatography-mass spectrometry (LC-MS) [23] in both single and combination dosage forms. Presently, regulatory authorities recommend manufacturers to employ sophisticated instruments such as HPLC for quantitative and qualitative estimation. Therefore, it is advantageous for analysts to utilize a developed and validated, sensitive method for the simultaneous estimation of ALO and DAPA via liquid chromatography in synthetic mixtures.

CHEMICALS AND MATERIALS

Various chemicals were utilized in the research, including acetonitrile, methanol, triple distilled water, and Ortho-phosphoric acid (OPA), all of which were of HPLC grade. These chemicals played crucial roles in the development and validation of the analytical method.

Both ALO and DAPA were obtained as gift samples from Piramal Pharmaceuticals, Ahmedabad, India and their identities were confirmed through melting point tests. This ensured the authenticity and quality of the drugs used in the present study.

The analysis was conducted using an HPLC 2030 model manufactured by Shimadzu Corporation, Japan, which provided the necessary instrumentation for precise and accurate measurements. Additionally, UV spectroscopy was performed using a Shimadzu UV-1800 spectrophotometer, also from Shimadzu Corporation, Japan. This instrument aided in determining the appropriate wavelengths through UV spectra, crucial for the accurate quantification of the drugs in the synthetic mixture.

Chromatographic Condition

The estimation was conducted using specific chromatographic conditions. A C18 column with dimensions of 250 mm × 4.6 mm and a particle size of 0.5 microns was employed for separation. The column oven temperature was maintained at 25°C throughout the analysis to ensure consistent conditions.

After multiple trials, the mobile phase was optimized to be methanol:water containing 0.1% OPA in a ratio of 95:05% v/v, with the pH adjusted to 3.5±0.02. This selection was crucial for achieving effective separation and accurate quantification of the target compounds.

During the analysis, an injection volume of 10 µL was used, and the flow rate was set at 1 ml/min. Both ALO and DAPA were monitored at a wavelength of 210 nm, which was determined to be optimal for their detection and quantification within the mixture. These carefully selected chromatographic conditions ensured reliable and precise results for the estimation of the drugs.

Preparation of Mobile Phase

The mobile phase was formulated by blending methanol with 0.1% OPA solution, adjusted to a pH of 3.5. This mixture was composed of methanol and the acid solution in a ratio of 95:05 %v/v.

To ensure homogeneity and remove any particulate matter, the prepared mobile phase underwent sonication and subsequent filtration through a 0.45 µm membrane filter. This meticulous preparation procedure was essential to guarantee the stability and purity of the mobile phase, thereby facilitating accurate chromatographic analysis.

Preparation of Standard Stock Solution

An accurate measurement of 1 mg of both ALO and DAPA drugs was precisely transferred into separate 10 ml volumetric flasks. Subsequently, each flask was filled to the mark with methanol to achieve a final concentration of 100 µg/ml for each drug. This meticulous procedure ensured the proper

preparation of standardized solutions for subsequent analytical processes, maintaining consistency and reliability in the experimental setup.

Preparation of Working Stock Solution

An aliquot of 3 ml was withdrawn from the standard stock solution containing ALO and DAPA and transferred into individual 10 ml volumetric flasks. The volume was then adjusted to the mark with the appropriate solvent, resulting in a final concentration of 30 µg/ml for each drug. This meticulous procedure ensured the accurate preparation of standardized solutions, maintaining consistency and reliability in the subsequent analytical processes.

EXPERIMENTAL CONDITIONS

Preparation of Calibration Curve (Linearity)

Working standard solutions were prepared by transferring aliquots of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, and 6 ml from the stock solutions into separate 10 ml volumetric flasks. This process resulted in solutions with concentrations ranging from 10 µg/ml to 60 µg/ml for both ALO and DAPA.

Subsequently, these prepared solutions were further diluted with the mobile phase to achieve the desired concentrations. Finally, each solution was injected into the chromatographic system for analysis. This methodical approach ensured the availability of a range of concentrations for calibration purposes, facilitating accurate quantification of ALO and DAPA in the subsequent analysis.

