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REVERSE PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN IN BULK AND SOLID DOSAGE FORM

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ABSTRACT

A simple, accurate, sensitive RP-HPLC method was developed for the determination of Dapagliflozin in bulk and it'sfilm coated tablet dosage form. The chromatographic separation was achieved on a reverse phase C_{18} Peerless Basic Column (250 mm×4.6 mm×5 µm). The mobile phase system consisting of acetonitrile and water (90:10v/v), was pumped using isocratic mode at 1mL/min flow rate. Detection of drug was made spectrophotometrically at a wavelength 223 nm using PDA detector and column temperature was adjusted at 30°C. The retention time was found to be 3.30 min. The method was validated to fulfil International Council on Harmonization (ICH) requirements and included specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness. The analyte response was instituted to be linear from range 10-60 µg/ml with a regression value of 0.997. % recovery was obtained as 100.59%, 100.45%, 101.29%. Limit of Detection and Limit of Quantification was found to be 2.91 µg/ml and 8.92 µg/ml. The method was found statistically accurate, precise with relative standard deviation (%RSD) values <2.0% and robust. Thus, proposed method is convenient, efficient and useful in routine analysis for estimation of Dapagliflozin in its bulk form and film coated tablet dosage form.

***Index Terms*: Dapagliflozin, Method Development and Validation, RP-HPLC.**

INTRODUCTION

Dapagliflozin is an Oral Antidiabetic drug that helps to control blood sugar levels. Dapagliflozin is a white crystalline powder having formula $C_{21}H_{25}ClO_6$. Molecular weight is 408.873g/mol. Dapagliflozin sold under the brand names of Farxiga, Oxra, Udapa etc [1]. Dapagliflozin is in a class of drug called SGLT2 inhibitors which is used for treating Type-2 diabetes. These drugs work by targeting and helping to stop sodium-glucose transport proteins from allowing glucose that has been filtered out of the blood by the kidneys to be reabsorbed back into the blood. The SGLT proteins are responsible for 90% of the glucose that is reabsorbed into the blood. By inhibiting the SGLT2 proteins, Dapagliflozin allows a significant amount of glucose in the blood to be removed by the kidneys and excreted in the urine. It is not suitable for treating type-1 diabetes [3],[4].

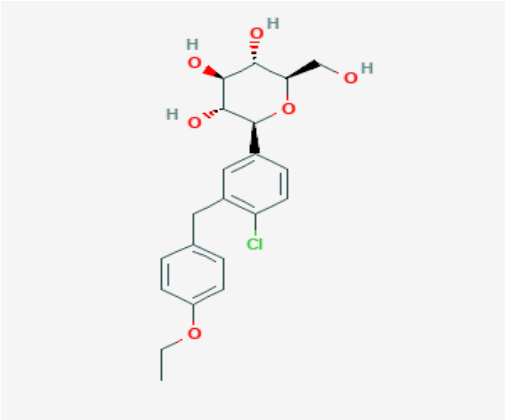

Figure 1: Structure of Dapagliflozin

Rationale of work

The objective of the research work to develop and validate a simple and accurate method of reverse phase chromatographic method to estimate amount of drug in dosage form. Literature survey reveals that there are limited literatures were available using PDA detector and mobile phase using acetonitrile and water.

Gouru Santosh reddy et al

Performed a linear, precise and accurate RP-HPLC (ReversePhase High-Performance Liquid Chromatography) method for the determination of dapagliflozin in the formulation. The method was accomplished on a C18 column (250×4.6mm; 5µm), & Samples were eluted using **acetonitrile: water (40:60%v/v)** deliveredat a flow rate of 1.0ml/min with a chromatographic run time of 10 min. The eluents were observed utilizing a UV detector with a wavelength set at 277nm. The method that was developed resulted in the retention of dapagliflozin at 7.029minutes. Dapagliflozin through current method has shown linearity ($r^2 > 0.999$) over the concentration range of 1-16 µg/ml. The percentage recovery was observed to be within the limits of 98-102%, demonstrating the accuracy of the method. Limit of detection (LOD) and limit of quantification (LOQ) were qualified at 0.049µg/ml and 0.1485µg/ml, respectively.[4]

Gunasekar Manoharan et al

RP-HPLC method has developed and validated for the analysis of Dapagliflozin in raw and tablet formulation. Separation of drugs products is developed on a C18 column reversed-phase using mobile phase composition of **Methanol: Water (75:25 v/v)**. The flow rate was adjusted to 1 mL/minute and the absorption maxima were observed at 230 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Linearity was obtained in the range of 5-25 µg/mL, for Dapagliflozin. The HPLC, Dapagliflozin tablet formulation assay shows percentage purity ranging from 99.98% to 100.12%. The mean percentage purity of Dapagliflozin is 100.22 %. The chromatographic retention time of Dapagliflozin was found to be 3.1 min.[6]

