JOURNAL OF PHARMACEUTICAL ANALYSIS

DEVELOPMENT AND VALIDATION FOR NEW ANALYTICAL METHOD OF DAPAGLIFLOZIN BY RP-HPLC

Anjali Chaudhary[1], Dr. Rita Saini[2], Dr. Shivanand Patil[3]
Master Scholar[1], Associate Professor[2], Director[3]
Department of Quality Assurance, Shree Dev Bhoomi Institute of Education, Science and Technology, Dehradun, Uttarakhand. Uttarakhand Technical University, Dehradun, India

Abstract
An innovative approach has been developed and validated for HPLC analysis of Dapagliflozin. This method was verified based on ICH guidelines, encompassing accuracy, linearity, precision, and robustness. The UV spectra of the standard solution were analyzed across the wavelength range of 200 nm to 400 nm, revealing the isobestic point at 205 nm. The Dapagliflozin peak was successfully separated using a mobile phase comprising 65:35 v/v of buffer and acetonitrile, with the buffer pH set to 3.8 using either orthophosphoric acid or sodium hydroxide, achieving optimal retention time, peak area, and symmetry.
All analytical parameters were appraised using a Shimadzu C18 column (15 cm × 4.6 mm internal diameter, 5 µm particle size), ensuring the Dapagliflozin peak was distinctly resolved at a wavelength of 205 nm. Dapagliflozin exhibited a retention time of 6.986 minutes, with a flow rate of 0.8 ml/minute and a sample volume of 10µl. The regression coefficients (r^2) were found to be 0.99985 for the standard solution and 0.99947 for the test solution, both meeting the acceptable criteria. The assay recovered rate assorted between 99.10% and 101.71%.
Index Terms: Dapagliflozin, RP-HPLC, Validation

1. Introduction:

One method for separating mixture components is chromatography. The procedure commences with the dissolution of the mixture in a substance referred to as the mobile phase, which subsequently carries it through another substance known as the stationary phase. Due to the differences in their speeds, the individual components of the mixture separate from each other as they traverse the stationary phase. The rate at which substances move, whether quickly or slowly, is influenced by the properties of the specific mobile and stationary phases, which also dictate the manner of their separation. The phrase "retention time" pertains to these differing travel durations. Chromatography serves as an analytical tool by directing its output into a detector that analyzes the contents of the mixture. Additionally, it can function as a purification method to isolate components of a mixture for application in other processes or experiments. In general, analytical chromatography necessitates significantly less material compared to chromatography aimed at isolating specific components or purifying a mixture.[1]
Liquid chromatography was created in 1906 by Russian botanist Mikhail Tsvet while he was trying to separate chlorophylls from plant extract. Because of this discovery, he was dubbed the Father of Chromatography—more accurately, the Father of Liquid Chromatography. He kept working with chromatography in the first decade of the 1900s,

mostly for the separation of pigments found in plants, like carotenes, xanthophylls, and chlorophyll. They called the technique "chromatography" or "color writing" because these components (yellow, orange, and green) have different colors. A new kind of chromatography emerged in the 1930s and 1940s and has persisted to this day, making it a practical and necessary technique for many types of separation and purification.[2]

2. Principle of HPLC

During the purification process, a separation column serves to separate the stationary and mobile phases. The stationary phase within a separation column consists of a granular material composed of small porous particles. The solvent or mixture of solvents that is propelled through the separation column under high pressure is referred to as the mobile phase. A syringe is utilized to transfer the sample into the mobile flow regime from the pump to the separation column through a valve that is connected to a sample loop, which is essentially a small capillary or stainless steel tube. Upon completion of this operation, a chromatogram is generated within the HPLC software. The various compounds can be identified and quantified due to the information provided by the chromatogram. The individual components of a mixture thus travel through the column at different rates, influenced by their interactions with the stationary phase. When a compound exits the column and is transmitted as a signal to the HPLC software on the computer, it is detected by an appropriate detector.[3]

3. Dapagliflozin

Dapagliflozin is utilized in conjunction with dietary modifications and physical activity, and occasionally alongside additional medications, to reduce blood sugar levels in both adults and children aged 10 years and older who are diagnosed with type 2 diabetes (a condition characterized by elevated blood sugar due to the body's inability to produce or effectively use insulin). [4]

Mechanism of Action

Dapagliflozin blocks the sodium-glucose cotransporter 2 (SGLT2), which is mostly located in the nephron's proximal tubule. Since 90% of glucose reabsorption in the kidneys is facilitated by SGLT2, its suppression encourages glucose excretion in the urine. People with type 2 diabetes mellitus may lose weight as an outcome of this process, which also helps to improve glycemic control.[5]

