Original Article

METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN BY RP-HPLC

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Abstract

A simple, precise and RP-HPLC method was developed and validated for the simultaneous determination of Dapagliflozin (DAP) in pharmaceutical dosage forms. The separation was achieved on an Inspire (4.6 x 150mm, 5µm)5micro column with isocratic flow. The mobile phase at a flow rate of 1.0mLmin consisted of Methanol and Water (80:20, v/v). The UV detection was carried out at 235nm. The retention times for DAP were 4.422 min, respectively. Parameters such as linearity, precision, accuracy, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines. A linear response was observed over the concentration range of 50-90µg/ mL for DAP. Limit of detection and limit of quantification for DAP were 2.98. The analysis concluded that the method was selective for Dapagliflozin which will help to improve quality control and contribute to stability studies of pharmaceutical tablets containing these drugs.

Keywords: Dapagliflozin, Validation, HPLC.

Introduction

Diabetes mellitus (DM) is a chronic disorder characterised by a high blood glucose level (hyperglycemia), whic h is caused by defects in insulin secretion, action, or both.

Diabetes is diagnosed using one of four plasma glucose (PG) criteria: elevated I fasting plasma glucose (FPG) (>126 mg/dL), (ii) 2 h PG during a 75g oral glucose tolerance test (OGTT) (>200 mg/dL), (iii) random PG (>200 mg/dL) with classic signs and symptoms of hyperglycemia, or (iv) haemoglobin A1C level >6.5%).

Biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP4) inhibitors, sodiu mglucose cotransporter (SGLT2) inhibitors, and glucosidase inhibitors are the most used oral antidiabetic drugs. The goal of this study was to look at the numerous analytical methods that have been established for determinin g antidiabetic medicines of the SGLT2 class in bulk and various pharmaceutical dose forms. The approach estab lished for estimating medicines separately or in combination formulations with other oral hypoglycemics was al so considered in this investigation.

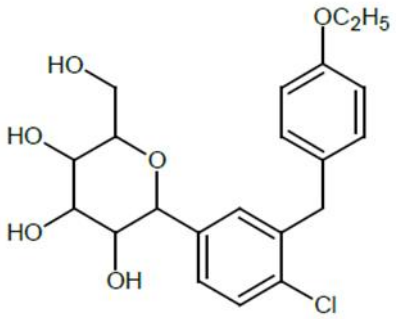
Materials & Method

Dapagliflozin (DAPA) belongs to the gliflozin class of drugs. Chemically, yes (2S, 3R, 4R, 5S, 6R) -2-(4-chloro phenyl -3-(4-ethoxybenzyl)-6-(hydroxymethyl) tetrahydro-2H-pyran

3,4,5triol (fig. 1) has the chemical formula C21H25ClO6 and has a molecular weight of 408.875g/mol. It's a whi te crystalline substance that dissolves in organic solvents such as DMSO dimethyl formamide and ethanol. It has a melting point of 55-60 degrees Celsius

Dapagliflozin is an antihyperglycemic drug that inhibits the sodiumglucose cotransporter subtype 2 (SGLT2). In comparison to SGLT1, which is a cotransporter of glucose in the gut, dapagliflozin inhibits SGLT2 specifically and potently. Dapagliflozin is a glucose blocker.

Fig. 1 Chemical structure of Dapagliflozin



Organoleptic properties of drug

The sample of Dapagliflozin was checked for organoleptic properties such as colour and odour.

Melting point determination

Identification of Dapagliflozin was done by checking its melting point and it was found in the range of 74 – 78 °C. Standard

Solubility analysis

Dapagliflozin is slightly soluble in Water and in aqueous solvents; and practically insoluble in toluene and n-hexane.

Fourier Transform Infra-red Spectroscopy (FTIR)

The IR study of pure drug was carried out by using Fourier transform infrared spectrophotometer (BRUKER). Infrared absorption spectrum of Dapagliflozin was recorded and interpreted over the wave number 4000 to 600 cm-1 using Fourier Transform spectrophotometer (Bruker, ECO- ATR) [1-5]

High Performance Liquid Chromatographic Method

Optimization of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of 10µg/ml of each of Dapagliflozin were prepared in methanol. After observing UV spectra of the drug, wavelength of 235 nm was selected for further study. [6-9]

Optimization of Chromatographic Conditions of Mobile phase for estimation of DAPA

A simple, rapid and cost-effective method has been developed for the determination of DAPA. The developed RP-HPLC method was optimized by changing various parameters such as ratio of mobile phase and flow rate to obtain sharp and symmetrical peaks with excellent baseline separation. The detection wavelength was set at 266 nm which show maximum absorption [10,11]

For the estimation of DAPA, Initially Acetonitrile and water in different proportion were tried however, DAPA poorly resolved, therefore ACN is replaced with methanol. When methanol and water were used in ratio of 50:50 v/v, DAPA peak obtained too earlier, no stable baseline and tailing peak, Further placing methanol and water at ratio of 50:50 v/v with flow rate 0.8 ml/min shows same result as shows earlier therefore [12], keeping the ratio of mobile phase same while change in flow rate with 0.2 ml/min (0.8ml/min) shows better baseline. Further changed ratio of mobile phase ratio methanol and water at ratio of 80:20 v/v with flow rate 1.0 ml/min. The problem of retention time and tailing peak has been resolved but peak get flattened. By preparing the dilution in absolute methanol shows good peak. The temperature of the column has a significant effect on the peak shape and retention time of the DAPA. Due to the reason, the temperature of the column was optimized by testing different temperatures (20 – 40ºC). The optimized temperature was selected as 40ºC obtained a better separation and shorten retention time. The flow rates of mobile phases varies between the 0.8 to 1.0 mL/min. the slower flow rate 0.8mL/min resulted in the peak with adequate separation and suitable retention time. Finally the developed RP-HPLC method was optimized and chromatographic separation of DAPA was performed on Shim-pack GIST C18 column using the mixture of methanol and water (80:20 v/v) with flow rate 1.0 mL/min, maintaining column temperature at 35ºC and detection at 235 nm.[13,14]

