

STABILITY INDICATING HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF DAPAGLIFLOZIN AND SITAGLIPTIN IN MARKETED FORMULATION**Mahima Vishwakarma^{*1}, Deepak Kumar Basedia¹, Sunil shah²****¹Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)****²TIT-College of Pharmacy, Bhopal (M.P.)*****Corresponding Author's E mail:**reenu.mishra.ap@gmail.com**Received 28 July. 2025; Revised 11 Aug. 2025; Accepted 17 Aug. 2025, Available online 15 Oct. 2025**

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ABSTRACT

A reliable and sensitive high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of Dapagliflozin (DAPA) and Sitagliptin (SITA) in tablet formulations. The method was optimized for accuracy, precision, linearity, and robustness. Both drugs exhibited excellent linearity with correlation coefficients (r^2) of 0.999. System suitability tests confirmed high performance with appropriate retention times and low tailing factors. Recovery studies at various concentration levels (80%, 100%, and 120%) showed high recovery rates with minimal deviation. Validation parameters, including repeatability, day-to-day variation, and analyst-to-analyst variation, demonstrated high precision with low % RSD values. The limit of detection (LOD) and limit of quantification (LOQ) were found to be sensitive, ensuring trace-level detection of both drugs. The method was successfully applied to the assay of tablet formulations, yielding assay values of 98.000% for DAPA and 99.300% for SITA. The results indicate that the developed HPLC method is suitable for routine quality control and stability studies of DAPA and SITA in pharmaceutical dosage forms.

Keywords: Dapagliflozin, Sitagliptin, HPLC, Linearity, System Suitability, Recovery Study, Validation.

INTRODUCTION

Dapagliflozin and Sitagliptin are prominent oral antidiabetic agents used in the management of type 2 diabetes mellitus. Dapagliflozin is an SGLT2 (sodium-glucose co-transporter 2) inhibitor that works by reducing glucose reabsorption in the kidneys, leading to lower blood glucose levels. Sitagliptin, a DPP-4 (dipeptidyl peptidase-4) inhibitor, helps in regulating blood sugar levels by enhancing insulin secretion and decreasing glucagon levels^{1,2}. The combination of these two drugs provides a complementary approach, making it effective in improving glycemic control in patients with type 2 diabetes³.

For quality assurance and regulatory compliance, it is critical to accurately quantify the active pharmaceutical ingredients (APIs) in their marketed formulations. Stability-indicating methods are vital for such quantification, as they allow for the determination of both the drug and its degradation products under various stress conditions, such as exposure to light, heat, humidity, or acidic/alkaline environments⁴. High-performance liquid chromatography (HPLC) has emerged as a reliable technique for such stability studies due to its high precision, sensitivity, and the ability to separate and quantify both the APIs and degradation products in a single run^{5,6}.

Various analytical techniques have been reported for the estimation of dapagliflozin and sitagliptin in pharmaceutical formulations; however, few methods are specifically stability-indicating, which is critical for long-term stability studies and quality control purposes. The objective of this study is to develop a stability-indicating HPLC method capable of simultaneously estimating dapagliflozin and sitagliptin in their combined pharmaceutical formulations, ensuring the method is robust, precise, and suitable for routine quality testing^{7,8}.

MATERIALS AND METHODS

Selection of mobile phase

Initially, several mobile phase ratios were tested to estimate Dapagliflozin and Sitagliptin in the fixed dosage form. After considering system suitability parameters such as retention time (RT), tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP), the most suitable mobile phase for the analysis was found to be a 50:50 v/v mixture of Acetonitrile and Methanol. The mobile phase was then filtered through a 0.45 µm filter paper to eliminate any particulate matter and degassed using sonication. The flow rate used for the analysis was set to 1.0 ml/min⁹.

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Preparation of Stock Solution

Accurately weighed 10 mg API of DAPA and SITA was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A).

Preparation of Sub Stock Solution

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (Acetonitrile) to give concentration of 100µg/ml of DAPA and SITA respectively (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for DAPA. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of SITA also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for DAPA and 5-25µg/ml for SITA were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of 5µg/ml for DAPA and 10µg/ml SITA was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 1 to 5 µg/ ml for DAPA) and (5 to 25µg/ ml for (SITA) and areas for each concentration were recorded three times and mean area was calculated¹⁰.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for SITA and 1, 2, 3, 4 and 5 µg/ml for DAPA indicates the precision under the same operating condition over short interval time.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for DAPA and SITA reported in table.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50% v/v) to (40:60% v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 5mg of DAPA and 10mg of SITA was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 5 µg/mL DAPA and 10 µg/mL SITA respectively. The amounts of DAPA and SITA in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation¹¹.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20 µl of each of forced degradation samples were injected.