System Suitability

System suitability studies were conducted in accordance with the guidelines outlined by the International Council for Harmonisation (ICH). The parameters including tailing factor, number of theoretical plates, and resolution were evaluated by injecting six replicates of a standard solution with a concentration of 30 µg/ml into the HPLC system.

This systematic approach ensured the assessment of the system's performance and suitability for the intended analysis. By analyzing multiple replicates of the standard solution, any variability in the chromatographic system could be identified, ensuring reliable and consistent results in subsequent analyses.

Specificity

Specificity was assessed by comparing the overlay spectra of the working standard solutions with those of the blank, which consisted of the diluent used for sample preparation. This comparison aimed to identify any potential interference or hindrance from the diluent, as well as any impurities originating from the synthetic process or formed during shelf life.

By visually inspecting the overlay spectra, any deviations or additional peaks could be detected, indicating the presence of impurities or interference. This analysis ensured that the method accurately measured the target compounds without interference from other components, guaranteeing the reliability and validity of the analytical results.

Limit of Detection and Limit of Quantitation

The linearity study was performed in triplicate to ensure the robustness and reliability of the results. To determine the limits of detection (LOD) and limit of quantification (LOQ), the average of the slope and the standard deviation of the intercept were utilized in the calculations.

This approach allowed for the accurate assessment of the minimum detectable concentration (LOD) and the minimum quantifiable concentration (LOQ) of the analytes in the sample matrix. By employing these calculations, the method's sensitivity and its suitability for the intended analysis were rigorously evaluated.

$LOD = 3.3 \times (\sigma / S)$
$LOQ = 10 \times (\sigma / S)$

Where,
σ = The standard deviation of Y-intercept of three calibration curves;
S = The mean slope of the three calibration curves

Accuracy

The accuracy of the method was verified through recovery studies conducted using the standard addition method. This involved comparing the theoretical amount of the analyte added to the sample with the actual amount recovered.

The accuracy assessment was performed by spiking the Active Pharmaceutical Ingredient (API) into tablet samples at specified levels. The study was carried out at three different concentration levels, that is, 50%, 100%, and 150% of the target concentration. Each level was analyzed in triplicate to ensure robustness and reliability of the results.

By comparing the theoretically added amount with the practically achieved amount, the accuracy of the method could be determined across various concentration levels, providing valuable insights into its performance and reliability in quantitative analysis.

Precision

Intraday precision was evaluated by conducting the analysis at different times within the same day, specifically at 10 am, 1 pm, and 4 pm. The concentration of the analytes was measured, and the percentage relative standard deviation (% RSD) was calculated to assess the variability of the results within the day.

Inter-day precision was assessed by analyzing standards of the same concentration on different days, specifically on day 1, day 3, and day 7. The % RSD was calculated to determine the consistency and reproducibility of the method over multiple days of analysis.

These precision studies provided valuable information about the method's reliability and consistency over time, helping to ensure the accuracy and robustness of the analytical procedure.

Robustness

Robustness testing was conducted by intentionally altering specific parameters such as the mobile phase ratio, wavelength, and flow rate. The %RSD was then calculated to assess the impact of these variations on the analytical results.

By systematically adjusting these parameters and measuring the resulting %RSD, the study aimed to evaluate the method's resilience to minor fluctuations in experimental conditions. This assessment provided valuable insights into the method's reliability and ability to produce consistent and accurate results under different operational settings.

Assay

For both dosage combinations, an assay procedure was conducted. Test solutions were prepared and injected into the chromatographic system after stabilizing the baseline. Subsequently, chromatograms were recorded to capture the response of the analytes.

The concentration of each test solution was determined using the slope and intercept values obtained from the calibration graph. This approach ensured accurate quantification of the analytes in the test solutions, enabling the assessment of their concentration levels in the samples.

RESULT AND DISCUSSION

Linearity

In the linearity assessment, a calibration curve was constructed by plotting concentration against the corresponding area obtained for both ALO and DAPA. The correlation coefficient for ALO was determined to be 0.999, while for DAPA, it was also found to be 0.999. These high correlation coefficient values indicate excellent linearity of the method across the tested concentration range.