Preparation of standard stock solutions

Accurately 10.0 mg weighed quantity of Dapagliflozin was transferred to 10.0 mL volumetric flask. That was dissolved by adding 1.0 mL mobile phase and then the drug solution was diluted up to the mark with methanol to get the stock solution of 1000 µg/mL of Dapagliflozin. The working standard solutions of drug were obtained by appropriate dilution of the respective stock solution with mobile phase.[15,16]

Mobile Phase Preparation

Prepare mobile phase by taking methanol and water (80: 20). Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min. [17]

Validation of Developed RP-HPLC Method

Linearity Procedure

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Test solutions of different concentration were injected separately and the chromatograms were recorded. A series of test preparations of Dapagliglozin (5-10µg/ml) were prepared by taking 0.5, 0.6, 0.7, 0.8, 0.9 ml from the stock solution in five 10 ml volumetric flask and final volume make up to the mark with methanol.[18,19]

Precision Study Procedure

Intraday and Interday Precision

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at two different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2.

Acceptance criteria:

The % RSD obtained should be NMT 2.0.

LOD and LOQ:

LOD and LOQ determined by the following formula by taking the standard deviation of y- intercept and slope from the linearity curves.[20]

Where σ is the standard deviation of the response and S is the slope of the calibration curve.

Accuracy

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated. For this study,

- Prepare three preparation of each 50 %, 100 % and 150 % level and inject in to the chromatography.
- Make the injection lowest concentration to highest concentration.
- Calculate individual recovery, mean recovery and %RSD.[21]

Acceptance Criteria:

- Individual and mean % recovery should be within 98.0 % to 102.0 %.

Repeatability

The value for %RSD (Relative Standard Deviation) less than 2 for five successive injections of the sample solution from the same homogenous mixture at working concentrations, which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results.[22]

Robustness

The robustness of an analytical procedure is an estimate of its capacity to last unchanged by slight but intentional change in the analytical method parameters. To assess HPLC method robustness some measurable factors were intentionally changed. The study was carried out solution (5 µg/mL) by varying the wavelength (230,235, and, 240 nm), flow rate (0.8,1 and 1.2 mL/min) and Temperature (35, 40, and 45 degree Celsius) respectively.

Ruggedness

The value for %RSD (Relative Standard Deviation) less than 2 for three successive injections of solution (5 µg/mL) by two different analysts of the sample solution from the same homogenous mixture at working concentrations, which indicate the method developed is rugged.

Results and discussion

Identification of drug:

Organoleptic properties of drug:

Table 1: Organoleptic properties of drug

Sr. No.	**Organoleptic Property**	**Dapagliflozin**

1	**Colour**	White crystalline powder
2	**Odor**	Odorless

Melting point of drug:

Table 2: Melting point of drug

Sr. No.	**Name of drug**	**M.P. (°C)**
1	**Dapagliflozin**	**74 - 78°C**

Solubility Study:

Solubility of Dapagliflozin was observed by dissolving them in different solvents and the observed results are given in the table no.3

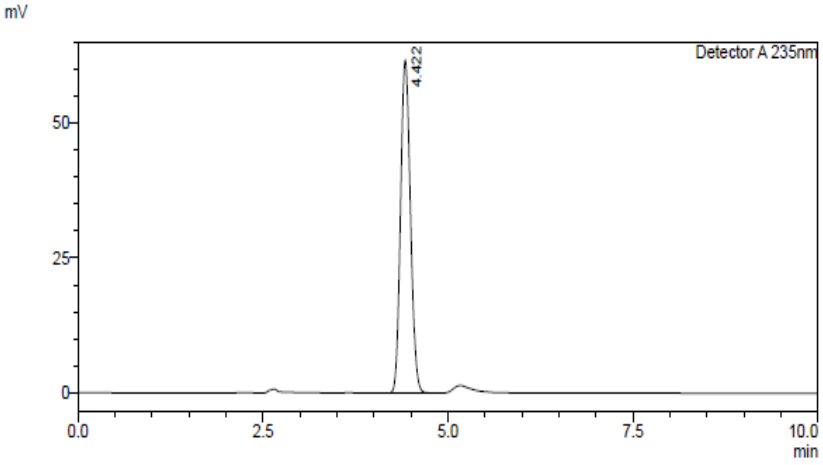
Table 3: Solubility Study of Dapagliflozin

Sr. No	**Solvents**	**Solubility**
1	Water	Soluble
2	Methanol	Soluble
3	Acetonitrile	Soluble
4	DMSO	Freely Soluble

Development of HPLC method for Dapagliflozin

High performance liquid chromatographic method was developed and validated for determination of Dapagliflozin in bulk form. Mobile phase consists of Methanol: Water (85:15 v/v). Chromatogram obtained was shows the maximum wavelength where the drug shows maximum response was 266 nm and is shown in Fig.2