Shakir Basha S et al

developed and validate a simple, selective, precise, and accurate method for the estimation of dapagliflozin using reversed-phase high performance liquid chromatography (RP-HPLC) technique. The proposed method utilizes chromatographic conditions hypersil BDS (250 mm
× 4.6 mm, 5 µ), mobile phase used **buffer : acetonitrile** (60:40) ratio, flow rate was maintained 1 ml/minute, column temperature was set at 30°C, detection wavelength was 245 nm, and diluent was mobile phase. The retention time was foundto be 2.789min. The linearity study was performed by taking 25-150% levels, and precision was found to be 0.5 for repeatability and 0.31 for intermediate precision. The % recovery wasfound to be 99.89%. Limit of detection and limit of quantitation were found to be 0.60 µg/ml and 1.81 µg/ml, respectively. The % purity was found to be 99.71%. Degradation study on dapagliflozin was performed and concluded that the purity threshold was more than purity angle and within the acceptable range.[7]

Jitendra Debata et al

describes a new, simple, selective, accurate, rapid and precise reversed-phase high-performance liquid chromatographic technique of Dapagliflozin was established as per ICH Guidelines. RP-HPLC was performed on a Waters C18, 5 µm particle size, 25 cm × 4.6 mm with **phosphate buffer and acetonitrile** in the ratio of **60:40 v/v** as a mobile phase and a flow rate of 1.0 ml min^{-1}. UV detection was performed at 237 nm. Total run time was 6.0 min. The retention time of Dapagliflozin was found to be 3.461 minutes. Validation of the developed method was done as per USP and ICH guidelines. Linear calibration plots were obtained in the concentration range of 10-60 µg/ml for Dapagliflozin. Limit of detection were 0.02 µg/ml and limit of quantification were 0.06 µg/ml for Dapagliflozin.[12]

METHODS

Instrument

HPLC JASCO 4000 Extrema with autosampler having Chromatopak Reverse phase C_{18} Peerless Basic Column (250 mm×4.6mm,5µm) equipped with PDA detector used for method development and validation studies. The chromatograms were recorded using ChromNav software.

Chemicals and Reagents

Dapagliflozin was obtained as gift sample from USV laboratories, Ltd. Dapagliflozin marketed product Udapa 10mg tablets were purchased from the local pharmacy in the market. HPLC grade acetonitrile, methanol and water were procured from Merck.

Chromatographic conditions

An HPLC system which is operated using a software ChromNav, fitted with Peerless Basic Column (250 mm×4.6mm,5µm) and PDA detector(at 223nm) was used for analysis. Isocratic run with a flow rate 1ml/min, temperature 30°C and injection volume 10 µl was preferred for resolving the drug.

Preparation of Mobile Phase

A mixture of Acetonitrile:Water (90:10) used as mobile phase.

Stock solution preparation (1000 µg/ml)

Weighed accurately about 10mg of dapagliflozin standard and transferred into a 10 ml volumetric flask. To this 7ml diluent (ACN) was added and sonicated for 10 minutes. Then further volume made with same diluent.

Standard solution preparation (30 µg/ml)

Pipette out 1 ml from stock solution and make up the volume to mark with diluent to give 100 µg/ml. From this pipette out 3 ml and transferred into 10 ml volumetric flask and make up volume with diluent to give 30 µg/ml.

Assay of Formulation

Triturate 10 tablets of drug DAPA, weigh quantity of tablet equivalent to 10 mg of drug into 10 ml of volumetric flask Add 7 ml of diluent disperse the tablet completely and sonicate for 10 min with intermittent swirling, cool the flask and dilute up to mark with diluent. Pipette out 0,3 ml in 10 ml of volumetric flask and make up volume with diluent

$$\text{ASSAY} = \frac{AT}{AS} * \frac{WS}{WT} * \frac{DT}{DS} * \frac{\text{AVERAGE WEIGHT}}{\text{LABEL CLAIM}} * \frac{P}{100} * 100$$

Where,

AT- Area of drug peak in chromatogram test solution

AS- Average area of the drug peak in chromatogram obtained for five replicate injection of standard solution WS- Weight of drug standard in mg used in preparation of standard solution

WT- Weight of sample, used in preparation of test sample, in mg

WS- Weight of standard, used in preparation of standard stock solution, in mg WT- Weight of sample, used in preparation of test sample, in mg

LC- Label claim of drug

P- % Purity of drug standard

METHOD DEVELOPMENT

Different proportions of water and methanol were tried as mobile phase to develop a simple reverse phase liquid chromatography method. But in this mobile phase peak does not have proper shape. So acetonitrile and water (90:10) was selected to get sharp peak with a flow rate 1.0ml/min. Peerless Basic Column (250 mm×4.6mm,5µm) and PDA detector (at 223nm) was used for analysis which gives lesser tailing. Maximum absorption wavelength 223nm was observed on UV spectrum. Therefore. 223nm was kept constant as the detection wavelength throughout investigation.