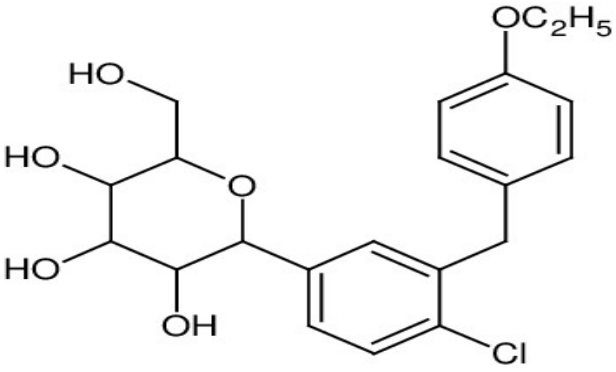


Figure: Dapagliflozin

4. Validation parameters recommended by FDA, USP and ICH are as follows

a. System Suitability

This is a crucial part of liquid chromatography methods that are used to test the chromatographic method's suitability, efficiency, resolution, and reproducibility for analysis. The system's suitability has been evaluated by calculating a number of factors, including peak resolution, peak tailing, number of theoretical plates, and efficiency.

b. Accuracy

The Accuracy of an analytical method signifies the near equivalence between the accepted true value or the acceptable reference value, and the obtained value. Accuracy is quantified as the recovery percentage, which is calculated by determining the known added analyte quantity (80%, 100%, 120%) within the sample, or by calculating the difference between the mean and the accepted true value based on the confidence interval. The International Conference on Harmonization (ICH) recommends measuring accuracy using a minimum of nine measurements across a minimum of three concentrations within the defined range (i.e., three concentrations and three replicates of each concentration).

c. Linearity

The ability of an analytical technique to yield test results that are exactly proportionate to the concentration of the sample analyte is known as linearity (within a specified range). The ICH Guideline suggests using at least five concentrations to assess linearity. The difference between the upper and lower levels of an analytical method is known as its range, and it is frequently measured using the method procedure with linearity, accuracy, and precision[7].

d. Linearity and range:

Analytical technique linearity is the potential (within a given range) to produce test results, which are directly proportional to the sample analyte concentration. The ICH Guideline proposes a minimum of five concentrations for determining linearity. The range of an analytical method is the difference between the upper and lower levels often seems to be measured with linearity, accuracy and precision using the method procedure[8].

e. Precision

The degree of agreement between a set of measurements derived from multiple samplings of the same homogeneous sample is known as the analytical method's precision. Repeatability: The repeatability of a test procedure is calculated by making full separate determinations of the same homogeneous batch of the material on specific samples and this would offer an indicator of the precision of the process under normal laboratory operating conditions. It is a short term analytical method under the same operations.

f. LOD

A single analytical limit is the lowest amount of an analyte in a sample that can be measured but is typically not quantified as the precise value. Usually expressed as the sample analyte concentration, the signal-to-noise ratio (S / N) (3:1) can be the focus of the LOD.

g. LOQ

The smallest quantity of the sample that can be quantitatively identified with suitable accuracy and precision is known as the quantification limit for a particular analytical technique. By injecting standards that yield this S/N ratio and also have an acceptable percent relative standard deviation, the LOQ is usually calculated from an S/N ratio determination (10: 1).[9]

h. Robustness

An analytical procedure's robustness is a measure of its ability to withstand minor but intentional changes in method parameters (pH, temperature, mobile phase composition, and instrumental settings) and to show its dependability under typical operating conditions. The methodical process of determining robustness involves assessing the device's suitability, examining the samples to hold a parameter, and figuring out how it affects the process[10].

4. Methodology

a. Mobile Phase: 0.01M Potassium Dihydrogen Orthophosphate and Acetonitrile (65:35 v/v)

b. Buffer:1.36 gm of potassium dihydrogen orthophosphate in 1000 ml HPLC water, add 1 ml of triethylamine and adjust pH 3.8 with dilute orthophosphoric acid.

c. Diluent: Water and Acetonitrile (50:50)

d. Preparation of Standard Solution: Weighed accurately 25 mg of Dapagliflozin working standard into a 50 ml volumetric flask. Dissolve and diluted to volume with diluent. Dilute 5 ml of this solution to 50 ml with same solvent mixture and mix. (0.05mg/ml)

e. Preparation of sample solution: 20 tablets were ground to obtain fine powder. Equivalent to 10 mg of dapagliflozin sample were weighed and transferred to 200 ml of volumetric flask and dissolved in diluent. The flask volume was made up to mark with diluent and was shaken. (0.05mg/ml)

f. Determination of Working Wavelength (λ max)

