**Scholarly Research Journal for Interdisciplinary Studies**
Online ISSN 2278-8808, SJIF 2026 = 8.727
https://www.srjis.com/issues_data/290
Peer Reviewed & Refereed Journal, MAR-APRIL 2026, Vol- 14/94

# RP-UPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF SITAGLIPTIN AND DAPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

**Swati Nitin Prabhu*[1], Surekha Kolhal[2], Nitin Prabhu[3] & Rajesh Samant [1]**

[1]*Department of Chemistry, K.C College, Mumbai. India*

**Department of Quality & Regulatory, Signet Excipients Pvt Limited*

***Corresponding author's E-mail:** swatinprabhu@gmail.com*

**Paper Received On**: 20 APRIL 2026

**Peer Reviewed On:** 24 APRIL 2026

**Published On:** 01 MAY 2026

***Abstract***

*An RP-UPLC method for the simultaneous determination of Sitagliptin and Dapagliflozin in tablets was developed and validated as per ICH & FDA guidelines. The separation was achieved with a 50 mm x 2.1 mm, 1.7 µm C18 column by using a simple linear gradient. Mobile phase A was buffer (0.05% trifluoracetic acid), and mobile phase B was acetonitrile. A simple gradient program was delivered at a flow rate of 0.3 mL/min. The column temperature was kept at 25°C. The detector was set at the wavelength of 210 nm. Injection volume was 2 µL. The gradient separation was achieved within 3 minutes.*

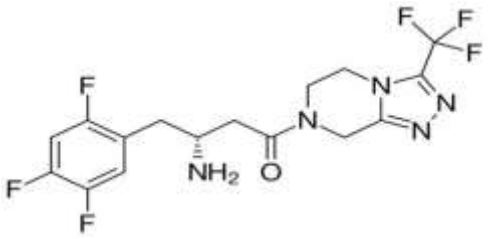
*The linearity of the proposed method was investigated in the range 0.25-0.75mg/mL (r2= 1.000) for Sitagliptin, and 0.025-0.075 mg/mL (r2= 1.000) for Dapagliflozin. The assay method is specific, as no blank and placebo interference was observed at the retention times of Sitagliptin and Dapagliflozin peaks. The developed method has an advantage that both drugs can be quantified alone or in combination using a single mobile phase, with time- and chemically efficient method.*

***Key words:** RP-UPLC, ICH, Validation, Sitagliptin, Dapagliflozin*

## INTRODUCTION

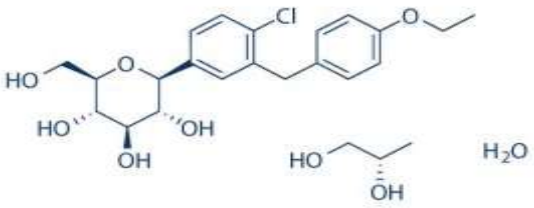
Sitagliptin contains a pyrrolidine ring with an amino group, a phenyl group, and a trifluoromethyl group.

Mechanism of Action: It is a DPP-4 (dipeptidyl peptidase-4) inhibitor, which helps increase the levels of incretin hormones, such as GLP-1 and GIP. These hormones stimulate insulin secretion and inhibit glucagon release, thereby lowering blood sugar levels.



Dapagliflozin is a synthetic C-aryl glucoside and belongs to the SGLT2 inhibitor class

Mechanism of Action: SGLT2 normally reabsorbs ~90% of filtered glucose. Dapagliflozin competitively binds to SGLT2, blocking glucose and sodium reabsorption.



The method was validated according to the present ICH guideline on validation of analytical procedure Q2A (R1). [18,19] Quantitation was achieved with UV detection at 210 nm based on peak area with linear calibration curves at different concentration ranges. The method was linear over a wide concentration range of 0.250-0.750 mg/ml for Sitagliptin and 0.025-0.075 mg/ml for Dapagliflozin. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100%, and 150% of target test concentration.

## MATERIALS AND METHODS

### Chemicals and reagents

Sitagliptin and dapagliflozin active pharmaceutical ingredients were obtained from local laboratories in Mumbai, India. Excipients were also obtained from local laboratories in Mumbai, India. A branded tablet formulation containing sitagliptin (100 mg) and dapagliflozin (10 mg) was procured from the local market. UPLC-grade acetonitrile and trifluoroacetic acid were procured from Merck (Mumbai, India). HPLC-grade water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were procured from Millipore (Mumbai, India). All reagents used were of analytical grade.