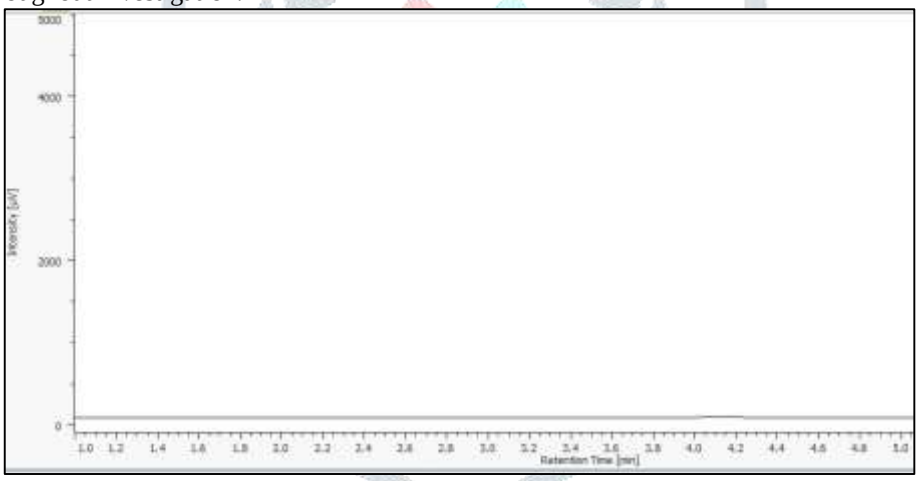

Figure2: Blank chromatogram of Dapagliflozin at 223nm

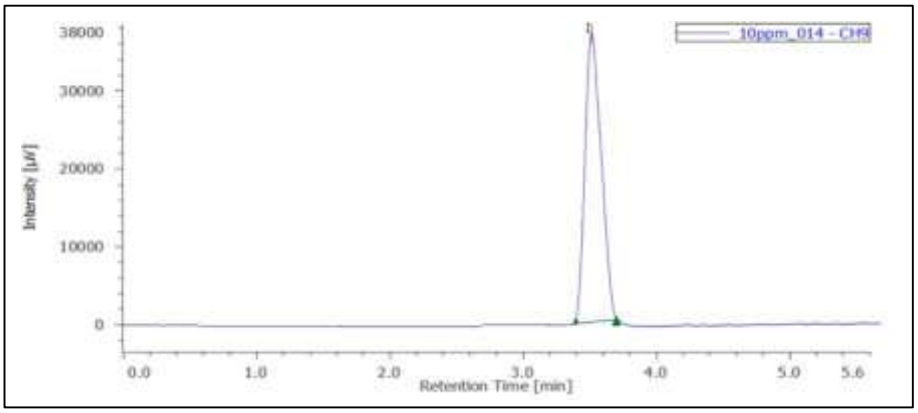

Figure 3: Standard chromatogram of Dapagliflozin at 223nm

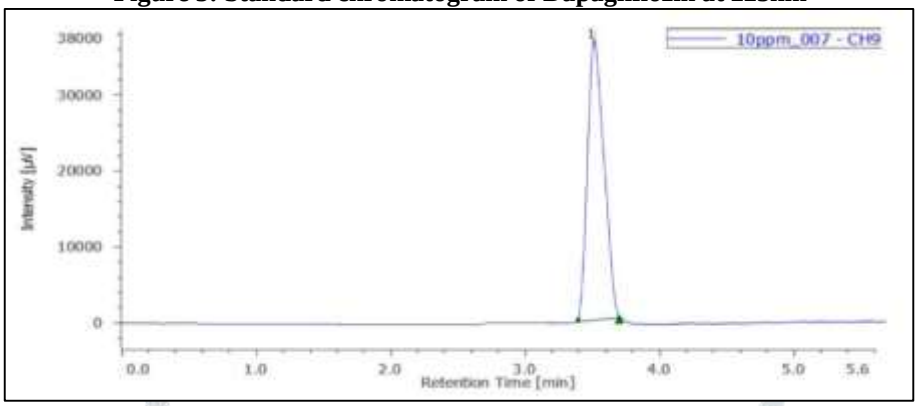

Figure No 4: Sample chromatogram of Dapagliflozin at 223nm

METHOD VALIDATION

Analytical method of developed method was validated using different parameters like system suitability, linearity, accuracy, precision, robustness, specificity, limit of detection, limit of quantitation as per ICH Q2R1 guidelines.

Assay of Marketed Formulation

Table 2: Assay of Tablet dosage form

Drug	Dapagliflozin
Label claim	10mg
Found content	9.97mg±0.8
% Assay	99.7%

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Inject blank, Standard and Sample preparation. Check any interference from diluents at the Drug peak.