Fig. 2: Typical chromatogram of Dapagliflozin



Validation Parameter

System suitability

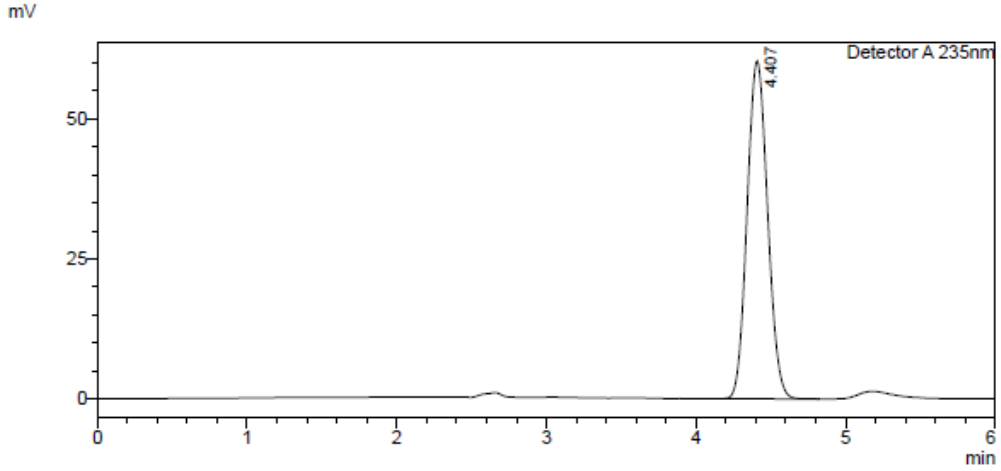
The system suitability test is an important aspect of method development since it ensures that the chosen chromatographic system will perform properly during the analysis. Table No. 4 summarizes the system suitability parameters as well as their acceptance requirements.

The efficiency of chromatographic column is expressed in terms of theoretical plates (N). The of number of theoretical plates for DAPA was found to be 4253. , thus exhibiting the good column efficiency N >2000). The tailing factor (T) for was 1.09 indicating symmetry of all peak (T < 2). The %RSD of the retention time and peak area was within 1% which indicates better efficiency of column (%RSD<1) the retention time. All the tested parameters were with acceptable limits, which indicates the suitability of the chromatographic system for further validation and analysis of sample.

Table 4: System Suitability Parameters

Parameters	**Conc. (20 µg/mL)**
Theoretical plate	4253
Tailing factor	1.09
Retention time	4.407
%RSD of the retention time	0.035
%RSD of the peak area	0.437

Fig. 3: Chromatogram of system suitability



Linearity

Linearity can be defined as the ability of an analytical method for procuring test results that are positioned directly proportional with respect to the concentration of the analyte in the sample. The high level values also referred to as the peak areas of the Dapagliflozin are plotted against the concentration and are put through the least square regression analysis for calculation of calibration equation and correlation coefficients. Test solutions of different concentration were injected separately and the chromatograms were recorded. The calibration curves of DAPA were linear over the tested concentration range of 50-90 µg/mL with a correlation coefficient (r 2) of 0.9998 and regression equations y = 258898x + 162730 in Table no.5.

Table 5: Data of calibration curve of Dapagliflozin by HPLC method

Sr. No.	**Conc. (µg/ml)**	**Area**
1	50	423098
2	60	684494
3	70	929854
4	80	1199678
5	90	1459996

Fig. 4: Calibration curve for Dapagliflozin

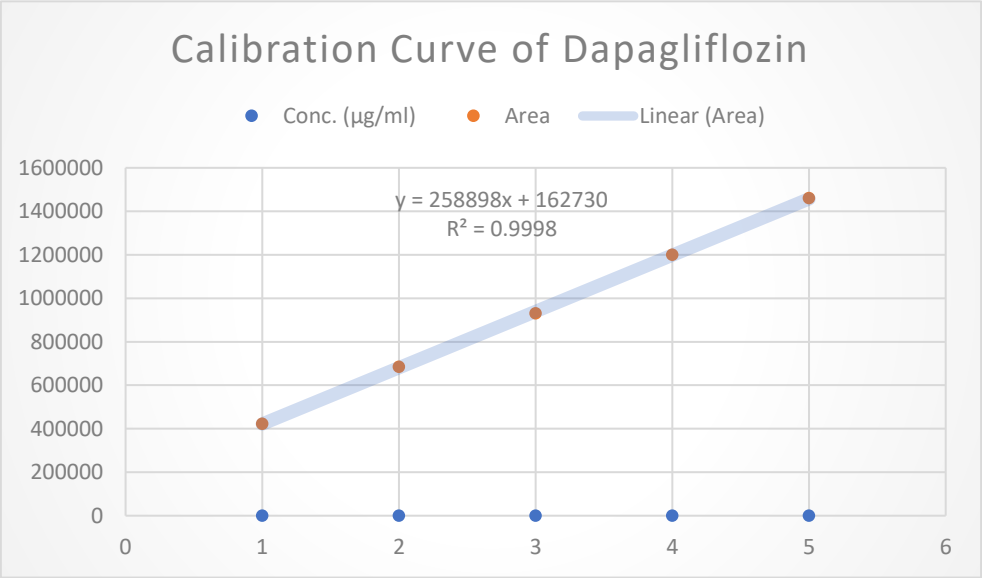


Table 6: Optical characteristics for Dapagliflozin

Sr. no	**Parameters**	**HPLC method**
1.	Lamda max	235nm
2.	Linearty	10-50
3.	Regression equation [y]	y = 258898x + 162730
4.	Slope[m]	363439
5.	Intercept[c]	110059
6.	correlation coefficient [r^2]	0.9998
7.	LOD	0.0817
8.	LOQ	0.247