**Selection of UV wavelength**:

50 ppm solution of each Sitagliptin and Dapagliflozin were prepared separately in Acetonitrile.

The UV scan of the above solutions was injected into the UPLC PDA system Model Empower 3 Acquity . The detection wavelength was set at 210nm because both components had higher responses. A separate chromatogram shown here for both components.

**Figure 1: Chromatogram of Sitagliptin with spectrum (200-400 nm) PDA UPLC system**

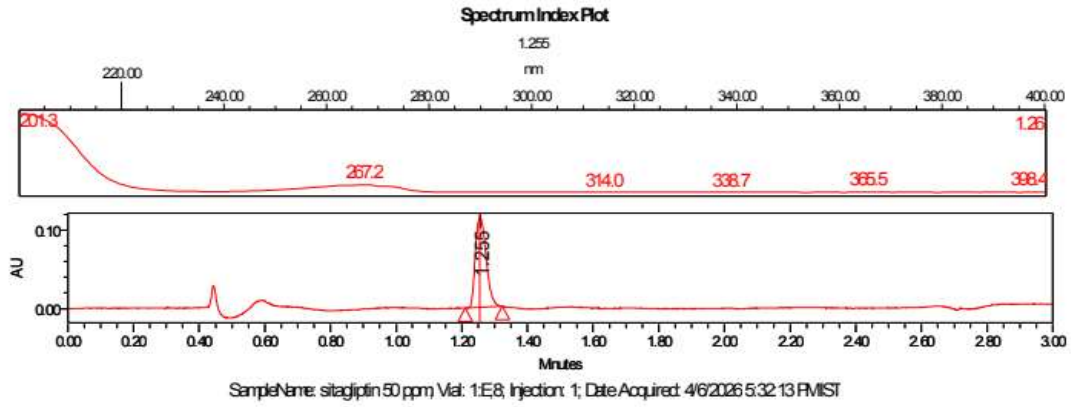
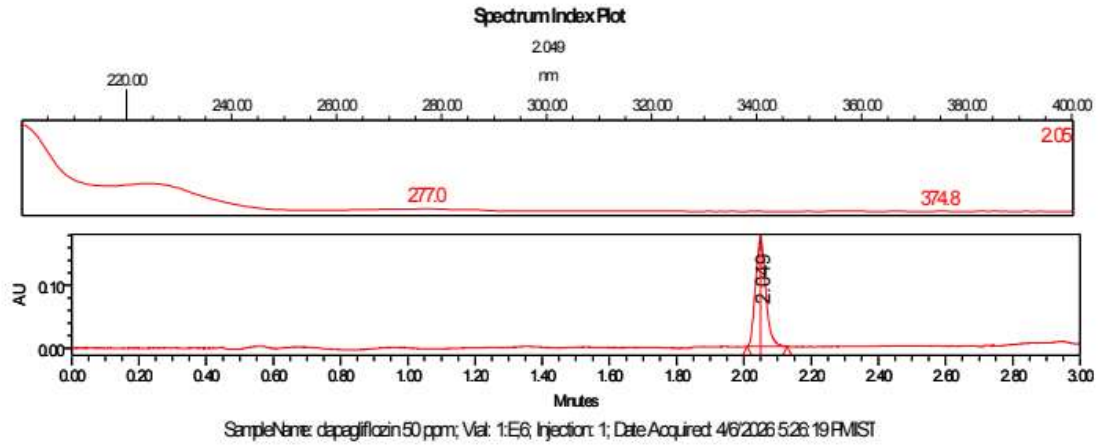



**Figure 2: Chromatogram of Dapagliflozin with spectrum (200-400 nm) PDA UPLC system**




**UPLC instruments and analytical conditions**

Chromatographic separation was achieved using UPLC System (Waters Acquity UPLC) containing binary solvent manager, an autosampler, and PDA detector. The output signal was monitored and processed using Empower software.