The linearity chromatogram is depicted in Figure 1, providing visual representation of the linear relationship between concentration and area for both the analytes. This graphical representation further supports the conclusion that the method exhibits satisfactory linearity, enhancing confidence in the accuracy and reliability of quantitative analysis.

System Suitability

The obtained data were analyzed to calculate the %RSD, resulting in values of 1.01% for ALO and 0.27% for DAPA. Both %RSD values were found to be less than 2% for both the drugs (Figures 2 and 3).

This indicates a high level of precision and reproducibility in the analytical method, with the variation in results being minimal. The low %RSD values further validate the reliability and consistency of the method in quantifying ALO and DAPA, enhancing confidence in the accuracy of the analytical data obtained.

Specificity

No interference was observed for excipients or any unwanted substances in the chromatogram. As a result, it was concluded that the proposed method exhibited specificity, effectively isolating and accurately quantifying the target analytes. This specificity is crucial for ensuring the reliability and validity of the analytical results, as it confirms that the method is capable of distinguishing and quantifying the analytes of interest without interference from other components in the sample matrix.

Limit of Detection and Limit of Quantification

The LOD was determined to be 0.9012 µg/ml for ALO and 0.3342 µg/ml for DAPA, while the LOQ was found to be 2.7310 µg/ml for ALO and 1.0128 µg/ml for DAPA. These low values indicate the high sensitivity of the method.

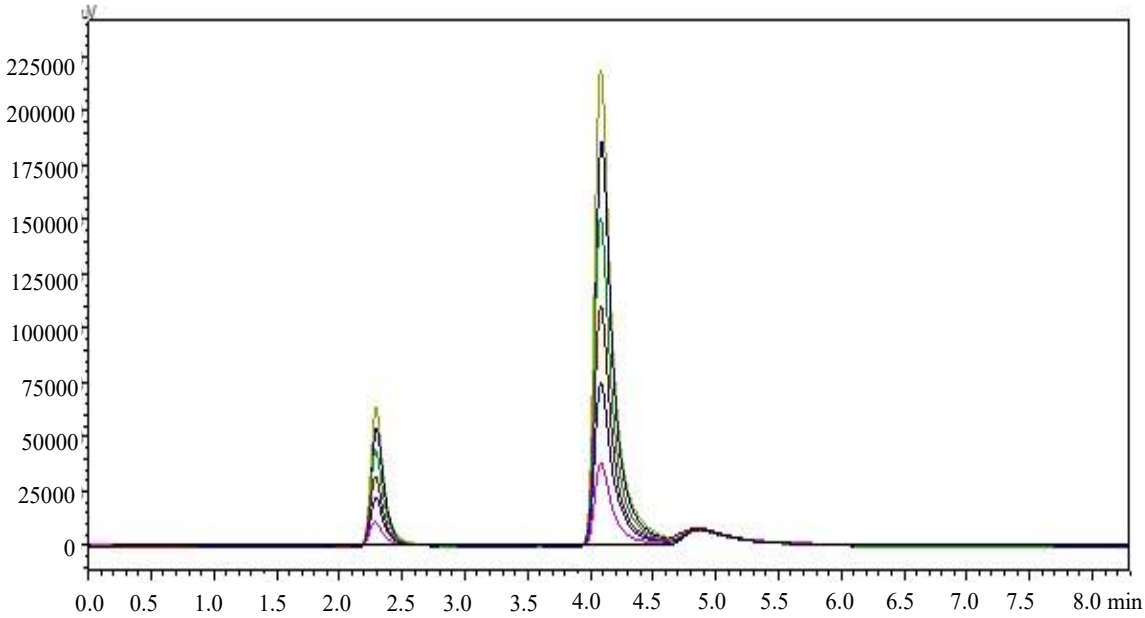

Figure 1. Chromatogram of linearity (Retention time for ALO: 2.1 min and DAPA: 3.9 min).