Fig. 5: Chromatogram of Linearity 50 µg/ml Dapagliflozin

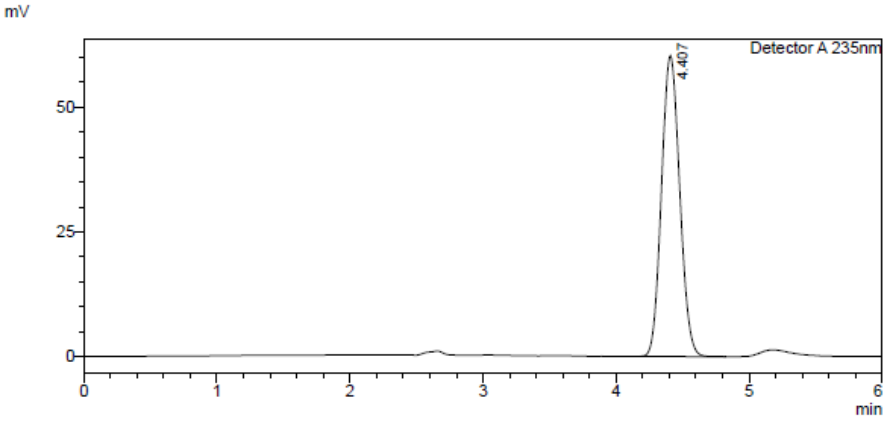


Fig. 6: Chromatogram of Linearity 60 µg/ml Dapagliflozin

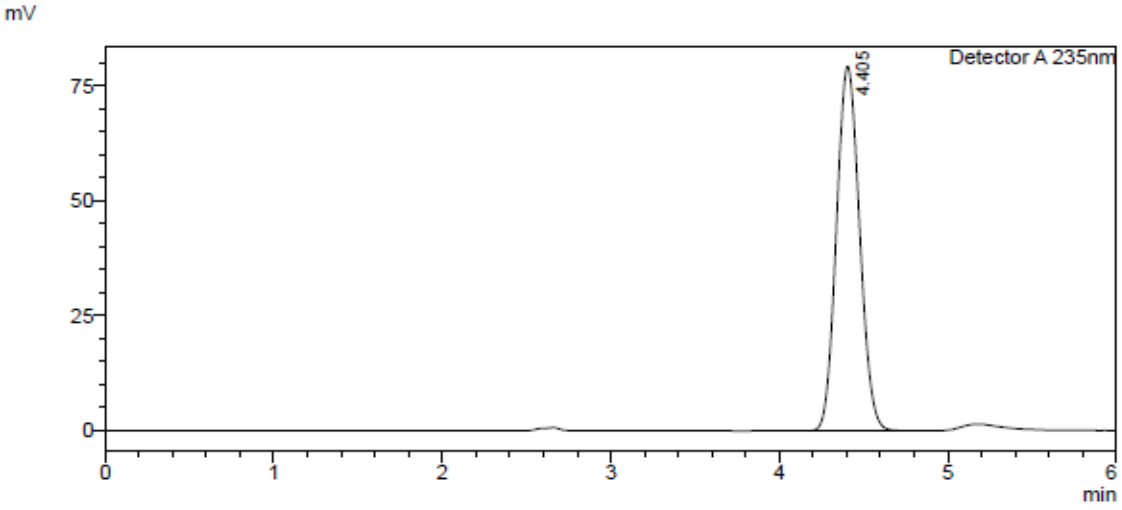


Fig. 7: Chromatogram of Linearity 70 µg/ml Dapagliflozin

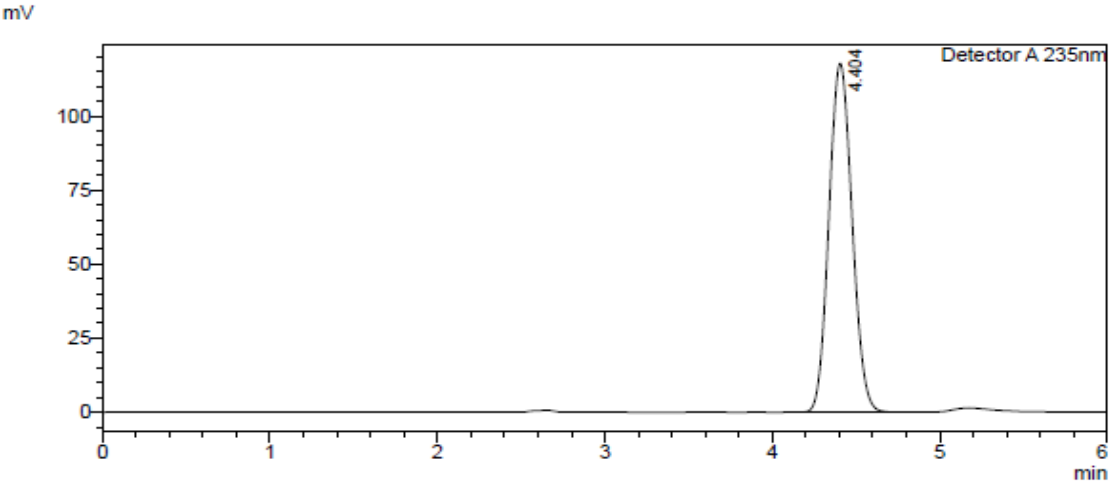


Fig. 8: Chromatogram of Linearity 80 µg/ml Dapagliflozin