Hemochrom C18 column (50 mm X 2.1 mm id and 1.7 µm particle size) was used as the stationary phase. Mobile phase A was Buffer (0.05 % Trifluoracetic acid) and Mobile Phase B was Acetonitrile. A simple gradient program was used, starting at 25:75 (A: B) changing to

80:20 at 3.0 minutes, then returning to 25:75 at 3.10 minutes and maintained for equilibration till 5.00 minutes. The mobile phase was delivered at a flow rate of 0.3 mL/min. The column was kept at ambient temperature. The detector was set to a wavelength of 210 nm. Injection volume was kept at 2 µL.

**Solutions and sample preparation**

For the system suitability test, the solution contains Sitagliptin (0.50 mg/mL) and Dapagliflozin (0.05 mg/mL) were used

For the linearity studies, the variable weight of compounds was weighed and diluted with the solvent to yield solutions at different concentrations.

For test sample solution, the content of sitagliptin and dapagliflozin simultaneously in a pharmaceutical dosage form, 20 tablets were accurately weighed and triturated to make a smooth powder. An accurately weighed portion of the powder equivalent to 100 mg of sitagliptin and 10 mg of dapagliflozin was transferred into 50 ml volumetric flask containing 20 ml of acetonitrile. The mixture was shaked manually for 10 minute to ensure contents are dissolved. Then volume was made upto the mark with diluent with intermittent shaking. The resultant solution was filtered through 0.45 µm membrane filter. Sample solution: Further 5 ml of the clear filtrate was taken into 20 ml volumetric flask and diluted upto the mark with diluent to get a final concentration of 500 ppm sitagliptin and 50 ppm dapagliflozin.

2 µL of these solutions were injected and the peak area was recorded from the respective chromatogram.

**Calculation**

All active ingredients were quantified with the following calculation:

$$\% \text{ Assay} = \frac{\text{Sample Area} \times \text{Standard dilution factor} \times 100}{\text{Standard area} \times \text{Sample dilution factor}}$$

## RESULTS AND DISCUSION

A literature survey revealed that no UPLC method is available in the official compendia for the simultaneous determination of sitagliptin and dapagliflozin in bulk and dosage forms. The proposed method was compared with reported methods in the literature, and the comparison is

shown in Table 1. Complete separation of the analytes was accomplished in less than 5 min, and the method can be successfully applied for the routine analysis of sitagliptin and dapagliflozin in bulk and commercially available dosage forms. Method Validation The developed RP-UPLC method was validated as per the International Conference on Harmonization (ICH) guideline, Validation of Analytical Procedures: Q2(R1) [18], for parameters such as system suitability, linearity and range, precision (repeatability), specificity, accuracy, and robustness. System Suitability The system suitability test was performed according to USP 37 [19]. The standard solution was injected six times, and the results were recorded to evaluate adequate peak separation (resolution), percentage relative standard deviation for area and retention time, peak asymmetry, and theoretical plates. The results obtained are compiled in Table 2.

**Table 1: System suitability**

Reference solution : Standard for n=6

| Parameter | Acceptance Criteria | **Sitagliptin** | **Dapagliflozin** |
|---|---|---|---|
| **%RSD** | Not less than 2.0 | 0.002 | 0.145 |
| **Resolution** | Not less than 2.0 | - | 4. 83 |
| **Symmetry Factor** | Should be between 0.8 – 1.5 | 1.26 | 1.16 |
| **Theoretical plates** | Not less than 1500 | 8723 | 9745 |

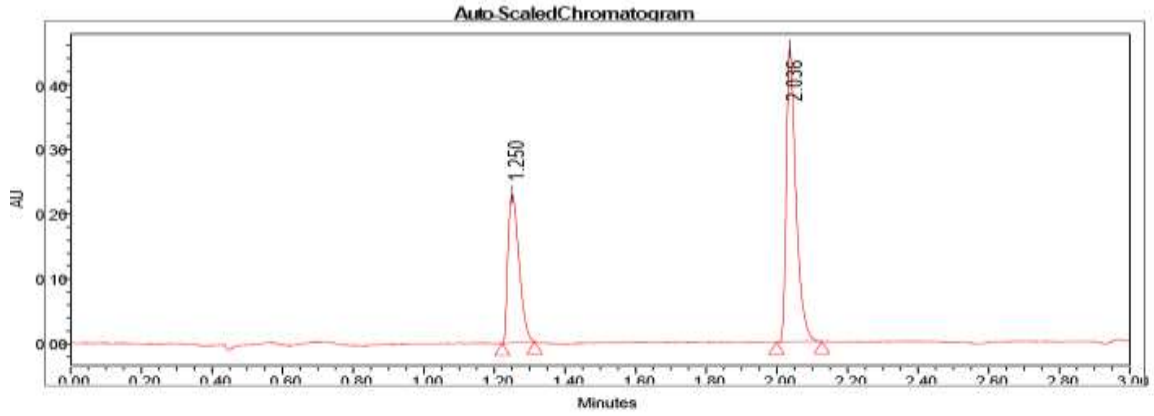
**Results:** It was observed that limits for percentage standard deviation for peak areas, symmetry factor and theoretical plates for all individual analytes were within the limit, which shows that the method has good system suitability.