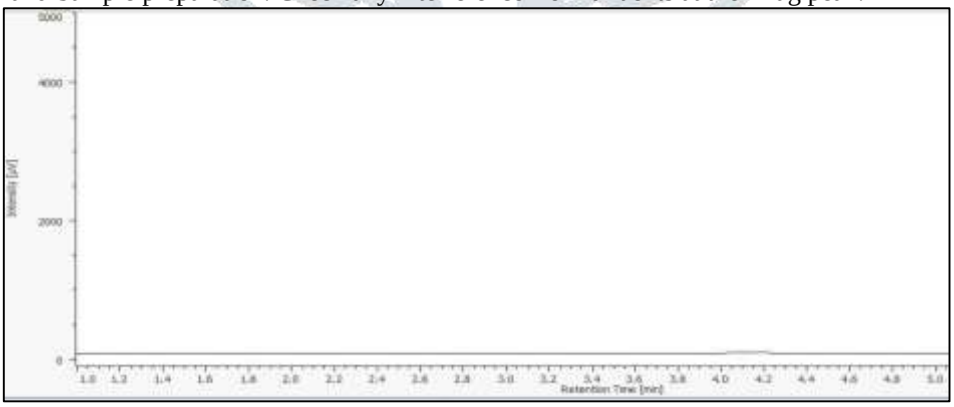

Figure 5: Blank Chromatogram for Specificity

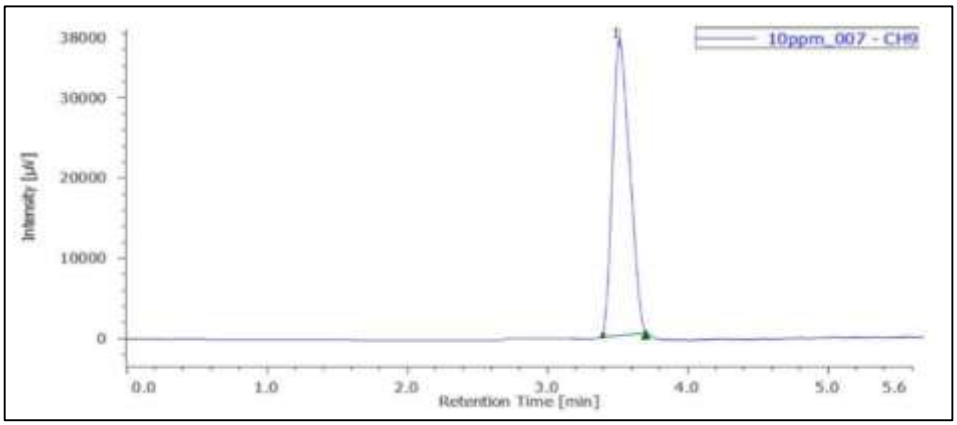

Figure 6: Standard chromatogram for Specificity

System suitability

A standard solution of dapagliflozin working standard was prepared and injected into the HPLC system. The parameters used in the system suitability are tailing factor, NTP.

Table 3: System Suitability Parameters

Parameters	Result
Symmetry factor	**1.1**
No. of theoretical plates	**3279**

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.To demonstrate linearity method, five standard solutions with concentrations of about 10- 60ppm was injected. The graph plotted between concentrations and peak area was shown in fig.5.

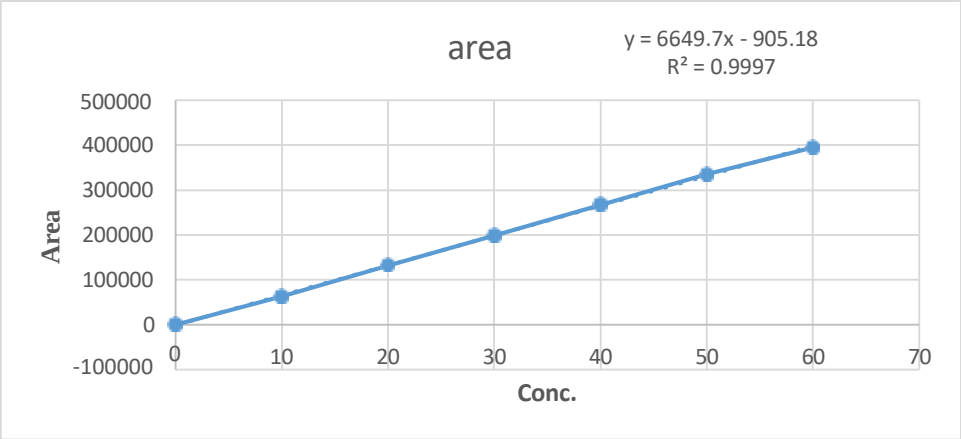

Figure 7: Standard curve linearity studies of dapagliflozin Table 4: Linearity results of Dapagliflozin

Sr No.	Concentrations (µg/ml)	Peak area
1	0	0
2	10	62953
3	20	132213
4	30	198697
5	40	267002
6	50	334658
7	60	394568
	Correlation coefficient	**0.999**
	Y-Intercept	**Y= 6649.7x – 905.18**