Acid degradation

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80°C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80°C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

Oxidative degradation

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation

50 mg of the drug sample was taken in to a petridish and kept in oven at 50°C for 4 weeks. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

RESULTS AND DISCUSSION

The results from the analysis of both Dapagliflozin (DAPA) and Sitagliptin (SITA) in tablet formulations demonstrate the reliability, accuracy, and precision of the developed HPLC method.

Both drugs exhibited excellent linearity, with correlation coefficients (r^2) of 0.999 for both DAPA and SITA, which indicates a strong linear relationship between concentration and the response (AUC). The slope (m) and intercept (c) values for DAPA and SITA suggest that the method is capable of detecting a wide range of concentrations with accurate quantification. These findings confirm the method's suitability for quantitative analysis of both drugs in the tablet formulations.

The system suitability results show consistent performance across the analysis for both drugs. For DAPA, the mean retention time (RT) was 4.116 minutes, and for SITA, it was 6.227 minutes, which is consistent across all replicates. The mean number of theoretical plates (2578.667 for DAPA and 3030.333 for SITA) is higher than the standard requirement, suggesting that the column provides adequate separation. Additionally, the mean tailing factors of 1.173 for DAPA and 1.137 for SITA

indicate that both drugs exhibit sharp peaks in the chromatograms, with minimal tailing, thus ensuring the system's efficiency.

The response ratio data shows that the mean response ratio for DAPA was 222.352, while for SITA, it was 156.423. The low % RSD values of 2.043% for DAPA and 0.893% for SITA indicate good precision in the response across multiple replicates. This suggests that the developed method has high accuracy and consistency, making it reliable for quantifying DAPA and SITA in formulations.

The recovery studies demonstrated that both drugs showed satisfactory recovery at different levels. At the 80% level, the mean recovery for DAPA was 98.47%, for DAPA at the 100% level it was 96.85%, and for the 120% level, it was 98.67%. Similarly, SITA at the 80% level showed a recovery of 98.36%. The % RSD values are very low, indicating that the method is precise and reliable across different levels of recovery for both drugs.

The validation parameters, including repeatability, day-to-day variation, analyst-to-analyst variation, and robustness, all showed excellent results for both drugs. The % RSD values for repeatability were 0.052 for DAPA and 0.055 for SITA, demonstrating high precision. The day-to-day variation results and the analyst-to-analyst variation also exhibited low % RSD, ensuring that the method is robust and reliable for routine analysis. The robustness data confirms that small variations in the method conditions (such as flow rate or mobile phase composition) do not significantly affect the results, reinforcing the method's robustness.

The limit of detection (LOD) and limit of quantification (LOQ) for DAPA were found to be 0.15µg/ml and 0.45µg/ml, respectively, while for SITA, the LOD was 0.85µg/ml, and the LOQ was 2.15µg/ml. These values suggest that the method is sensitive enough to detect trace amounts of both drugs in the formulation, making it suitable for routine analysis of DAPA and SITA.

The assay results for the tablet formulation showed that DAPA had an assay value of 98.000% and SITA had an assay value of 99.300% with very low % RSD of 0.125% for DAPA and 1.125% for SITA. These results indicate that the developed method is highly accurate and can be used for the quality control of commercial tablet formulations containing these drugs.

