WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 15, Issue 3, 845-862. Research Article ISSN 2277–7105

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF DAPAGLIFLOZIN AND SITAGLIPTIN IN PURE PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

**Suresh Kannan V., Ragunath Samiyappan*, Vijayamirtharaj Ram Aswamy and Senthilkumar Natesan**

Department of Pharmaceutical Analysis, JKKMRF-Annai J.K.K. Sampoorani Ammal College of pharmacy, Komarapalayam, Namakkal, Tamil Nadu, India (Affliated to The Tamil Nadu Dr. M. G. R. Medical University, Chennai, Tamil Nadu, India).

Article Received on 26 Dec 2025,
Article Revised on 15 January. 2026,
Article Published on 1 February 2026,

https://doi.org/10.5281/zenodo.18430417

***Corresponding Author**
**Ragunath Samiyappan**

Department of Pharmaceutical Analysis, JKKMRF-Annai J.K.K. Sampoorani Ammal College of pharmacy, Komarapalayam, Namakkal, Tamil Nadu, India (Affliated to The Tamil Nadu Dr. M. G. R. Medical University, Chennai, Tamil Nadu, India).

**How to cite this Article:** Suresh Kannan V, Ragunath Samiyappan*, Vijayamirtharaj Ram Aswamy and Senthilkumar Natesan. (2026) Analytical Method Development And Validation For Simultaneous Determination of Dapagliflozin and Sitagliptin In Pure Pharmaceutical Dosage Form By Rp-Hplc. "World Journal of Pharmaceutical Research, 15(3), 845–862.


## ABSTRACT

The objective of this study was to develop a new simple, rapid, sensitive, and validated reversed phase-high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Dapagliflozin and sitagliptin in tablet dosage form. By utilizing RP-HPLC, this method fills a gap in the existing literature. Optimizing various parameters of the chromatographic process was key to developing an effective method for separating and detecting drugs. International guidelines and regulatory requirements were followed for the validation of the method, including specificity, linearity, accuracy, Precision, robustness, and suitability for the system. For analysis of mixed solutions containing Dapagliflozin and Sitagliptin, a Chromatographic separation was achieved on a FLOWROSIL C 18 column (150x4.6mm), 5µm, a mobile phase ratio consisting of Buffer: ACN: Methanol (50:50) at flow rate 1.0ml/min, and total run time 10 min, The injection volume 20µl. The detection wavelength is 210nm. Approximately 2000 plates were found, indicating a successful chromatographic separation. With a tailing factor of less than 2

and a well-resolved peak, the peaks appear symmetrical and well-resolved. In order to ensure there were no interfering peaks, retention times for Dapagliflozin and Sitagliptin were found to be 6.897 and 4.584min. As for Dapagliflozin, the correlation coefficient ($R^2$) is 50% and Sitagliptin is 150%. This indicates a good linear relationship between drug concentrations and peak areas based on the high correlation coefficients. For Dapagliflozin and Sitagliptin, the %RSD values were 0.12% and 1.31%, respectively, below the acceptable limit of 2%. As a result, the method is accurate and repeatable. In the study of Dapagliflozin, the mean percent recovery was 98.74%, while in the study of Sitagliptin, the mean percent recovery 98.57%. Under varied conditions, both Dapagliflozin and Sitagliptin showed %RSD values within the Acceptable range of 2%, demonstrating the robustness and reliability of the method. This Study concluded that valuable insight is provided into the use of validated RP-HPLC methods in this study, which contributes significantly to the evolution of pharmaceutical analytical techniques.

**KEYWORDS:** Dapagliflozin and sitagliptin, RP-HPLC, Method development, Method validation.

## INTRODUCTION

Dapagliflozin **(Figure 1)** is given as a crystalline solid. Dapagliflozin decreases renal glucose reabsorption through the solid-glucose co-transporter (SGLT) and provides an insulin-free option for regulating blood glucose levels in type 2 diabetic patients. Dapagliflozin is a first-generation SGLT inhibitor that selectively targets SGLT2 over SGLT1.Dapagliflozin is a first-generation SGLT inhibitor that selectively targets SGLT2 over SGLT1.

Sitagliptin (**Figure 2)** It works by inhibiting the enzymes DPP-4, which typically breaks down certain gastrointestinal hormones called incretins (like GLP-1 and GIP). By preventing the breakdown of these incretins, sitagliptin increase their active levels in the bloodstream. Higher incretin levels lead to increased insulin release from the pancreas and reduced glucagon (a hormone that raise blood sugar) production by the liver, in a glucose -dependent manner.

Dapagliflozin and Sitagliptin are used to treat type 2 diabetes. The combined mode of action of Dapagliflozin and Sitagliptin, as well as their outstanding efficacy and safety profiles, support the utilization of this fixed-dose combination as a therapeutic option for T2DM patients.

The literature review reveals that several methods have been reported for estimating Dapagliflozin and Sitagliptin alone or in combination with other drugs in their pharmaceutical dosage forms, but none of these methods are available for estimating these drugs in the selected pharmaceutical dosage form. In the investigation of formulations containing two or more pharmaceuticals, one drug may interfere with the estimation of another. To avoid this, component combinations frequently separate by extraction, which makes the process time consuming, difficult, and often inaccurate. As a result, it was considered worthwhile to create an analysis approach capable of estimating both medications in combination without the need for separation. As a result of the literature review, it was believed to design a precise, accurate, easy, and reliable method for estimating medication in tablets utilizing the following technology of RP HPLC method for Dapagliflozin and Sitagliptin. The technique was confirmed, and recovery tests were undertaken using ICH criteria and many statistical metrics.