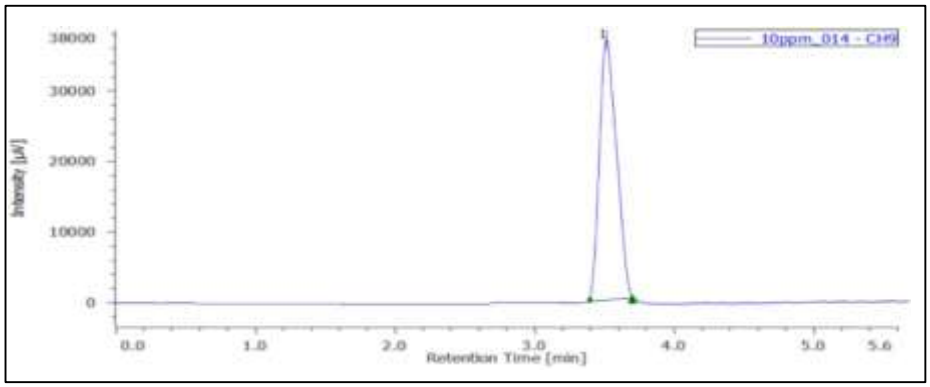

Figure 8: Chromatogram of Dapagliflozin for Linearity 10 µg/ml

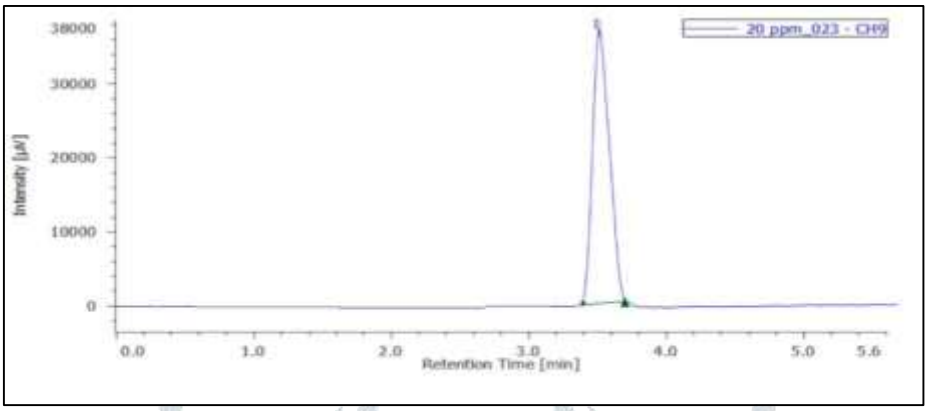

Figure 9: Chromatogram of Dapagliflozin for Linearity 20 µg/ml

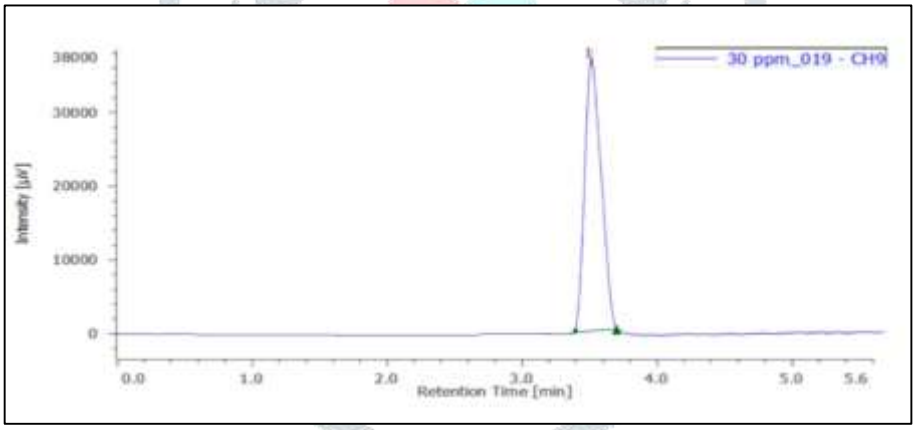

Figure 10: Chromatogram of Dapagliflozin for Linearity 30 µg/mL

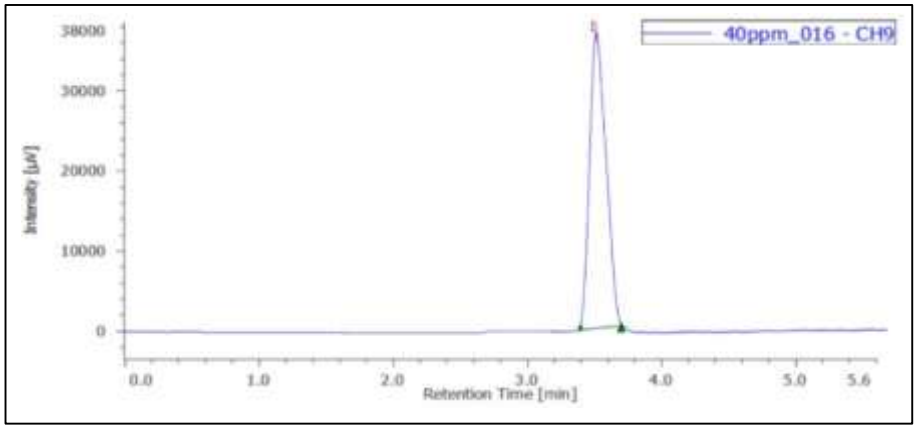

Figure 11: Chromatogram of Dapagliflozin for Linearity 40 µg/ml

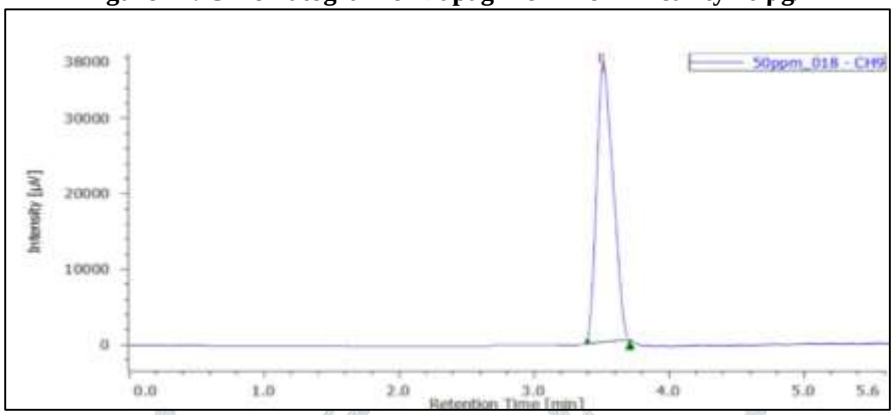

Figure 12: Chromatogram of Dapagliflozin for Linearity 50 µg/ml

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

System Precision

Repeatability is the fixed amount of drug was repeated at 6 times. The %RSD was calculated and presented in table III.

Intermediate precision

The intermediate precision were calculated by measuring the responses of standard peak on the same day and another day of the same solution concentration.

Table 5: Repeatability results of Dapagliflozin

HPLC INJECTION OF DAPAGLIFLOZIN (20 µg/ml)	RETENTION TIME (min)	PEAK AREA
1	3.017	132456
2	3.016	133445
3	3.017	135678
4	3.018	137898
5	3.016	132456