In a 100ml volumetric flask, 10mg of the standard drug dapagliflozin is taken, dissolved in diluent, and the volume is adjusted to the mark. 1ml of this solution was pipetted into a 10ml volumetric flask, and the volume was adjusted with water to give a concentration of 10µg/ml. A diluent is used as a blank when scanning the above-prepared solution in the UV range of 200–400 nm. 205nm was determined to be the λmax. A trail using a mobile phase mixture of buffer: acetonitrile (65:35% v/v) was deemed satisfactory following multiple initial trails using mixture of acetonitrile and buffer in different combinations and proportions. There were sharp peaks at a flow rate of 1ml/minute. Shimazu C18 column with Lab Solution software (150 x 4 x 6 mm internal diameter, 5µparticle size).

g. Optimum Chromatographic Conditions

- **Column:** Shimadzu C_{18} Column (150mm ×4.6mm, internal diameter: 5µ particle size)
- **Mobile Phase:** Buffer and Acetonitrile(65:35% v/v)
- **Buffer:** 1.36gm Potassium Dihydrogen Phosphate in 1L water and 1ml Triethylamine and pH adjusted to 3.8
- **Flow Rate:** 0.8 ml/minute
- **Detector:** UV-Detector
- **Injection Volume:** 10µl
- **Wavelength:** 205nm
- **Oven Temperature:** 30ºC

5. Results and Conclusion:

The standard solutions' UV spectra were examined between 200 and 400 nm in wavelength. The drug's absorptivity values at the optimal wavelength were computed, and the isobestic point was established at 205 nm. The data on linearity were provided below.

S.N.	Concentration (µg/ml)	Absorbance
1.	10	0.060
2.	20	0.117
3.	30	0.184
4.	40	0.244
5.	50	0.301

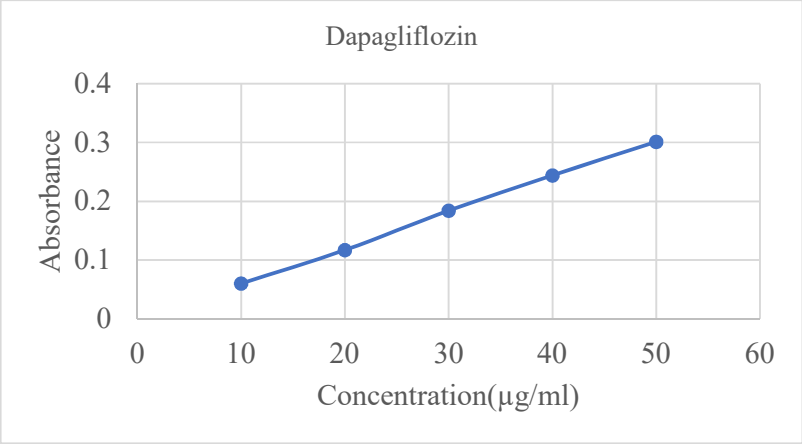


Fig.1: Calibration graph for Dapagliflozin

The analysis of the previously discussed experimental results leads to the conclusion that the newly developed method for the simultaneous estimation of Dapagliflozin is simple, accurate, and demonstrates high resolution. The objective was to establish an HPLC method for the quantitative assessment of Dapagliflozin. The subsequent section presents a discussion of the findings and results of the study.

The Dapagliflozin standard sample was analyzed using a UV Spectrophotometer, scanning wavelengths from 200 nm to 400 nm, with a fixed wavelength of 205 nm. In the context of HPLC, various column configurations are available. For our study, we selected the Shimadzu C18 Column (150mm × 4.6mm, with an internal diameter of 5µ particle size) over other options, as it provided excellent resolution for the Dapagliflozin peak at 205 nm.

Specificity: The absence of a discernible peak on the bands when utilizing the formulation's diluent and excipients suggests a significant level of specificity for the proposed method

Linearity: Linearity was noted across the spectrum of 80% to 120% (specifically at 80%, 90%, 100%, 110%, and 120%). The regression coefficients (r2) were found to be 0.99985 for the

Standard Solution and 0.99947 for the Test Solution, both of which fall well within the acceptable limits.

Accuracy: The percentage recovery of Dapagliflozin was found to be between 99.19% and 101.71%, which falls within the acceptable range, and it was noted that there was virtually no interference from the drug with the excipients or other medications in the formulation.

Intermediate Precision: The assay percentages reported by three analysts were 103.80%, 104.29%, and 104.96%, respectively. The repeatability was measured at 0.958, while the intermediate precision was recorded at 1.953, both of which fall within the established acceptance criteria.

Robustness: This represents an additional validation parameter for the HPLC method, where a minor variation in the drug's retention time was noted, yet it still conformed to the acceptance criteria.