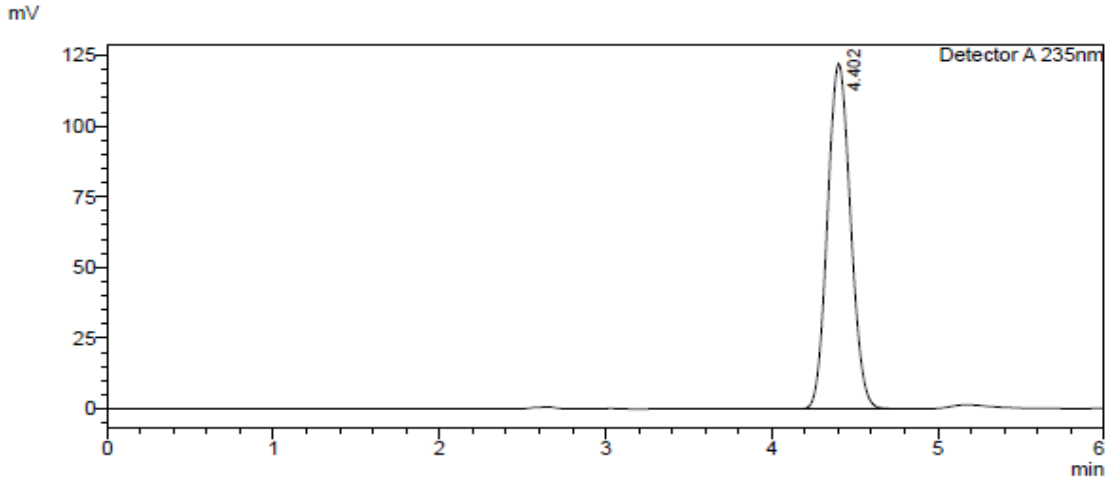
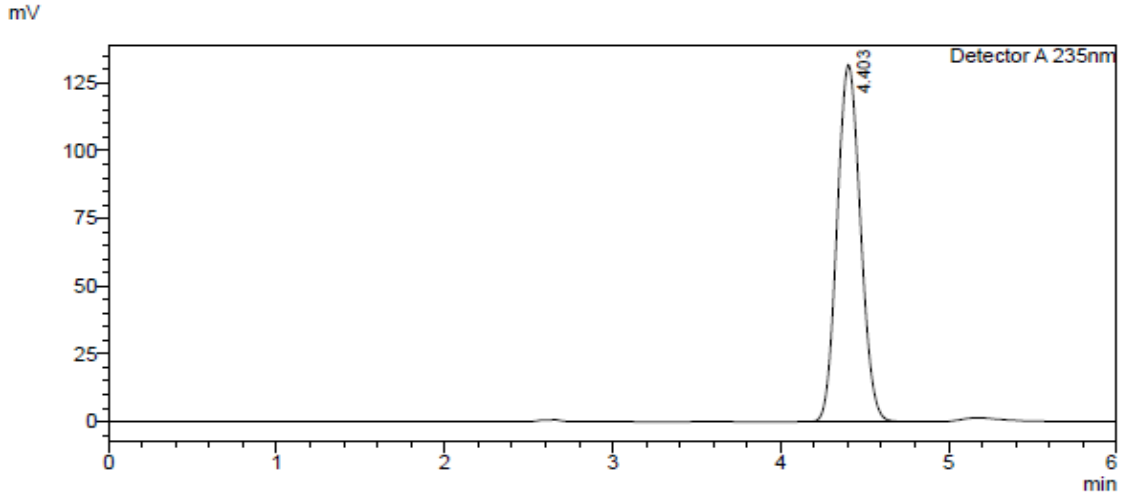


Fig. 9: Chromatogram of Linearity 90 µg/ml Dapagliflozin



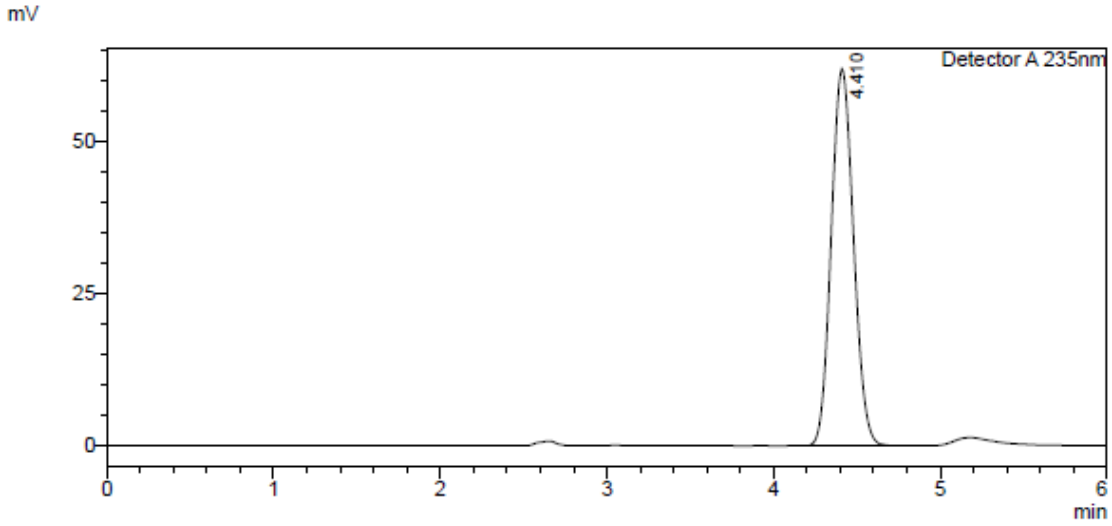
Repeatability

Repeatability expresses the precision under the operating. Conditions over a short interval of time. Data is summarizes in Table No. 7.