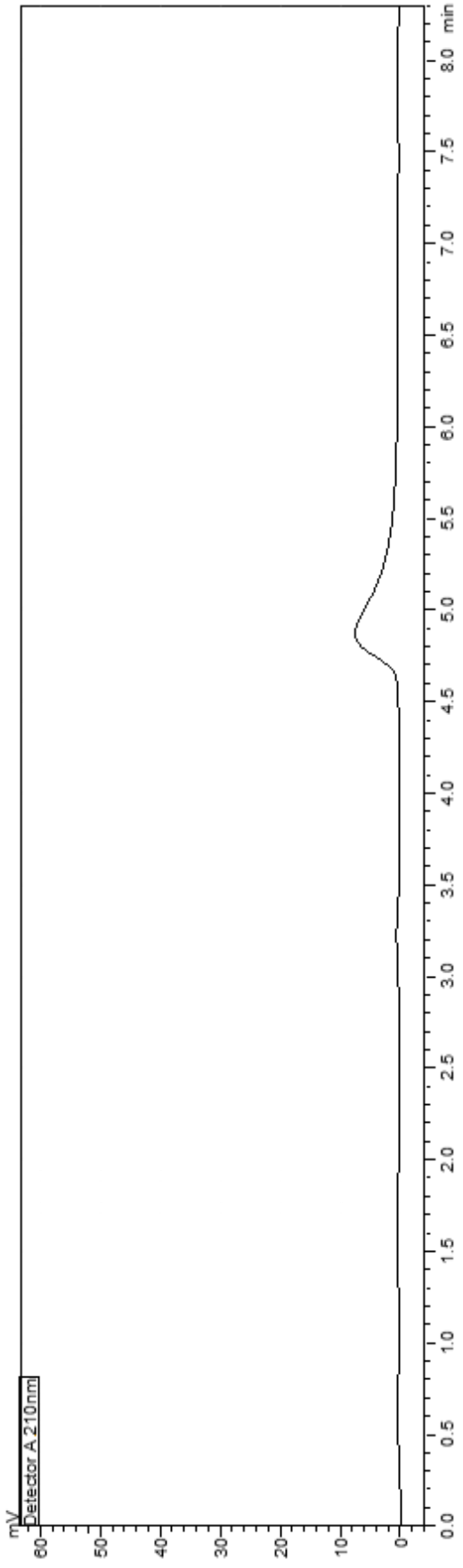

Figure 2. Chromatogram of blank.