## MATERIALS AND METHODS

### Chemicals and Reagents

Potassium phosphate, Acetonitrile, Ortho phosphoric acid (AR Grade), Ammonium Acetate, Triethylamine, NEBULAE HI-TECH LABORATORIES Chemicals Pvt. Ltd. Pharmaceutical grade Dapagliflozin+ Sitagliptin Sun pharmaceutical laboratories Pvt. Ltd (OXRA-S 10mg/100mg), Sun pharmaceutical laboratories Pvt. Ltd., Mumbai, India respectively.

### Instruments

The analysis was performed by using the Shimadzu digital electronics balance, pH meter - Elico Pvt. Limited, India, Jasco V-600 UV/ Vis- spectrophotometer, Shimadzu LC2010 CHliquid chromatograph system with UV – VISIBLE detector and auto sampler injector. Chromatograms were recorded and integrated on PC installed with Lab solutions chromatographic software. Shimadzu liquid chromatograph equipped with LC – 10 AT VP pump, SPDM10A VP diode array detector and rheodyne 7725 *i* injected with a 20 µl loop. Chromatograms were recorded and integrated on PC installed with LC solutions chromatographic software.

### Chromatographic Conditions

Waters Corporation (Milli – Q -Water) was used for method development, forced degradation and method validation. This system is comprised of a ternary gradient pump and auto sampler

(2487 Separation module), column oven and a photo diode array detector. Inspire (C18 x 150x 4.6mm, 5µm) column was used. The instrumental settings were a flow rate of 1 mL/min, a column temperature at 40°C and a detector wavelength of 210nm. The injection volume was 20 µL. Data acquisition was made with the software LC Software 1000 (Thermo Separations Products, Riviera Beach, FL).

## REAGENTS AND SOLUTIONS

### Preparation of Mobile Phase

**a.Selection of mobile phase**

Solvent type, solvent strength, strength of buffer and optimum pH were optimised to get the chromatographic conditions that gave best separation.

The mobile phase was made up of buffer and acetonitrile in a 50:50 ratio (v/v). The pH of the mobile phase was adjusted to 3.0 using sodium hydroxide. The buffer employed in the mobile phase consisted of 0.1% of orthophosphoric acid in double-distilled water. The mobile phase was premixed, filtered using a 0.45-µm filter, and degassed.

**b. Preparation of stock solution**

10mg of Dapagliflozin and 100mg of Sitagliptin were accurately weighed and transferred in to a separate 50 ml volumetric flask and sufficient mobile phase was added to dissolve the drug. The final volume was made up to 50 ml with mobile phase (primary stock solution). Pipette out 2ml from the above stock solution into a 50ml volumetric flask and the final volume was made up to the mark with the mobile phase.

### Preparation of Sample solution

20 tablets were weighed and powdered, tablets powder equivalent to 100mg of Dapagliflozin and 10 mg of Sitagliptin was transferred in to a 50 ml volumetric flask, sufficient amount of mobile phase was added and dissolved by 20 minutes ultrasonication. Then made the volume up to the mark with the mobile phase and filtered with 0.45 µ filter paper. Pipette out 2 ml from the above solution and diluted to 50ml with the mobile phase

### Recording the chromatogram

A steady baseline was recorded with the fixed chromatographic conditions and 20 µg of standard drug solutions and sample solutions were injected and chromatograms were

recorded. Calibration curve was plotted using the standard drug peak area versus concentration of standard solutions.

**METHODS DEVELOPMENT**

The developed method was fully validated for the parameters as per ICH guidelines.

**SYSTEM SUITABILITY STUDIES**

About 642.4 mg of working standard of Dapagliflozin and Sitagliptin was weighed and transferred into a clean and dry 50 mL standard flask, the sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase.

0.5 mL of the stock solution was pipetted into a 10 mL standard flask and diluted to mark with mobile phase and filtered through 0.45µ filter.

**LINEARITY**

Linearity was assessed by performing measurement at several analyte concentrations. A minimum five concentrations were recommended for linearity studies.

The linearity of an analytical method is its ability to show test results that is directly proportional to the concentration of analyte in sample with in a given range. The linearity of an analytical method was determined by mathematical treatment of test result obtained by analysis of samples with analyte concentration across claimed range of peak area Vs concentration is plotted and percentage curve fitting is calculated.

**ACCURACY: (RECOVERY)**

A study of accuracy was conducted. Drug assay was performed in triplicate as per test method by spiking the Dapagliflozin and Sitagliptin drug substance to the placebo equivalent to 80%, 100%, and 120% of the labelled amount as per the test method.

The average % recovery of Dapagliflozin and Sitagliptin was calculated. Separately inject the blank, placebo, Dapagliflozin and Sitagliptin in to the chromatograph.

## SPECIFICITY/ SELECTIVITY

The specificity of the method was evaluated by analysing the sample solution spiked with the excipients at appropriate levels. The assay result was unaffected by the presence of extraneous materials.

## ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides on indication of its reliability during normal usage.

## LIMIT OF DETECTION

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value, under the stated experimental conditions. The detection limit is usually expressed as the concentration of analyte (percentage parts per million) in the sample.

## LIMIT OF QUANTIFICATION

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantification limit is expressed as the concentration of analyte (e.g: % ppm) in the sample. Which can be quantitated with suitable precision and accuracy.

## RESULTS AND DISCUSSION

A simple Reverse phase high performance liquid chromatographic method has been developed and subsequently validated for Dapagliflozin and Sitagliptin tablets.