Table 7: Data for Repeatability study of Dapagliflozin by HPLC method

Sr.No.	**Repeatability**	**Area**
1.	50µg/mL	573509
2.	50µg/mL	572825
3.	50µg/mL	572952
4.	50µg/mL	573018
5.	50µg/mL	572928
6.	Mean	573046.4
7.	SD	267.7467087
8.	%RSD	0.046

Fig. 10: Chromatogram of Reputability (05µg/ml) of Dapagliflozin



Accuracy

Accuracy was studied by % recovery found was with acceptable limits. Result of accuracy study are summarized in Table no. 19 the data reviled that all three different levels, the mean percentage recovery was within fixed limits of 98 to 102 %. The accuracy of developed method was verified by %RSD which was less than 2 %.

Table 8: Statistical validation of Dapagliflozin by HPLC method

Levels	% Mean recovery	SD	%RSD
50%	100.1	0.0124	0.0123
100%	99.98	0.0188	0.0188
150%	100	0.0311	0.0318

*****Average of three determination

Fig. 11: Chromatogram of % Recovery of Dapagliflozin (Level 50)

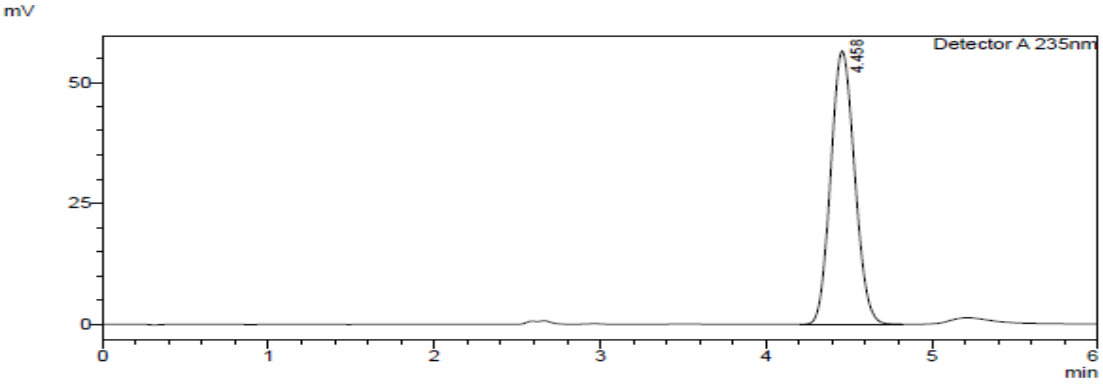
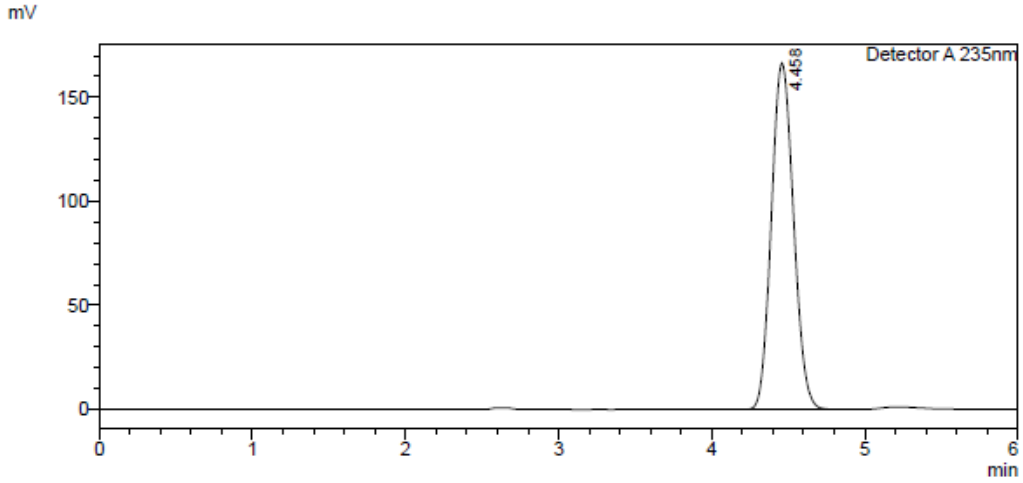


Table 9: Data for intraday precision of Dapagliflozin by HPLC method

Conc. (µg/mL)	0 Hours		2 Hours		4 Hours		Mean	SD	RSD
	Peak Area	Retention time	Peak Area	Retention time	Peak Area	Retention time			
50	572597	4.409	572412	4.399	572532	4.393	572513	93.852	0.0163
60	753391	4.409	753142	4.396	753366	4.394	753299	137.11	0.0182
70	1129799	4.405	1129841	4.396	1130145	4.392	112992	188.81	0.0167

Fig. 12: Chromatogram of % Recovery of Dapagliflozin (Level 100)



Precision

The method also shows its affinity to the agreement between a series of measurement obtained via numerous samplings of the same sample under same analytical conditions. The outcome of the intraday and inter day accuracy represented in terms of %RSD for determining Dapagliflozin was also shown in Table No. 9. The values for intraday accuracy lied between 0.99 to 0.464 %. The same for inter day precision laid between 0.94 to 0.256 %. The values in both of the conditions lie below 2% showing the high accuracy of the method.