**Specificity**

Specificity was performed to detect the presence of interference peak (blank) at the retention time of analyte peak. The specificity of the method was checked by comparison of chromatograms obtained from test sample solution. The interference of placebo was detected by preparing placebo solution equivalent to about the weight in proportion of tablet preparation as per test method and was injected into the UPLC system. The interference of blank was detected by injecting diluent as per test method.

The representative chromatogram obtained for Sitagliptin and Dapagliflozin are shown in Figure-2.

**Figure 3: Typical Chromatograms of Standard Solution containing Sitagliptin and Dapagliflozin.**




Peak Results

| | Name | RT | Area | Height | Amount | Units |
|---|---|---|---|---|---|---|
| 1 | Sitagliptin | 1.250 | 501679 | 225276 | | |
| 2 | Dapagliflozin | 2.036 | 853104 | 451988 | | |

**Results:** No interference from diluent, excipients or any other peak was found at retention time of Sitagliptin and Dapagliflozin.

**Precision**

Method precision was evaluated by carrying out six different test sample solution preparations Assay of these samples were determined. Precision of the method was evaluated by calculating the %RSD. The values are given in Table-3.

**Table 2: Precision and Intermediate Precision results**

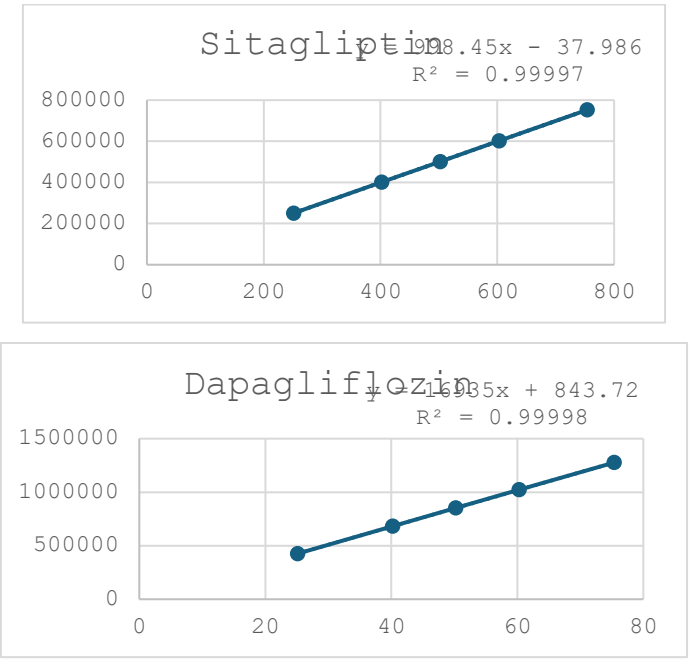
| | Sitagliptin | Dapagliflozin |
|---|---|---|
| **Average Assay (%)** | 100.00 | 99.97 |
| **%RSD** | **0.003** | **0.128** |

**Results:** 2.00 % RSD

**Linearity**

Linearity of detector response was determined by preparing a series of solution of working standards (mixture of all both ingredients) over the range of 50% to 150% of targeted concentration. These solutions were injected and response area was recorded. Calibration curve was constructed by plotting areas against concentration and regression equations were computed. The linearity plots with values are shown in Figure-3.