The separation was carried out by using a Buffer (0.02 Ammonium dihydrogen phosphate): Acetonitrile (50:50). The detection was carried out at 210 nm. The column was Flowrosil C18, 250x4.6mm, 5µm. The flow rate was selected as 1.0 mL/min.

The retention time of Dapagliflozin and Sitagliptin was found to be 6.897 and 4.584. **TABLE.29** The asymmetry factor or tailing factor of Dapagliflozin and Sitagliptin was found to be 0.956 and 1.522, **TABLE .29** which indicates symmetrical nature of the peak. The number of theoretical plates of Dapagliflozin and Sitagliptin was found to be 20417 and 15128, which indicates the efficient Performance of the column. These parameters represent the specificity of the method.

From the linearity studies, specified concentration levels were determined. it was observed that Dapagliflozin and Sitagliptin were linear in the range of 50% to 150% for the target concentration by RP-HPLC. The linearity range of Dapagliflozin and Sitagliptin 50% to 150% was found to obey linearity with a correlation coefficient to. The validation of the proposed method was verified by system precision and method precision by RP-HPLC. The %RSD of system suitability for Dapagliflozin and Sitagliptin was found to be 0.12 and 0.31

**TABLE.51**

The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The robustness studies were performed by changing the flow rate, filters and wavelength. The ruggedness study was also performed. The analytical method validation was carried out by RP-HPLC as per ICH guidelines and given below are the tables are the summary of the results.

**Table no.1: System Suitability parameters for sitagliptin.**

| Injection | Retention time | Peak area | USP Plate count | USP Tailing factor |
|---|---|---|---|---|
| 1 | 4.334 | 4635907 | 4380 | 1.359 |
| 2 | 4.337 | 4637821 | 4384 | 1.360 |
| 3 | 4.335 | 4653532 | 4369 | 1.363 |
| 4 | 4.336 | 4645846 | 4360 | 1.366 |
| 5 | 4.334 | 4660297 | 4347 | 1.370 |
| 6 | 4.335 | 4674036 | 4329 | 1.373 |
| Mean | | 4651239 | | |
| RSD | | 0.31 | | |

**Table no.2: System suitability for Dapagliflozin.**

| Injection | Retention time | Peak area | USP plate count | Tailing factor |
|---|---|---|---|---|
| 1 | 5.351 | 2370981 | 9340 | 1.283 |
| 2 | 5.352 | 23668706 | 9305 | 1.284 |
| 3 | 5.351 | 2373320 | 9320 | 1.287 |
| 4 | 5.350 | 2369221 | 9288 | 1.288 |
| 5 | 5.348 | 2374651 | 9239 | 1.295 |
| 6 | 5.349 | 2371503 | 9219 | 1.294 |
| Mean | | 237109 | | |
| RSD | | 0.12 | | |

**Table no.3: Linearity for Dapagliflozin.**

| S. No | Volume of stock solution taken (ug/mL) | Volumetric flask taken (mL) | Concentration of solution (%) |
|---|---|---|---|
| 1 | 75 | 10 | 50 |
| 2 | 112.5 | 10 | 75 |
| 3 | 150 | 10 | 100 |
| 4 | 187.5 | 10 | 125 |
| 5 | 225 | 10 | 150 |

**Tableno.4: LinearityforSitagliptin.**

| S. No | Volume of stock solution taken (ug/mL) | Volumetric flask taken (mL) | Concentration of solution (%) |
|---|---|---|---|
| 1 | 50 | 10 | 50 |
| 2 | 75 | 10 | 75 |
| 3 | 100 | 10 | 100 |
| 4 | 125 | 10 | 125 |
| 5 | 150 | 10 | 150 |

**Table no.5: Accuracy for Dapagliflozin.**

| Injection | Retention time | Peak area | USP plate count | Tailing factor |
|---|---|---|---|---|
| 1 | 4.400 | 4122564 | 5001 | 1.371 |
| 2 | 4.385 | 4168688 | 4883 | 1.375 |
| 3 | 4.375 | 4209276 | 4827 | 1.379 |
| 4 | 4.361 | 4579687 | 4527 | 1.396 |
| 5 | 4.365 | 4602075 | 4505 | 1.394 |
| 6 | 4.358 | 4620963 | 4474 | 1.397 |
| 7 | 4.352 | 50265214 | 4182 | 1.404 |
| 8 | 4.349 | 5038029 | 4149 | 1.406 |
| 9 | 4.345 | 5062524 | 4155 | 1.412 |
| Mean | | 4651239 | | |
| RSD | | 0.30 | | |

**Table no. 6: Accuracy for Sitagliptin.**

| Injection | Retension time | Peak area | USP plate count | Tailing factor |
|---|---|---|---|---|
| 1 | 5.378 | 2009395 | 9826 | 1,299 |
| 2 | 5.368 | 2001674 | 9703 | 1.299 |
| 3 | 5.360 | 2022108 | 9608 | 1.298 |
| 4 | 5.348 | 2326911 | 9377 | 1.299 |
| 5 | 5.353 | 2348117 | 9392 | 1.299 |
| 6 | 5.348 | 2351290 | 9321 | 1.299 |
| 7 | 5.336 | 2773985 | 9052 | 1.298 |
| 8 | 5.341 | 2767794 | 9113 | 1.295 |
| 9 | 5.336 | 2773985 | 9052 | 1.298 |

**Table no. 7: Specificity/(selectivity).**

| Dapagliflozin | Sitagliptin | Peak found |
|---|---|---|
| 1 | 1 | No |
| 2 | 2 | No |
| 3 | 3 | No |