The technique has been found to be user-friendly, accurate, precise, and suitable for the routine analysis of Dapagliflozin. The developed method is straightforward and easy to apply for assessing the quality and stability of Dapagliflozin Tablets.

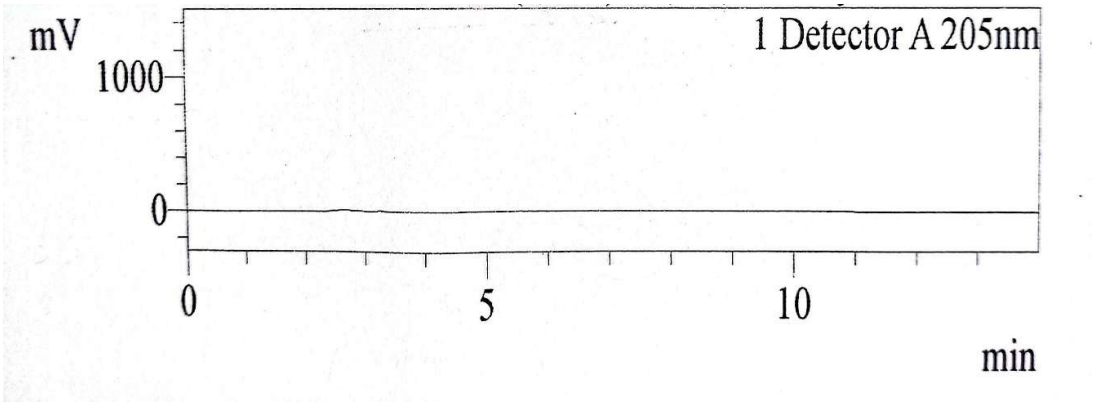