**Figure 4: Calibration curves showing linearity -Sitagliptin & Dapagliflozin**




**Results:** The correlation coefficient values were within the limit 0.998 and Y-intercept values were within ± 2 %.

**Accuracy (Recovery)**

To study the accuracy of the method recovery experiments were carried out. The accuracy of the test method was determined by varying weights of crushed test sample at the level of 50%, 100% and 150% of targeted concentration. The recovery samples were prepared in triplicate at each level. The samples at different levels were chromatographed and the percentage recovery for the amount added was calculated. The values were given in Table-4.

**Table 3: Accuracy (Recovery)**

| Active Ingredient Name | Concentration (%) | Amount Added (mg/mL) | Amount found (% ) | Mean Recovery (%) | Average Recovery (%) |
|---|---|---|---|---|---|
| **Sitagliptin** | 50 | 0.245 | 50.002 | 100.0 | 99.9 |
| | 100 | 0.515 | 100.002 | 99.71 | |
| | 150 | 0.765 | 150.002 | 99.99 | |
| **Dapagliflozin** | 50 | 0.0247 | 49.97270702 | 99.95 | 99.86 |
| | 100 | 0.0521 | 99.79234622 | 99.79 | |
| | 150 | 0.0745 | 149.7533451 | 99.84 | |
| **Acceptance criteria** | **The mean and individual recoveries should be within 98.0 – 102.0%** | | | | |

**Results:** Accuracy results obtained shows that the mean and individual recoveries were in range of 98.0 – 102.0%

**Robustness**

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase
2. Percentage of trifluoracetic acid

**Table 4: Robustness results**

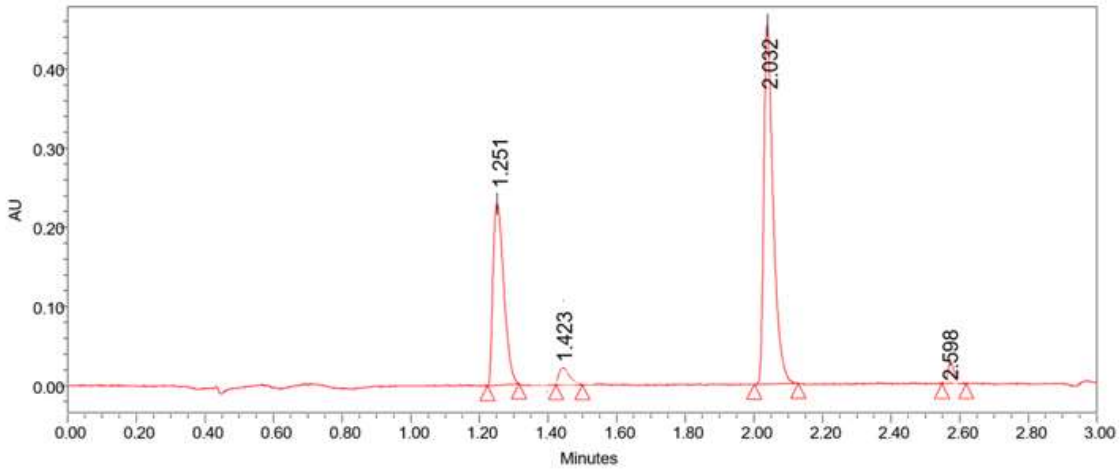
| **Drug** | **Area at Flow ( 0.3 ml, 0.33ml and 0.27 ml)** | **Area at Mobile Phase (0.05% TFA, 0.055% TFA, 0.045% TFA)** |
|---|---|---|
| sitagliptin | 501019 | 500675 |
| sitagliptin | 501011 | 500709 |
| sitagliptin | 501017 | 500686 |
| Average | 501015.7 | 500690.0 |
| SD | 4.16 | 17.35 |
| **%RSD** | **0.001** | **0.003** |
| dapagliflozin | 848810 | 849943 |
| dapagliflozin | 848555 | 849094 |
| dapagliflozin | 848810 | 848246 |
| Average | 848725 | 849094.3333 |
| SD | 147.2243186 | 848.5000491 |
| **%RSD** | **0.017** | **0.100** |

**Results:** From variation in, flow rate, and percentage trifluoracetic acid, it was observed that there were no marked changes in the chromatograms, which demonstrated that the method developed is robust.