**Table no. 8: Robustness for Dapagliflozin.**

| Sample Name | Peak Name | Retention Time | Area | USP Plate Count | USP Tailing Factor | USP Resolution |
|---|---|---|---|---|---|---|
| Comp-1 | Dapagliflozin | 6.543 | 2926709 | 9801 | 1.308 | 4.163 |
| Comp - 2 | Dapagliflozin | 4.489 | 2000093 | 8466 | 1.284 | 3.966 |

**Table no.9: Robustness for Sitagliptin.**

| Sample Name | Peak Name | Retention Time | Area | USP Plate Count | USP Tailing Factor | USP Resolution |
|---|---|---|---|---|---|---|
| Comp-1 | Sitagliptin | 5.342 | 5664247 | 4682 | 1.428 | 4.845 |
| Comp -2 | Sitagliptin | 3.652 | 3927318 | 4129 | 1.369 | 4.489 |

**Table no. 10: Limit of detection.**

| Injection | Retention time | Peak area | USP plate count | Tailing factor |
|---|---|---|---|---|
| 1 | 4.348 | 320324 | 8492 | 1.474 |
| 2 | 5.339 | 57835 | 10848 | 1.302 |

**Table no. 11: Limit of Quantification.**

| Injection | Retention time | Peak area | USP plate count | Tailing factor |
|---|---|---|---|---|
| 1 | 4.349 | 699969 | 8299 | 1.410 |
| 2 | 5.341 | 183392 | 10633 | 1.303 |

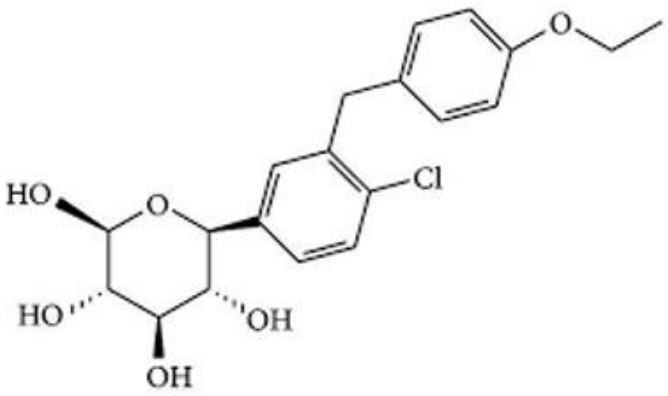



**Figure.1: Dapagliflozin.**

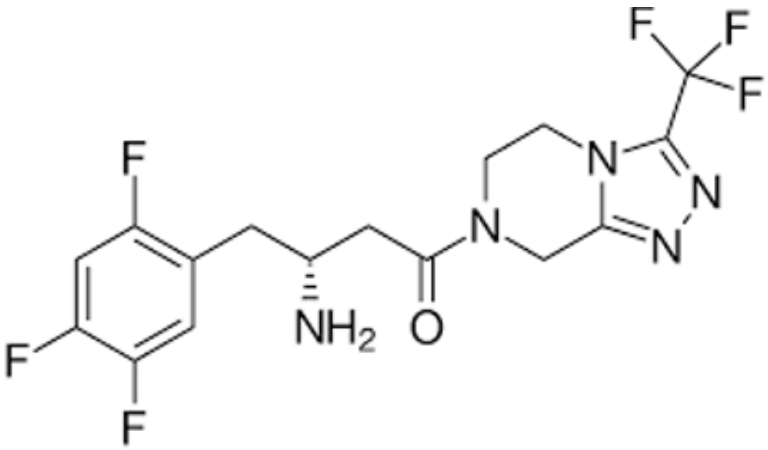
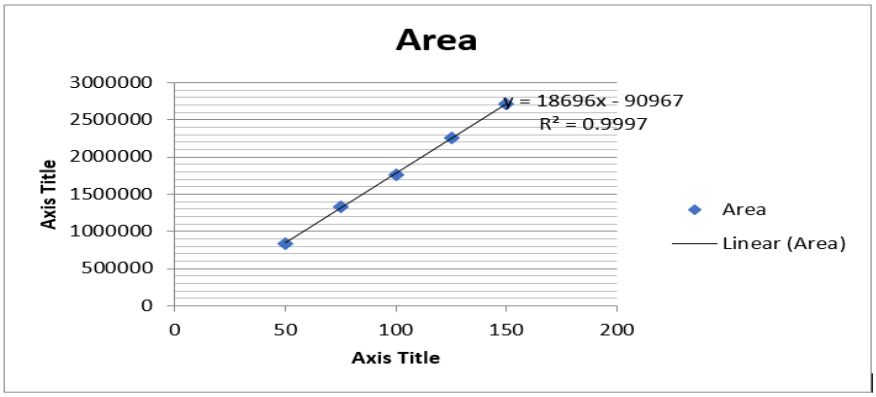
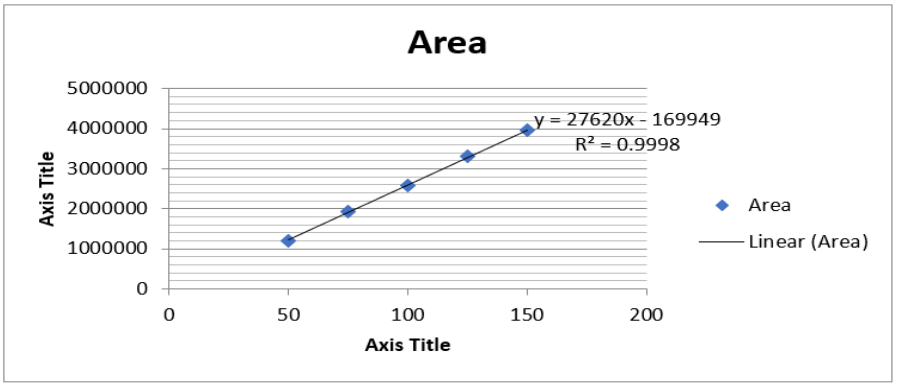