Fig.2: Diluent of Dapagliflozin

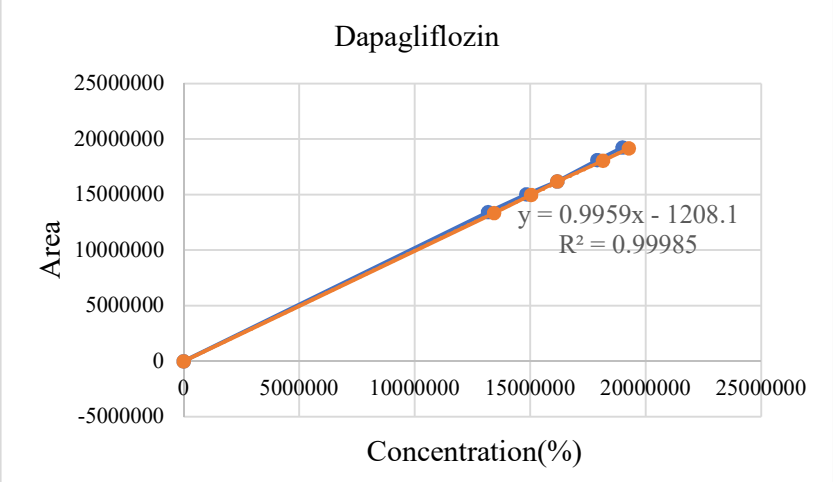


Fig. 3: Linearity Curve of Standard

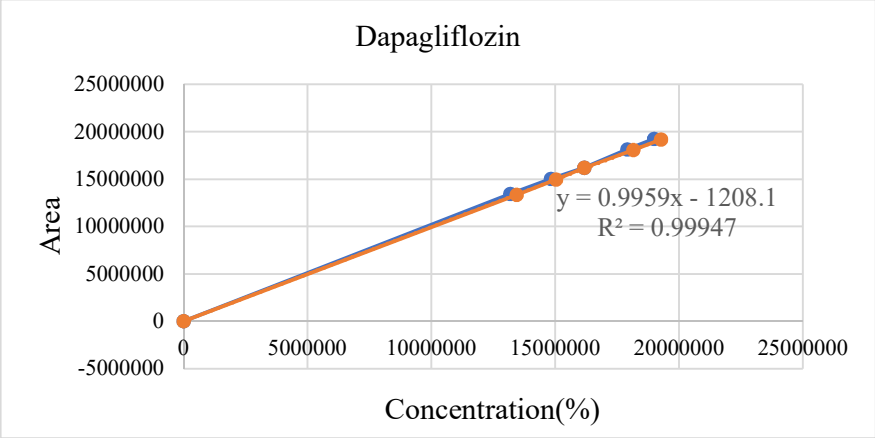
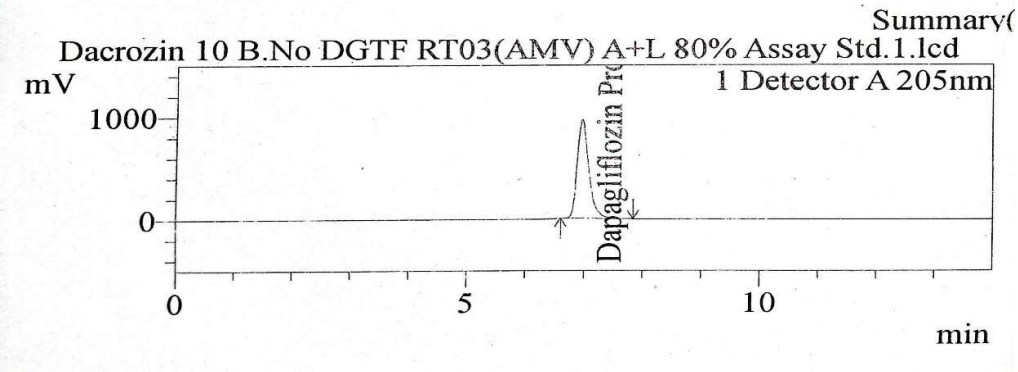
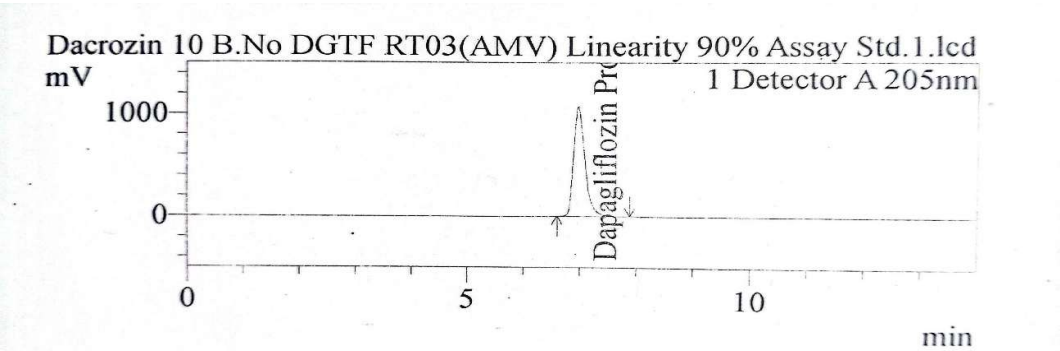
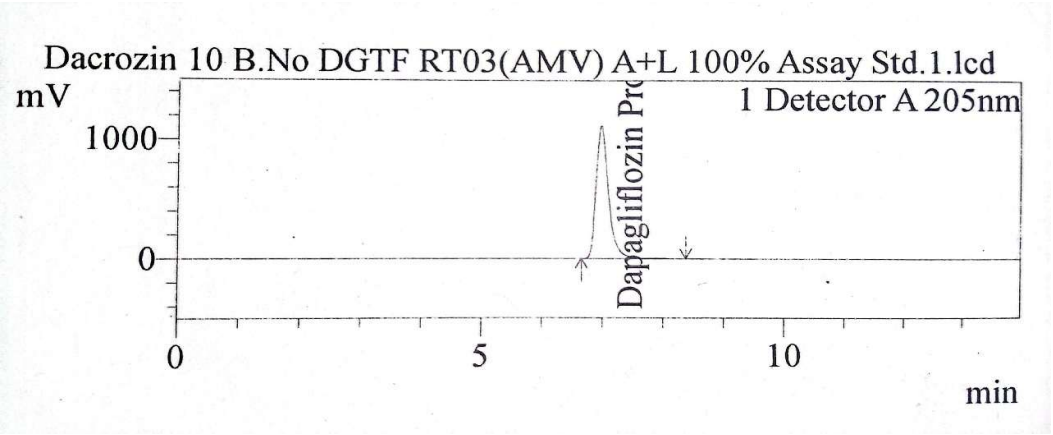