6	3.017	133445
Average	**3.01683**	**134229.7**
Standard deviation	**0.0075**	**2148.373**
%RSD	**0.0249**	**1.60**

Table 6: Intermediate results of Dapagliflozin

Conc. in (µg/ml)	Area	Mean	Std Dev.	%RSD
20 (µg/ml)	133245 132231 133434	132970	646.932	0.48
30 (µg/ml)	198697 198567 198667	198643.7	68.068	0.03
40 (µg/ml)	267052 267001 267002	267018.3	29.160	0.01

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy was performed for 80%, 100% and 120% for dapagliflozin. The %RSD was calculated should be ≤2%.

Table 7: Accuracy results of Dapagliflozin

Level	Stock Added (µg/ml)	Std. Added (µg/ml)	Total Conc. (µg/ml)	AUC	Conc. (µg/ml)	%recovery	%mean recovery
80%	20	16	36	240412	36.29	100.8	
80%	20	16	36	239149	36.1	100.27	**100.59**
80%	20	16	36	240212	36.26	100.72	
100%	20	20	40	270003	40.74	101.85	
100%	20	20	40	262755	39.65	99.12	**100.45**
100%	20	20	40	266146	40.16	100.4	
120%	20	24	44	292612	44.14	100.31	
120%	20	24	44	296602	44.74	101.68	**101.29**
120%	20	24	44	297200	44.83	101.88	

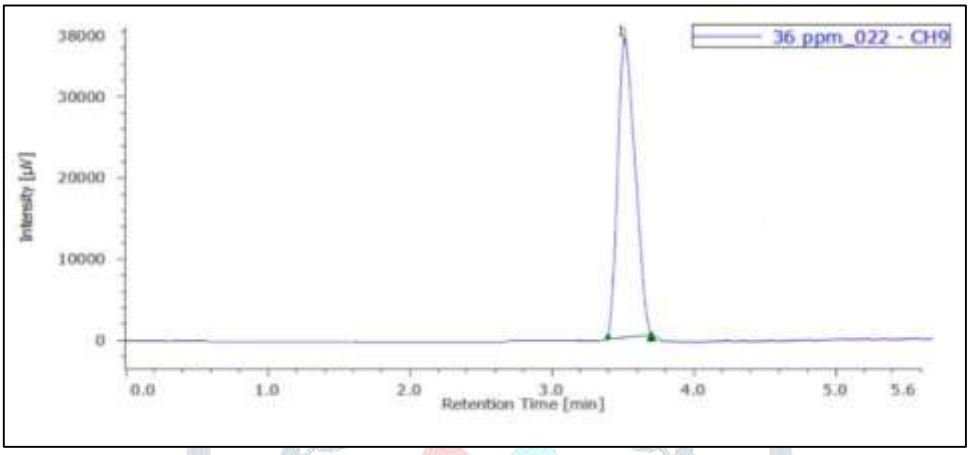

Figure 13: Chromatogram of Dapagliflozin for Accuracy (80%)