Fig. 13: Chromatogram of % Recovery of Dapagliflozin (Level 150)

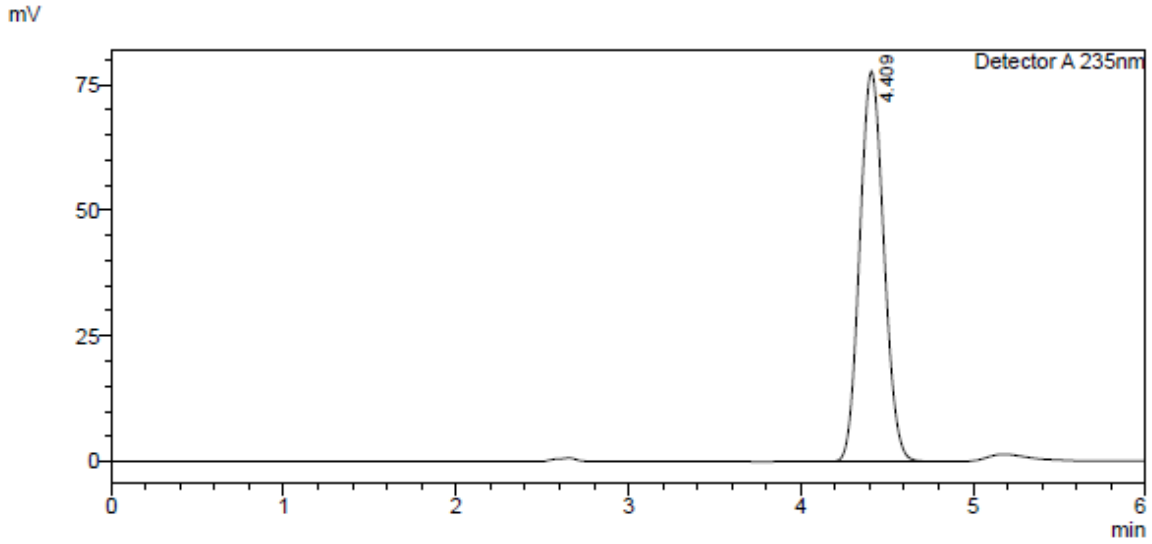


Fig. 14: Chromatogram of Intraday Precision of 60 µg/ml Dapagliflozin

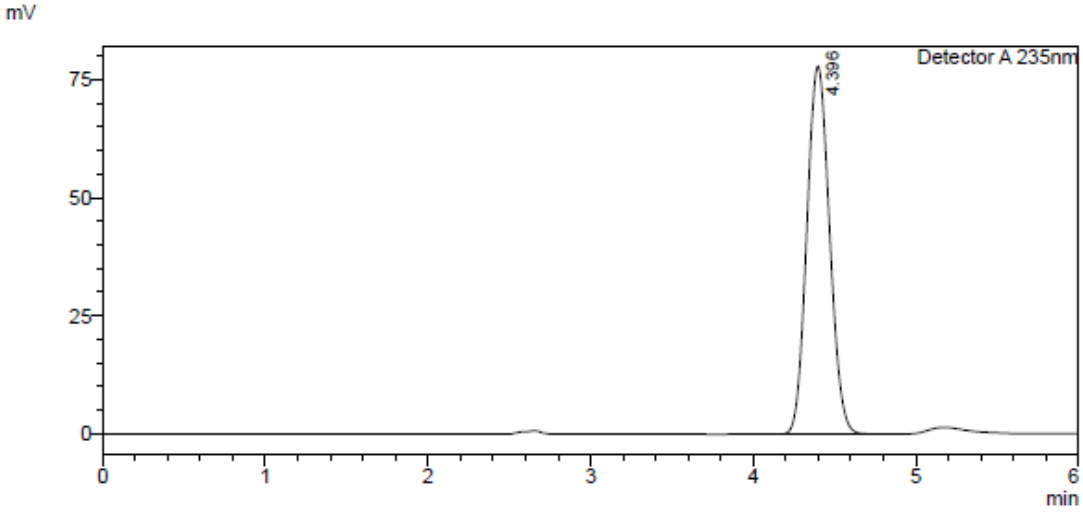


Fig. 15: Chromatogram of Intraday Precision of 70 µg/ml Dapagliflozin

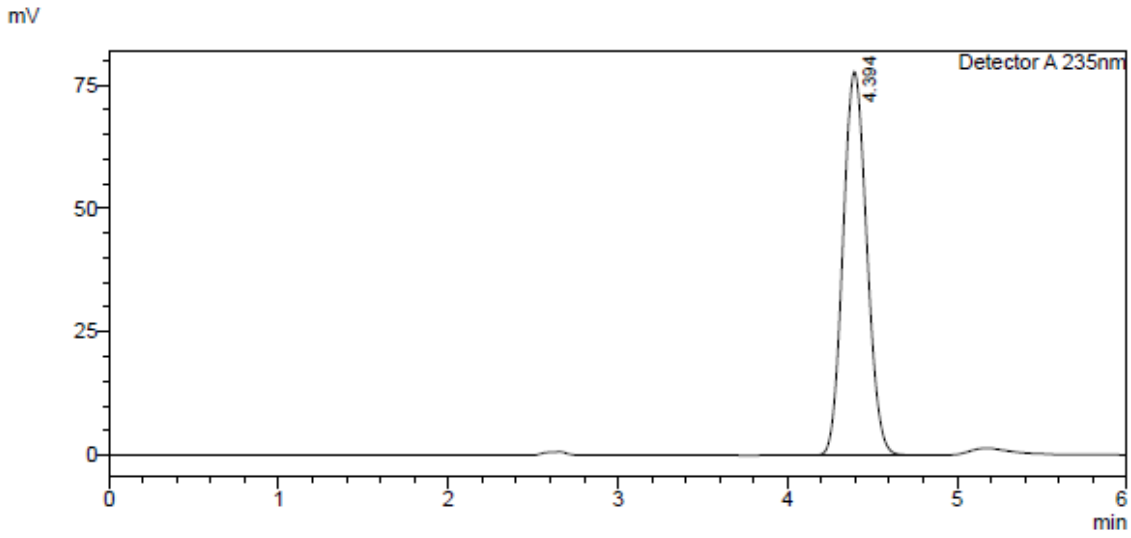


Fig. 16: Chromatogram of Interday Precision of 20 µg/ml Dapagliflozin