**Forced Degradation Condition:**

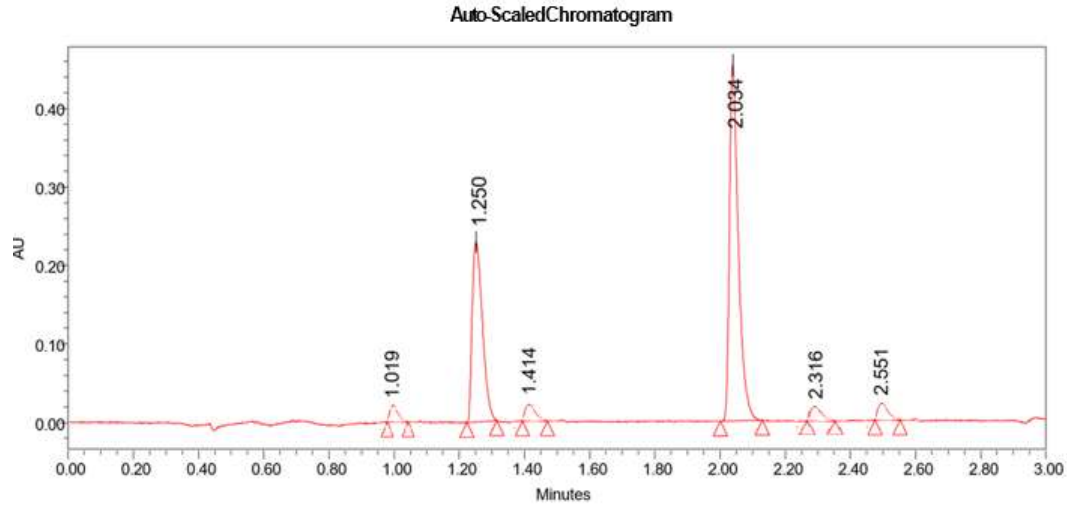
**Acid degradation Standard:** 5 ml of Standard stock solution was taken into 20 mL volumetric flask. 1 ml 1 N HCl was added into the flask. The flask was refluxed at 60 °C 12 hours. Solution was then allowed to cooled down and then neutralized with ml 1 N NaOH. Volume was made upto the mark with diluent and injected**.**

**Figure 5: Chromatograms of acid degradation of Sitagliptin and Dapagliflozin.**




**Base Degradation:** 5 ml of standard stock solution was taken into 20 mL volumetric flask. 1 ml N HCl was added into the flask. The flask was kept at room temperature for 12 hours. Solution was then neutralized with 1 ml NaOH. Volume was made upto the mark with diluent and injected.

**Figure 6: Chromatograms of base degradation of Sitagliptin and Dapagliflozin.**




**Oxidative Degradation:** 5 ml of Standard stock solution was taken into 20 mL volumetric flask. 1 ml 3% H2O2 was added into the flask. The flask was kept at room temperature for 12 hours. Volume was made upto the mark with diluent and injected.