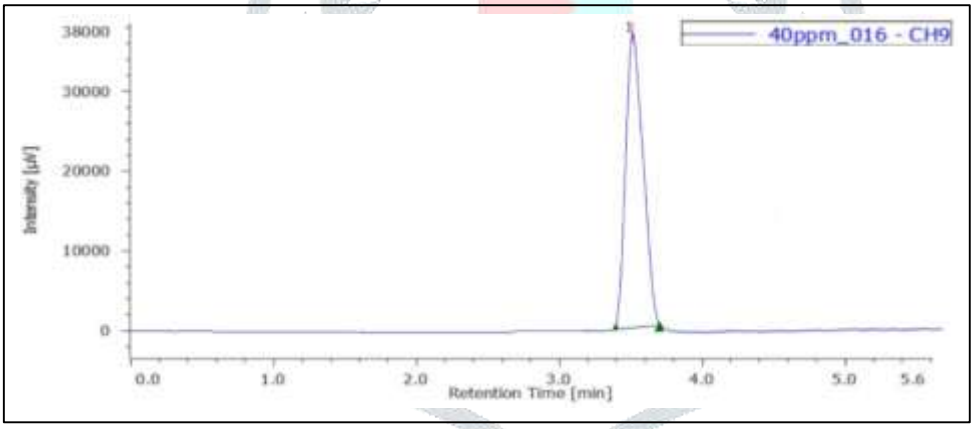

Figure 14: Chromatogram of Dapagliflozin for Accuracy (100%)

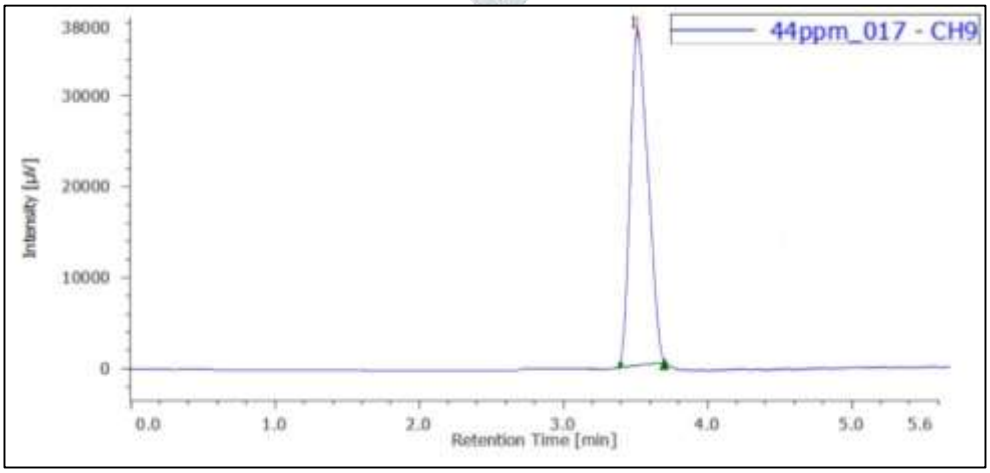

Figure 15: Chromatogram of Dapagliflozin for Accuracy (120%)

Robustness

Robustness is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The analysis was performed by changing flow rate, temperature, wavelength and mobile phase composition. The results were calculated as %RSD and were given in Table. No. 8

Table 8. Robustness results of Dapagliflozin

Parameter	Variation	Peak area	Peak area	Peak area	Average	Std. Dev	%RSD
Flow rate	0.9	198465	198467	198465	198465	0.577	0.0002
	1.0	198564	198567	198564	198565	1.73	0.0008
	1.1	198543	198678	198544	198588	77.65	0.03
Wavelength	222	196462	196463	196467	196464	2.645	0.001
	223	198667	198666	198667	198666	0.577	0.0002
	224	198657	198567	198657	198627	51.96	0.02

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 σ/S

σ =standard deviation of response S = the slope of linearity plot

LODwas found to be**2.91 µg/ml.**

Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOQ = 10 σ/S

σ =standard deviation of response S = the slope of linearity plot LOQ was found to be **8.82µg/ml.**

DISCUSSION

HPLC method development and validation has been important in pharma industry in recent years because of their importance in the quality of drugs and drug products. The goal of this study is to develop and validate a RP-HPLC method for the determination of Dapagliflozin in bulk and it's tablet dosage form. The main objective of method development was to determine the drug content in the formulation and it's % purity. Few methods were reported for analytical method development and validation by RP-HPLC. The chromatographic conditions like mobile phase, flow rate, temp was optimized and validated successfully. There has been many mobile phases were tried to gave a sharp and symmetrical peak, but finally acetonitrile : water (90:10v/v) was selected which gave a single sharp peak with retention time 3.30min. The mobile phase is simple to prepare and run time was less than 6 min which consumes only less than 6ml of mobile phase shows that method was economical.