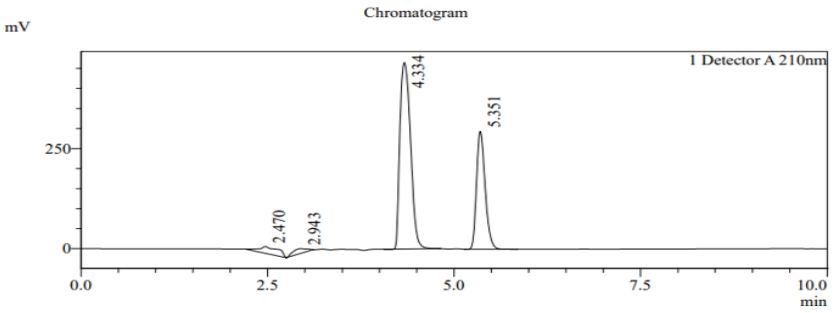

**Figure.2: Sitagliptin.**

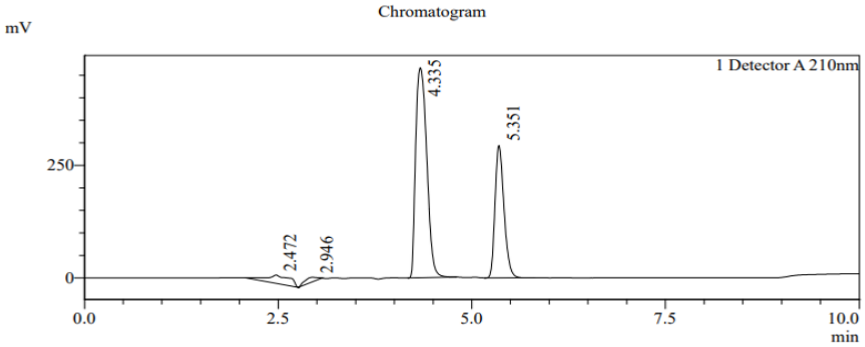


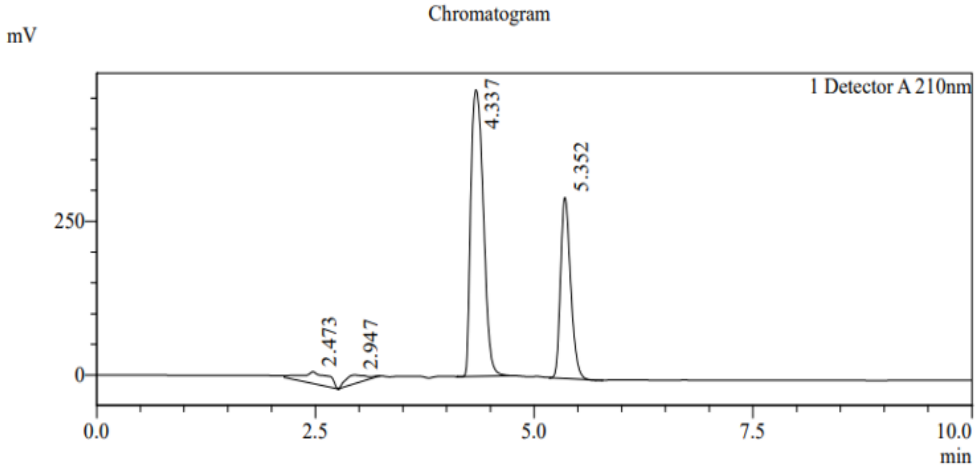

**Linearity of Dapagliflozin.**

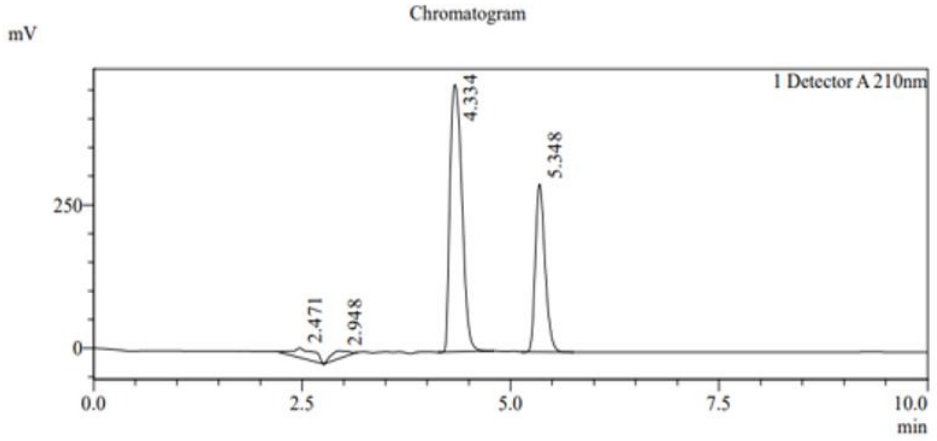


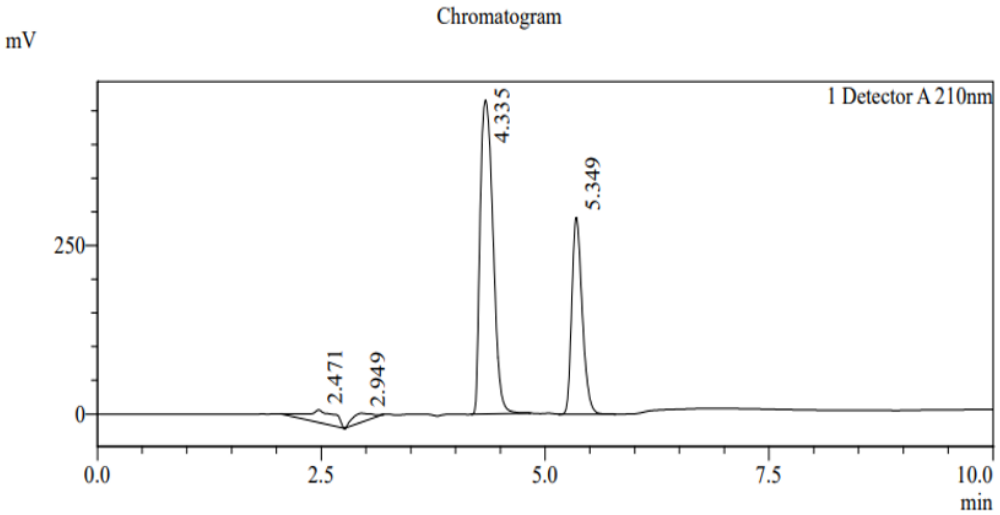

## System Suitability



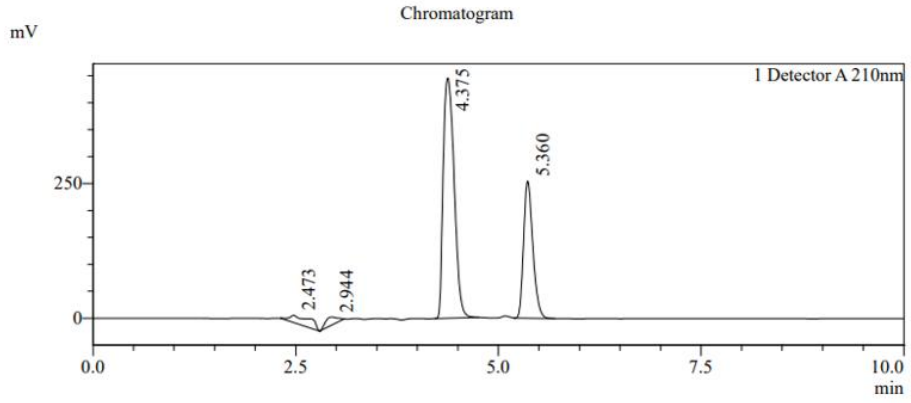