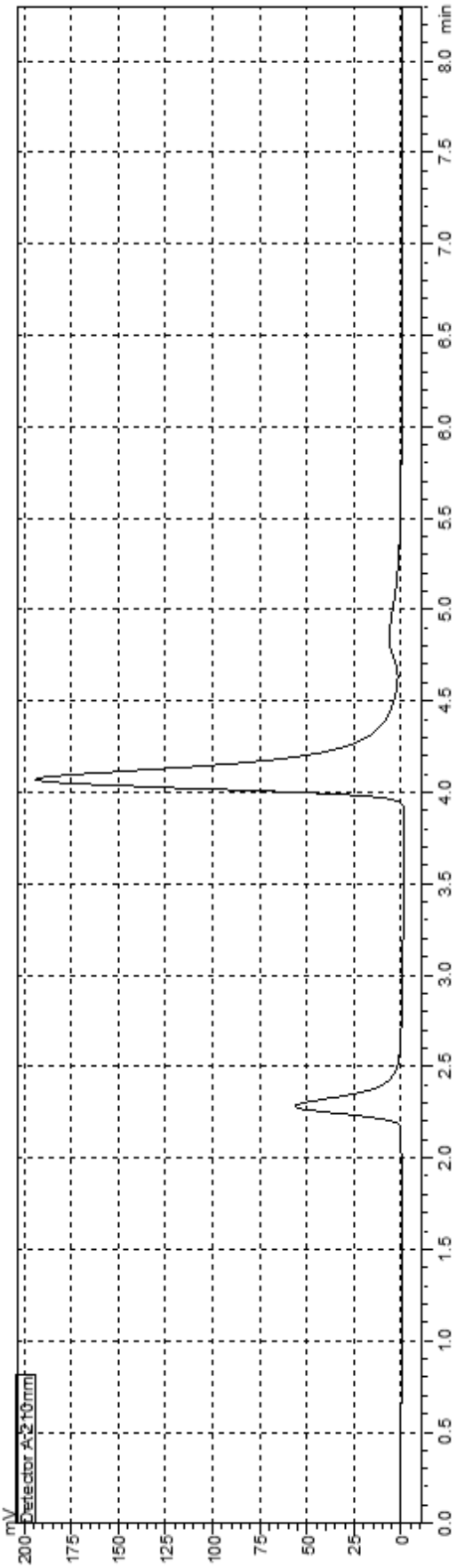

Figure 3. Chromatogram of standard (ALO: 2.1 min and DAPA: 3.9 min).

The ability to detect and quantify ALO and DAPA at such low concentrations underscores the method's sensitivity, further affirming its capability to accurately measure trace amounts of the analytes. This sensitivity is essential for ensuring the method's effectiveness in practical applications, particularly in detecting and quantifying analytes in complex matrices at low concentrations.

Accuracy

Various samples were injected, and the data were analyzed to determine the percentage recovery. The mean percentage recovery of ALO and DAPA was found to be within the range of 99–101%. This indicates that the method demonstrated accuracy in quantifying the analytes.

Table 1 likely presents the detailed results of the percentage recovery analysis, corroborating the accuracy of the method across multiple samples. The ability to achieve close to 100% recovery suggests that the method effectively quantified the target analytes, further enhancing confidence in the reliability and validity of the analytical results.

Table 1. Result of system suitability.

Concentration (µg/ml)		Mean ± SD		%RSD	
ALO	***DAPA***	***ALO***	***DAPA***	***ALO***	***DAPA***
30	30	408788.7 ± 4139.228	1899464 ± 5155.931	1.01	0.27

ALO: Alogliptin; DAPA: Dapagliflozin; %RSD: relative standard deviation.

Precision

The %RSD for intra-day and inter-day precision was determined to be less than 2% for both ALO and DAPA. These precision measurements were employed to evaluate the reproducibility of the developed method.

The data obtained from the precision studies indicated that the method exhibited precision and reproducibility, as the %RSD values for both ALO and DAPA fell within the acceptable range. This demonstrated the method's capability to generate consistent and reliable results across multiple analyses, providing assurance of the method's robustness and suitability for quantitative analysis of the analytes.

Robustness

Minor changes in chromatographic conditions, such as variations in the mobile phase composition, wavelength, and flow rate, were assessed to determine the robustness of the method. The data obtained from the robustness study indicated that the method exhibited robustness.

This assessment involved evaluating the method's performance under slightly altered conditions to gauge its resilience and ability to produce consistent results. The findings from the robustness study confirmed that the method maintained its accuracy and reliability despite minor variations in chromatographic parameters. Thus, demonstrating the method's suitability for practical application in routine analytical procedures.

Assay

The validated HPLC method was effectively utilized to determine the concentrations of ALO and DAPA in formulations. The results obtained were 98.93% for ALO and 99.96% for DAPA, respectively, which closely matched the corresponding label claim values.

Table 1 likely presents a detailed comparison between the measured concentrations and the label claim values for both ALO and DAPA in the formulations. The high degree of agreement between the measured values and the label claim values indicates the accuracy and reliability of the developed HPLC method for quantifying ALO and DAPA in the formulations.

CONCLUSION

An analytical technique for quantifying ALO and DAPA in synthetic mixture form was developed and validated following the guidelines outlined in the ICH Q2(R1). The proposed method offers the advantage of simplicity, as it can be implemented using a straightforward HPLC approach. Through meticulous validation, the proposed study demonstrated precision, accuracy, and sensitivity.

Furthermore, based on the validation results, the developed method was deemed to be not only precise, sensitive, and accurate but also robust. These qualities affirm the reliability and consistency of the method across various conditions. The validated methods are anticipated to be seamlessly integrated into routine analyses, including those for ALO and DAPA in commercial formulations, in the future.

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