CONCLUSION

A simple, precise and selective analytical method has been developed by RP-HPLC technique as per ICH Q2 (R1) guidelines in bulk and tablet formulation. The method has several advantages including, simple mobile phase, rapid analysis, simple sample preparation method and improved selectivity as well as sensitivity. The %RSD values are less than 2% which shows high degree of precision of the proposed method. The method was found to be robust as there was no significant change in the peak area and retention time. The system suitability tests were performed to assess the quality performance of the method. So the proposed method was found to be more specific, robust, rugged and most suitable for routine analysis. By performing degradation studies, concluded that developed method was found to be most stable in base and thermal condition.

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REFERENCES

1. Drug Profile;2008.http://www.https://go.drugbank.com/drugs/DB06292(Last accessed on April 21,2021)
2. SreenivasaS, Modalavalasa R, Kothapalli C. (2018) Validation of a new developed stability indicating rp-liquid chromatographic method for the quantitative determination of dapagliflozin. Der PharmaChemica, 10(1):93-102
3. Pathak, S.; Mishra, P. (2020) A Review on Analytical Methods of Dapagliflozin: An Update. IJPQA.,11, 355-360.
4. Reddy G, Bera A. Basak M, Peraman P, Nagappan k.(2020) A Quantitative, sensitive and rapid validated analytical rp-hplc method for the estimation of dapagliflozin in bulk and pharmaceutical dosage formulations. Int.J.Res.Pharm.Sci., 11(2):2543-2548.
5. ICH 2005. Validation of analytical procedures: text and methodology Q2 (R1). International conference on harmonization, pages 11– 12.
6. Manoharan G, Ismaiel A, M Zeyad.(2018) Stability-indicating rp-hplc method development for simultaneous determination and estimation of dapagliflozin in raw and tablet formulation. Chemistry Research Journal, 3(2): 159-164.
7. Basha S, P Sravanthi. (2017) Development and validation of dapagliflozin by reverse-phase high performance liquid chromatography method and it's forced degradation studies. Asian J pharm Clin Res.,10(11):101-105.
8. Sangapati M, Dhanlakshmi K, Reddy N, Sreenivasa S. (2014) Development and validation of stability indicating RP-HPLC method for determination of Dapagliflozin. Journal of Advanced Pharmacy Education &Research. 4(3): 350-353.
9. Jeyabaskaran. M, C Rambhau, Dhanlakshmi B. (2013) RP-HPLC method development and validation of dapagliflozin in bulk and tablet formulation. Int.J.of Pharmacy and Analytical research.2(4)**:** 221-226.

10. B Reddy Padmaja et al. (2018) A Highly Validated RP-HPLC Method for the simultaneous estimation of Dapagliflozin and Saxagliptin in Tablet Dosage Forms. Int. J. Pharm. Sci. Drug Res., 10(5): 372-378.
11. ManasaS, Dhanalakshmi K, Reddy N, Sreenivasa S. (2014) Method development and validation of dapagliflozin in api by rp-hplc and uv spectroscopy. Int. J. Pharm. Sci. Drug Res., 6(3): 250-252.
12. Debata J, Kumar S, Jha S, Khan A. (2017) A new RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. Int J Drug Dev& Res.**,** 9(2): 48-51.
13. Verma M, Patel C. (2017) Development and stability indicating HPLC method for Dapagliflozin in API and pharmaceutical dosage form. Int J Appl Pharm. 9(5): 33-41.
14. Deepan T, Rao M.V. Dhanaraju M.D. De(2017) Development of validated stability indicating assay method for simultaneous estimation of metformin and dapagliflozin by RP-HPLC. Europeon Journal of Applied Sciences. 9(4): 189-199.
15. Kalyan S, Parle A. (2020) Dapagliflozin: An Anti-diabetic drug with cardiovascular benefits. International Journal of Pharmaceutical Sciences and Research. 11(5): 1986-1993.
16. Olokoba, A. B., Obateru, O. A., Olokoba, L. B. (2012) Type 2 Diabetes Mellitus: A Review of Current Trends. Oman Medical Journal. 27(4): 269–273.
17. Available URL from https://pubchem.ncbi.nlm.nih.gov/ compound/Dapagliflozin.
18. Ashwini R, Eswarudu MM, Srinavasa BP. A Review on Analytical Methods for Estimation of Dapagliflozin and Saxagliptin in Bulk and in Pharmaceutical Dosage forms. International Journal of Research in Pharmacy and chemistry, 8(3), 460-468.