Chromatogram
mV
1 Detector A 210nm
4.337
5.352
250
2.473
2.947
0
0.0
2.5
5.0
7.5
10.0
min

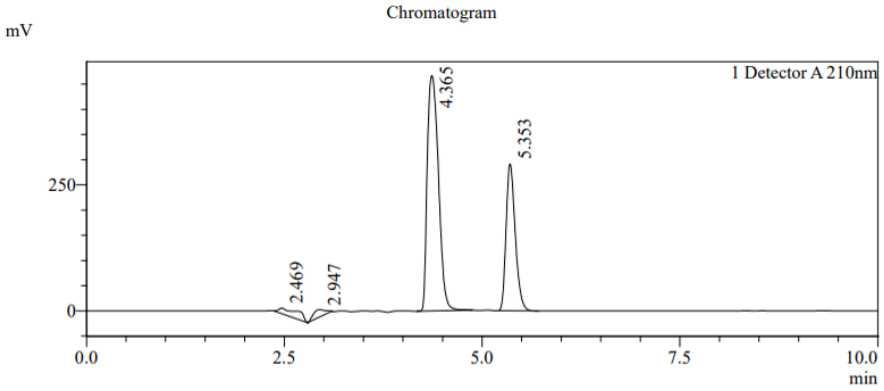

Chromatogram
mV
1 Detector A 210nm
4.334
5.348
250
2.471
2.948
0
0.0
2.5
5.0
7.5
10.0
min


Chromatogram
mV
1 Detector A 210nm
4.335
5.349
250
2.471
2.949
0
0.0
2.5
5.0
7.5
10.0
min