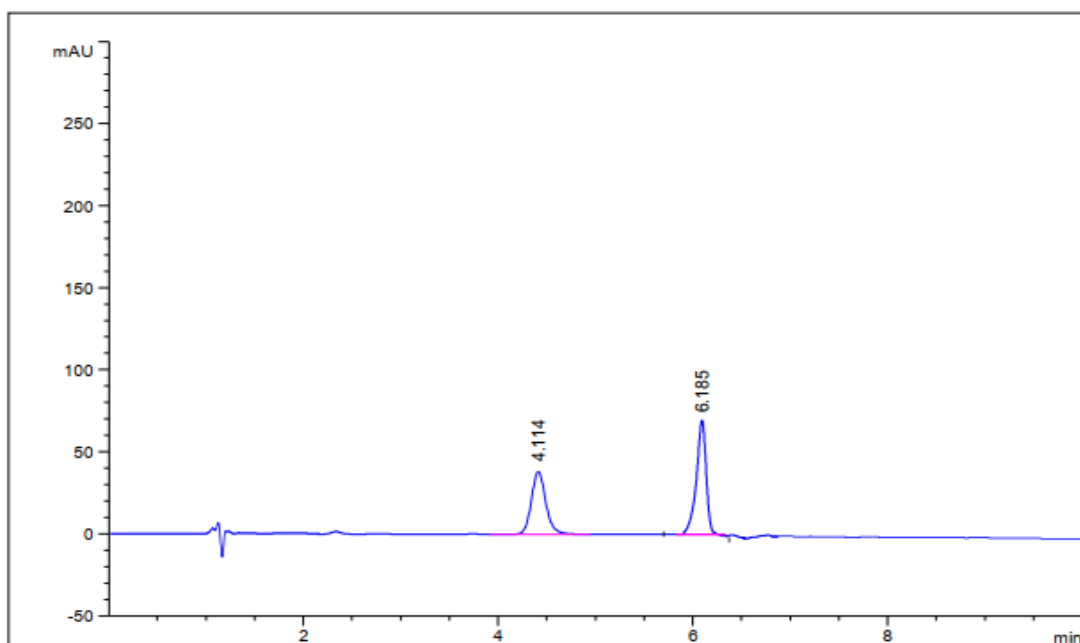


Figure 1: Chromatogram of Both the drug

Table 1: Results of Linearity

Parameter	DAPA	SITA
Correlation Coefficient (r^2)	0.999	0.999
Slope (m)	43.57	157.95
Intercept (c)	6.888	13.334

Table 2: Results of System Suitability parameters

Parameters	DAPA	SITA
Mean RT (min)	4.116	6.227
Mean AUC	1089.788	1542.915
Mean AUC	1089.788	1542.915
Mean RT (min)	4.116	6.227
Mean AUC	1089.788	1542.915
Mean No. of Theoretical Plates	2578.667	3030.333
Mean Tailing Factor	1.173	1.137

Table 3: Results of Response Ratio data for linearity

Parameters	DAPA	SITA
Mean Response Ratio	222.352	156.423
Standard Deviation (SD)	4.542	1.397
% Relative Standard Deviation (%RSD)	2.043	0.893

Table 4: Results of Recovery study

Recovery Study	DAPA (80% Level)	DAPA (100% Level)	DAPA (120% Level)	SITA (80% Level)
Mean % Conc.	98.47	96.85	98.67	98.36
Standard Deviation (SD)	0.196	0.516	0.634	0.219
% Relative Standard Deviation (%RSD)	0.199	0.533	0.643	0.222

Table 5: Results of validation parameters

Parameters	Mean	Standard Deviation	% RSD
Repeatability of DAPA	97.71	0.050	0.052
Repeatability of SITA	99.361	0.054	0.055
Day-to-Day variation of DAPA	97.10	0.069	0.071
Day-to-Day variation of SITA	99.055	0.065	0.066
Analyst to Analyst variation of DAPA	99.527	0.045	0.045
Analyst to Analyst variation of SITA	97.48	0.052	0.053
Robustness of DAPA	97.44	0.059	0.061
Robustness of SITA	99.232	0.063	0.064

Table 6: LOD and LOQ of DAPA and SITA

Name	LOD (µg/ml)	LOQ (µg/ml)
DAPA	0.15	0.45
SITA	0.85	2.15

Table 7: Result of assay of tablet formulation

	DAPA*	SITA*
Label Claim (mg)	5mg	10mg
% Found (mg)	4.9	9.93
% Assay	98.000	99.300
% RSD	0.125	1.125

*Average of three determination

CONCLUSION

The developed HPLC method for the simultaneous estimation of DAPA and SITA in tablet formulations has demonstrated excellent linearity, precision, accuracy, and robustness. The method is suitable for routine analysis in quality control laboratories, as it meets the validation criteria and provides reliable results for both the drugs. The assay values and the recovery studies further confirm the method's capability to accurately measure the drug content in pharmaceutical formulations, ensuring consistent product quality.

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