**Figure 7: Chromatograms of oxidative degradation of Sitagliptin and Dapagliflozin.**

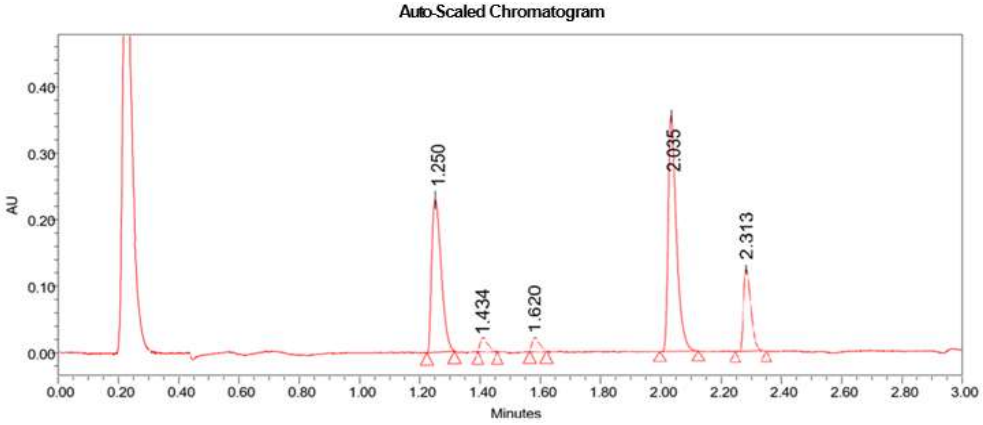
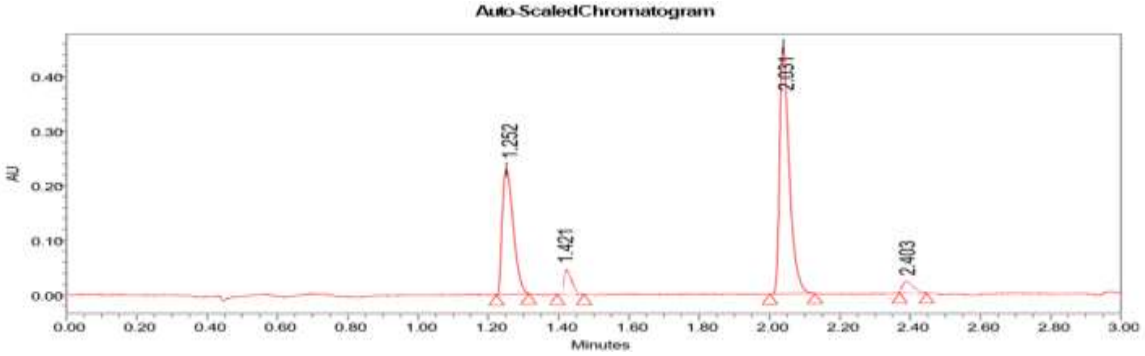


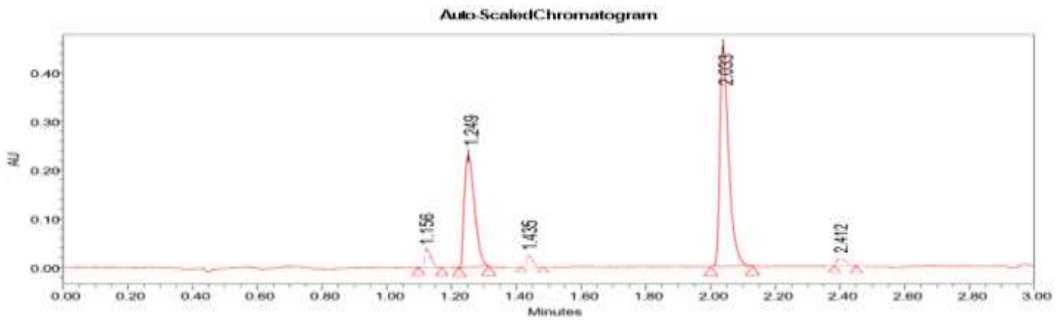

**Photo Degradation:** APIs were kept into sunlight for 72 hours. Solutions were made as per test method and then injected.

**Figure 8: Chromatograms of photo degradation of Sitagliptin and Dapagliflozin.**




**Thermal Degradation:** APIs were kept into hot air oven at 60 °C for 48 hours and then solutions were made asper test sample preparation and injected.

**Figure 9: Chromatograms of Thermal degradation of Sitagliptin and Dapagliflozin.**



## CONCLUSION

Sitagliptin improves glycemic control by increasing insulin secretion and reducing hepatic glucose production. It acts by prolonging the activity of incretin hormones, namely GLP-1 and GIP. Elevated incretin levels enhance glucose-dependent insulin release and suppress glucagon secretion from pancreatic alpha cells, thereby decreasing hepatic glucose output. Dapagliflozin lowers blood glucose levels by inhibiting the sodium–glucose co-transporter 2 (SGLT2) in the kidney. This inhibition reduces glucose reabsorption in the renal tubules, leading to increased urinary glucose excretion and a consequent reduction in blood glucose levels. A gradient RP-UPLC method has been developed and validated for the analysis of sitagliptin and dapagliflozin in tablet dosage forms. The results of method validation revealed that the assay method was specific, selective, linear, accurate, and robust. The validation performed further provides documented evidence that the analytical method for the simultaneous estimation of sitagliptin and dapagliflozin by RP-UPLC in tablet dosage forms can consistently quantify these drugs in combined and single dosage forms and can be used for routine analysis in quality control and R&D laboratories.

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