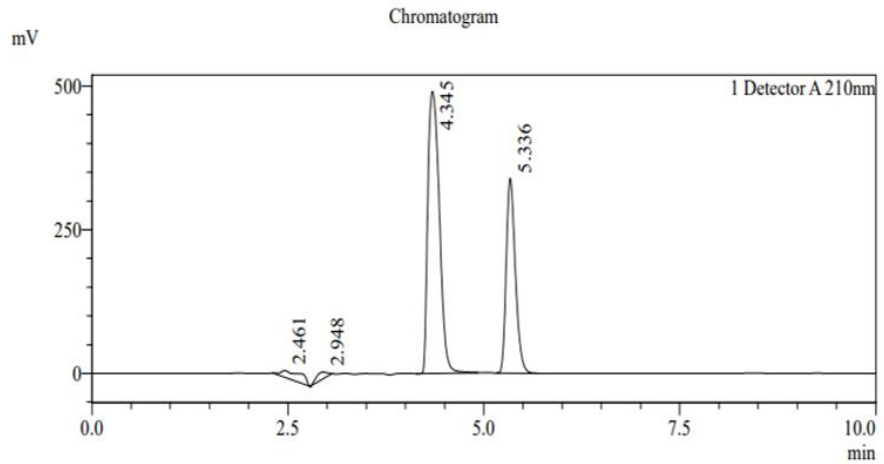
**Accuracy 80%**




**Accuracy 100%**

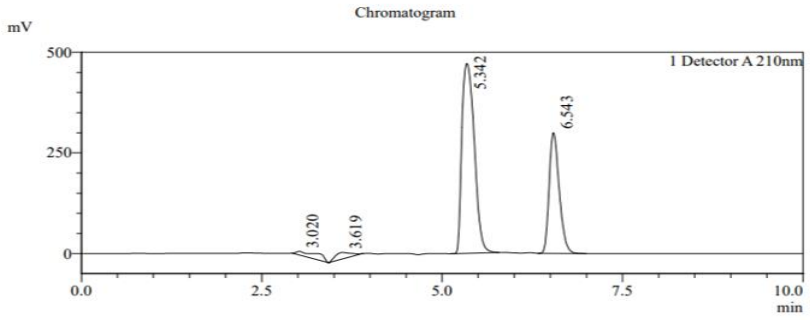



**ACCURACY 120%**

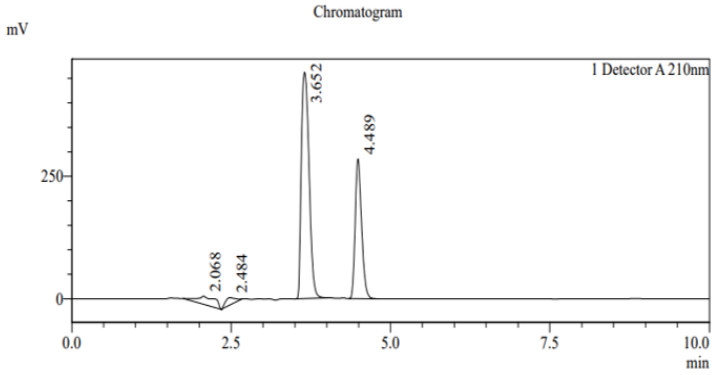


## ROBUSTNESS

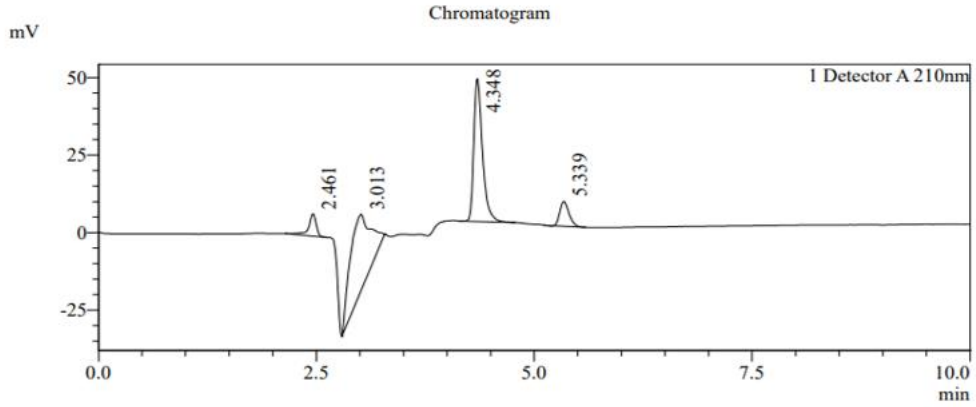
### FLOW SAMPLE - 1



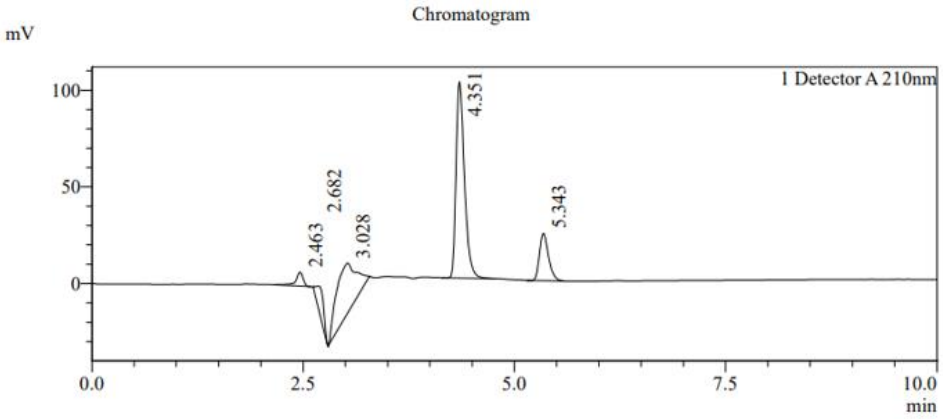

### FLOW SAMPLE – 2




## LIMIT OF DETECTION



## LIMIT OF QUANTIFICATION




## CONCLUSION

A RP - HPLC method for Dapagliflozin and Sitagliptin were developed and validated in tablet dosage form as per ICH guidelines. The results are found to be complying with the acceptance criteria for each of the parameter.

RP HPLC - SHIMADZU LC2010 CHT with Flowrosil($C_{18}$, 250 x 4.6 mm, 5µm) Packed Column, Injection volume of 10 µL is injected and eluted with the Mobile phase (Buffer (0.02 Ammonium dihydrogen phosphate): Acetonitrile (50:50). Which was pumped at a flow rate of 1.0 mL at 220 nm. The peak of Dapagliflozin and Sitagliptin was found well separated at. The developed method 3.033 and 13.408 was validated for various parameters as per ICH guidelines like system suitability, accuracy (recovery), precision (repeatability), specificity (interference), robustness, limit of detection, limit of quantitation, linearity and range.