Fig. 4: Linearity Curve of Test







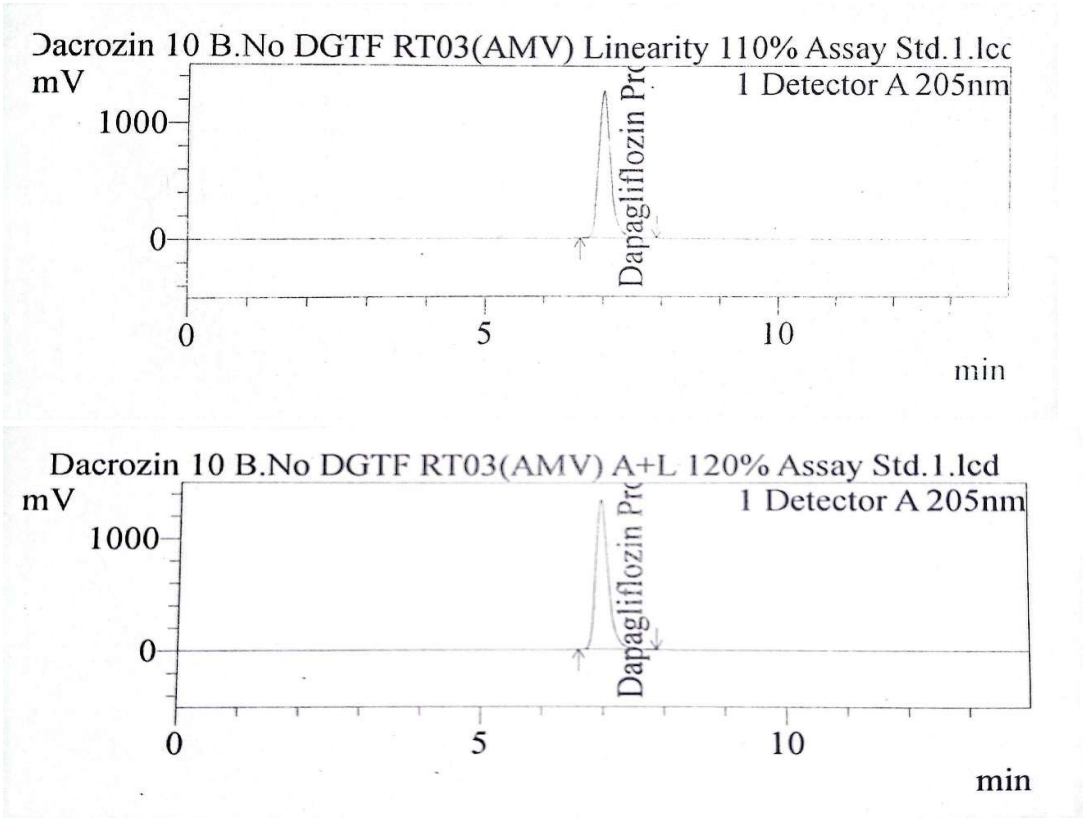


Fig.5: Linearity of standard at 80%, 90%, 100%, 110% & 120%

A completely new HPLC method has been created and approved for the analysis of Dapagliflozin in both bulk and pharmaceutical formulations. The method has demonstrated robustness, accuracy, linearity, specificity, and precision. Its suitability for estimating Dapagliflozin in pharmaceutical formulations was confirmed by low % RSD values and satisfactory recovery percentages. This technique achieves the highest recovery rates, with an average recovery nearing 100% for each component. The method's precision and accuracy were found to be acceptable. It is characterized as straightforward, accurate, and precise, making it suitable for routine analysis of dosage forms and stability studies involving Dapagliflozin. The developed method is user-friendly and facilitates the regular determination of Dapagliflozin and its stability.

7. References:

1. Ali AH. HighPerformance Liquid Chromatography (HPLC): A review. Ann Adv Chem. 2022; 6: 010-020.
2. Gurudeep C and Sham KA. Instrumental methods of Analysis. 5th ed. Mumbai: Himalaya publishing home; 2005. 108-109.
3. Douglas SA, James F and Timothy N. Principle of instrumental analysis 5th ed. Singapore: Thomas Brooks Cole; 2004. 739-740