Hence it is concluded that the assay method is found to be valid in terms of reliability, accuracy, precision and specificity. Hence it is suitable for routine analysis as well as for stability analysis.

## REFERENCE

1. Yesha Darshak Patel, Pinak Rameshbhai Patel, Jigna Bhatt, Binny Mehta, Krunal Detholia., Quantitative computation and stability evaluation of phase III composition comprising sitagliptin and dapagliflozin propanediol monohydrate by RP-HPLC., 2022.
2. Mohsin Kazi, Abdulmohsen A. Alqahtani, Badr S. Alsaadi, Musaed Alkholief & Fars K. Alanazi., UHPLC Method Development for Determining Sitagliptin and Dapagliflozin in

Lipid-Based Self-Nanoemulsifying Systems as Combined Dose in Commercial Products and its Application to Pharmacokinetic Study of Dapagliflozin in Rats

3. Ajay Gupta, Dr S K Mishra., A Novel Analytical Method for Simultaneous Quantification of Dapagliflozin and Sitagliptin y Reverse Phase High Performance Liquid Chromatography
4. So-Jin Kong, Joo-Eun Kim., Development of Clinically Optimized Sitagliptin and Dapagliflozin Complex Tablets: Pre-Formulation, Formulation, and Human Bioequivalence Studies
5. Pinak Patel, Yesha Patel, Shivani Jani and Krunal Detholia., Quantitative Computation of Synthetic Mixture Comprising Sitagliptin and Dapagliflozin from Synthetic Mixture by Vierdot's Method.
6. Mahesh Attimarad a, Mohammed Monirul Islam b, Sheeba Shafi c, Marysheela David c, Aminur Rahman b, Effren II Plaza Molina d., Smart eco-friendly mathematically manipulated UV spectroscopic methods to resolve severely overlapped spectra of a binary mixture of dapagliflozin with sitagliptin and vildagliptin
7. Biotech AP. Reversed Phase Chromatography: Principles and Methods. Amersham Pharmacia Biotech AB, Uppsala, Sweden, 1999.
8. Zzaman MT, Gilani SJ, Nagarajan K, Siddiqui SA. HPLC Method Development and Validation for Pharmaceutical Analysis-A Review. Internationale Pharmaceutica Sciencia, 2012; 2(3): 13-21.
9. Skoog DA, West DM, Holler FJ. SR crouch: Fundamentals of analytical chemistry. USA: Nelson Education, 2013.
10. Swartz ME, Krull IS, editors. Analytical method development and validation. CRC press, 2018.
11. ICH Q2A. Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
12. Q2B CH. Validation of analytical procedures: methodology. In International Conference on Harmonization, Geneva, Switzerland, 1996 Mar.
13. European Medicines Agency. ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology, 1995.
14. Borman P, Elder D. Q2 (R1) validation of analytical procedures. ICH Quality guidelines, 2017 Sep 29; 5: 127-66.

15. Gurdeep R, Chatwal S, Anand K. Instrumental methods of chemical analysis. Himalaya publishing house, 2016.
16. Ahuja S, Rasmussen H, editors. HPLC method development for pharmaceuticals. Elsevier, 2011.
17. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry: Quantitative Analysis. University of London, Athlone Press, 1962.
18. Zotou A. An overview of recent advances in HPLC instrumentation. Open Chemistry. 2012; 10(3): 554-69.
19. Rao TN. Validation of analytical methods. Calibration and Validation of Analytical Methods—A Sampling of Current Approaches. 2018.
20. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. John Wiley & Sons. 2012.
21. Eldin AB. Validation of an Analytical Procedure. Quality Control of Herbal Medicines and Related Areas, 2011: 17.
22. Papadoyannis IN, Samanidou VF. Validation of HPLC instrumentation. Journal of liquid chromatography & related technologies, 2004; 27(5): 753-83.
23. Majors R. Current trends in HPLC column technology. 2012.
24. Kirkland JJ. Development of some stationary phases for reversed-phase HPLC. Journal of Chromatography A., 2004; 1060(1-2): 9-21.
25. Saha P, Pandey MM. Design of Experiment (DoE)-Approach Based RP-HPLC Analytical Method Development and Validation for Estimation of Efavirenz in Bulk and Formulations. Journal of Chromatographic Science, 2022 Jan; 60(1): 35-44.
26. FDA. Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability," Federal Register (Notices), 2000; 65(169): 52776–52777.
27. Ganorkar SB, Shirkhedkar AA. Design of experiments in liquid chromatography (HPLC) analysis of pharmaceuticals: Analytics, applications, implications and future prospects. Reviews in Analytical Chemistry, 2017; 36(3): 1-43.
28. Awotwe-Otoo D, Agarabi C, Faustino PJ, Habib MJ, Lee S, Khan MA, Shah RB. Application of quality by design elements for the development and optimization of an analytical method for protamine sulfate. Journal of pharmaceutical and biomedical analysis, 2012; 62: 61-7.

29. Siregar C, Prabaningdyah NK, Choiri S, Riyanto S, Rohman A. Optimization of HPLC using central composite design for determination of curcumin and demethoxycurcumin in tablet dosage form. Dhaka University Journal of Pharmaceutical Sciences, 2017; 16(2): 137-45.
30. Lestari AD, Rohman A, Martono S. Central Composite Design Applied in HPLC Optimization for Analysis of Tartrazine and Auramine O in Powder Drinks. International Journal of Applied Pharmaceutics, 2019; 7: 211-5.