4. Malviya, Rishabha & Bansal, Vvipin & Pal, Om & Sharma, Pramod. (2010). High-performance liquid chromatography: A short review. Journal of Global Pharma Technology. 2. 22-26.
5. ICH Q2 (R1) validation of analytical procedures: Text and methodology [online]. 2005[cited2014November]; Available from: URL:http://www.ich.org/fileadmin/public website/ICH products/ Guidelines Quality/ Q2 R1 Guidline.pdf
6. Sible, Alexandra M. PharmD; Nawarskas, James J. PharmD; Alajajian, David MD; Anderson, Joe R. PharmD Cardiology in Review 24(1): p 41-47, January/February 2016. | DOI: 10.1097/CRD.0000000000000093
7. Douglas SA, James F and Timothy N. Principle of instrumental analysis 5th ed. Singapore: Thomas Brooks Cole; 2004. 739-740
8. Devala Rao GA. Text book of Pharmaceutical Analysis. 4th Ed. New Delhi: Birla publications; 2005. 5-10.
9. Sanagapati M, Dhanalakshmi K, Reddy NG and Sreenivasa S., Development and Validation of Stability-Indicating RP-HPLC Method for Determination of Dapagliflozin, J. Adv. Pharm. Edu. Res., 2014, 4(3), 350-353
10. Jani BR, Shah KV and Kapupara PP: Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture. International Journal of Research and Development in Pharmacy & Life Sciences 2015; 4(3): 1569-76.
11. Bhavyasri K, Surekha: bioanalytical method development and validation of dapagliflozin and metformin hydrochloride in combined dosage form using uv spectroscopy. Gedrag & Organisatie Review 2020; 33.
12. Mehulkumar, Ramesh , Vinay kumar , R Srinivas, and Prakash V Diwa Asian J. Research Chem. 2(2): April.-July, 2009, pg. no. 127- 30
13. Gurudeep C and Sham KA. Instrumental methods of Analysis. 5 th ed. Mumbai: Himalaya publishing home; 2005. 108-109.
14. Novartis Pharmaceuticals Australia Pty Limited, ABN 18 004 244 160, 54 Waterloo Road, Macquarie Park NSW 211
15. Bari, S.B.; Kadam, B.R.; Jaiswal, Y.S.; Shirkhedkar, A.A. “Impurity profile: Significance in Active Pharmaceutical Ingredient”, Eurasian Journal of Analytical Chemistry, 2007, 2, 32-54
16. ICH Q2 (R1) validation of analytical procedures: Text and methodology [online]. 2005[cited2014November]; Available from: URL:http://www.ich.org/fileadmin/public website/ICH products/ Guidelines Quality/ Q2 R1 Guidline.pdf
17. ICH, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Current Step 4 Version, Parent Guideline, 1994, 630.
18. Skoog, D. A.; Holler, F. G.; Nieman, T. A. Principles of Instrumental Analysis. 5th Edition, Thomson Brooks/Cole Asia Pvt. Ltd., Singapore. 2004, 4-7.
19. Drug Profile and Information of Dapagliflozin Propanediol Monohydrate", October 2023, Available from:
20. Sharma A, Jain V and Prabhu V: Stability–indicating RP_HPLC method development and validation for simultaneous estimation of teneligliptin hydrobromide hydrate and

metformin hydrochloride in combination tablet dosage form. European Journal Biomedical Pharm 2018; 5(5): 453-62.
21. SB Wankhede, SB Wadkar, KC Raka, SS Chitlange, Indian Journal of Pharmaceutical Sciences, Year : 2009, Volume : 71, Issue : 5, Page : 563-567
22. Karuna PC, China E, and Basaveswara Rao MV., Unique UV spectrophotometric method for reckoning of Dapagliflozin in bulk and pharmaceutical dosage forms, J. Chem. Pharm. Res., 2015, 7(9), 45-49.
23. Mohammad Y, Gowri DS. A validated stability indicating HPLC method for simultaneous determination of metformin hydrochloride and dapagliflozin in bulk drug and tablet dosage form. A J Pharm Clin Res.2015; 8:320-326.
24. Kant R, Bodlarb, Kapoor G and Bhutani R: optimization of a single HPLC-PDA method for quantifying metformin, gliclazide, pioglitazone, dapagliflozin, empagliflozin, saxagliptin, linagliptin and teneligliptin using cent
25. Obermeier M, Yao M, Khanna A, Koplowitz B, Zhu M, Li W, et al. In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium-glucose cotransporter type II inhibitor, in animals and humans. Drug Metab Dispos 2017;38:405-14.
26. Manasa S, Dhanalakshmi K, Reddy GN, Sreenivasa S. Method development and validation of dapagliflozin in API by RPHPLC and UV-spectroscopy. Int J Pharm Sci Drug Res. 2014; 6(3):250- 252.
27. V. Gaikwad. H.; Bassett, J.; Mendham, J.; Denney, R. C. Vogel's Textbook of Quantitative Chemical Analysis. 5th Edition, Addison Wesley Longman Inc, Singapore. 2018, 4-5
28. British Pharmacopeia. Vol II. London: Medicines and Healthcare products Regulatory Agency; 2016. 1064-1065.
29. Chatwal, G. R.; Anand, S. K. Instrumental Methods of Chemical Analysis. 5th Edition, Himalaya Publishing House, New Delhi. 2007, 2.624-2.629
30. Patel KJ, Chaudhary AB. Stability Indicating RP-HPLC Method Development and Validation for Estimation of Dapagliflozin and Metformin HCL. World Journal of Pharmacy and Pharmaceutical Sciences.2017; 6(9): 796-809
31. Ramachandra, Neha MS, Sonali DS, Vishal T, Pawan K. Development and validation of stability indicating HPTLC method for simultaneous estimation of Sacubitril and Valsartan in pharmaceutical dosage form. European Journal of Biomedical and Pharmaceutical Sciences 2017; 4(8): 369-374
32. O. Sagirli, A. Önal, S. E. Toker and D. Şensoy, Chromatographia, Volume 66, Numbers 3-4 / August, 20, Springer Link Date Tuesday, May 05, 2009 Pages. 213-218 07
33. Afshan Jabeen et al Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Sacubitril in pharmaceutical dosages form. IAJPS 2018, 05 (07), 7079-7087.
34. N Anjaneyulu, Professor, Department of Pharmaceutical Analysis, Geethanjali College of Pharmacy, Cheeryal(V), Keesara(M), Medchal (Dist), Telangana, India. Development and Validation of A RP- HPLC Method for the Simultaneous Estimation of Dapagliflozin in Rat Plasma ISSN:2573-2250.
35. C. J. Patel, Amrohi, Prashanth Kumar.K, Nivedita.G, Pradeep Kumar.T, Dibyalochan Mohanty and Prakash.V. Diwan, Stability indicating RP-HPLC method for simultaneous

estimation of dapagliflozin in bulk and combined pharmaceutical dosage form. World J. Of Pharmacy and Pharm. Sci 6(4): 1714-1728

36. Mahabole G. R.; Anand, S. K. Instrumental Methods of Chemical Analysis. 5th Edition, Himalaya Publishing House, New Delhi. 2024, 2.624-2.629
37. Afshan Jabeen et al Indicating RP- HPLC Method for Simultaneous Estimation of Dapagliflozin in pharmaceutical dosages form. IAJPS 2018, 05 (07), 7079-7087.
38. Patel KH, Luhar SV, Narkhede SB (2016) Simultaneous estimation of dapagliflozin in the synthetic mixture by RP-HPLC method. J Pharm Sci Biosci Res 6(3): 262-269.
39. Manjiri Shinde, Dr. Satish Kumar, Dr. Arunabha Mallik, Dr. Jyoti, A review on stability indicating HPLC method development. EP-RA international Journal of Research and Development. Vol.6/Issue:10/ October 2021.
40. Zhanna Kobalava, Yulia Kotovskaya, Oleg Averkov, Elena Pavlikova, Valentine Moiseev, Diego Albrecht, Priya Chandra, Surya Ayalasomayajula, Margaret F. Prescott, Parasar Pal, Thomas H. Langenickel, Pierre Jordaan, and Iris Rajman Cardiovasc Ther. 2016 Aug; 34(4): 191–198. Published online 2016 Jul 6.
41. SS Chitlange , Kiran Bagri and DM Sakarkar, Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. Asian J. Research Chem. 1(1): July-Sept. 2008
42. Farheen Begum, S.H. Rizwan Stability indicating analytical method development and validation for estimation of Sacubitril and Valsartan in bulk and pharmaceutical dosage form using RP-HPLC Int. J. of Farmacia, 2016; Vol-(2) 1: 79-87.
43. International conference on harmonization, "Q2A- Validation of analytical Procedures: Methodology", IFPMA, Genevia, 1995, 2-3-1 to A-3.
44. Patel KH, Luhar SV, Narkhede SB (2016) Simultaneous estimation of sacubitril and valsartan in the synthetic mixture by RP-HPLC method. J Pharm Sci Biosci Res 6(3): 262-269.
45. ICH Q2 (R1) validation of analytical procedures: Text and methodology [online]. 2005[cited2014November]; Available from: URL:http://www.ich.org/fileadmin/public website/ICH products/ Guidelines Quality/ Q2 R1 Guidline.pdf
46. British Pharmacopeia. Vol II. London: Medicines and Healthcare products Regulatory Agency; 2016. 1064-1065.
47. Wate SP, Nimje H, Dharkar DP, Razdan R. Simultaneous RP-HPLC detrermination of metformine and Dapagliflozin in tablets. Indian Journal of Pharmaceutical Sciences 2007; 69(2): 281-283.
48. Lakshmi S, Devarajan. Spectrophotometric and HPLC method for simultaneous estimation of dapagliflozin from tablets. International Journal of Chemtech Research 2009; 1(1): 96-102.
49. Samel A, Raman B. Simultaneous estimation of related impurities of Dapagliflozin in its API by RP-HPLC. Asian Journal of Chemistry 2009; 21(1): 176-182.
50. Chandrashekhar N, Patel HR, Karvekar MD, Suresh BAR. Simultaneous estimation of Dapagliflozin and metfoemine in their combined dosage forms by spectrophotometric and RP-HPLC method. Journal of Analysis and Bioanalytical Techniques 2011; 2(4): 1-5

51. Mohamed AH, Fathalla B, Mahmoud AO, Sayed D, Saha Z, Safaa FS. Simultaneous determination of dapagliflozin in tablet and biological fluids using micellar liquid chromatography. Journal of Chromatographic Science 2017; 1-9.