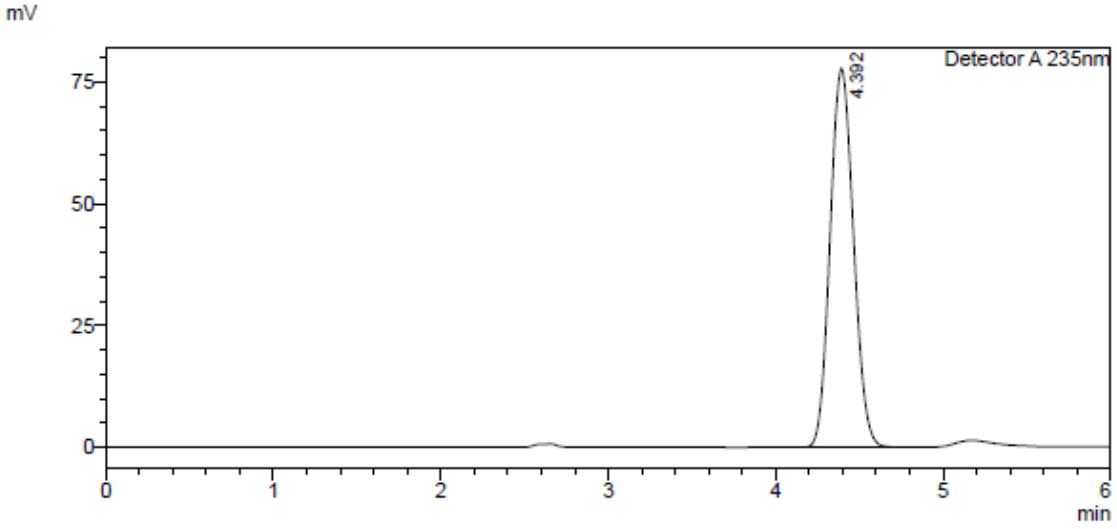
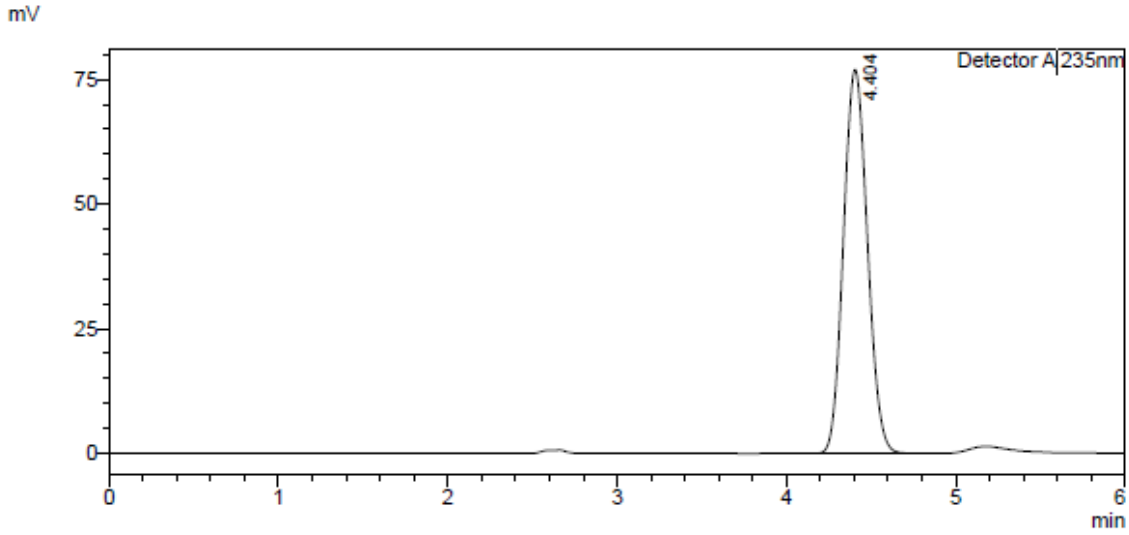


Fig. 17: Chromatogram of Interday Precision of 60 µg/ml Dapagliflozin



Limit of detection and limit of Quantitation

The results of LOD and LOQ are presented in Table No. 10

Table 10: Results of LOD and LOQ values of Dapagliflozin

Sr. No.	Parameters	Values
1.	LOD	0.0817
2.	LOQ	0.247

Robustness

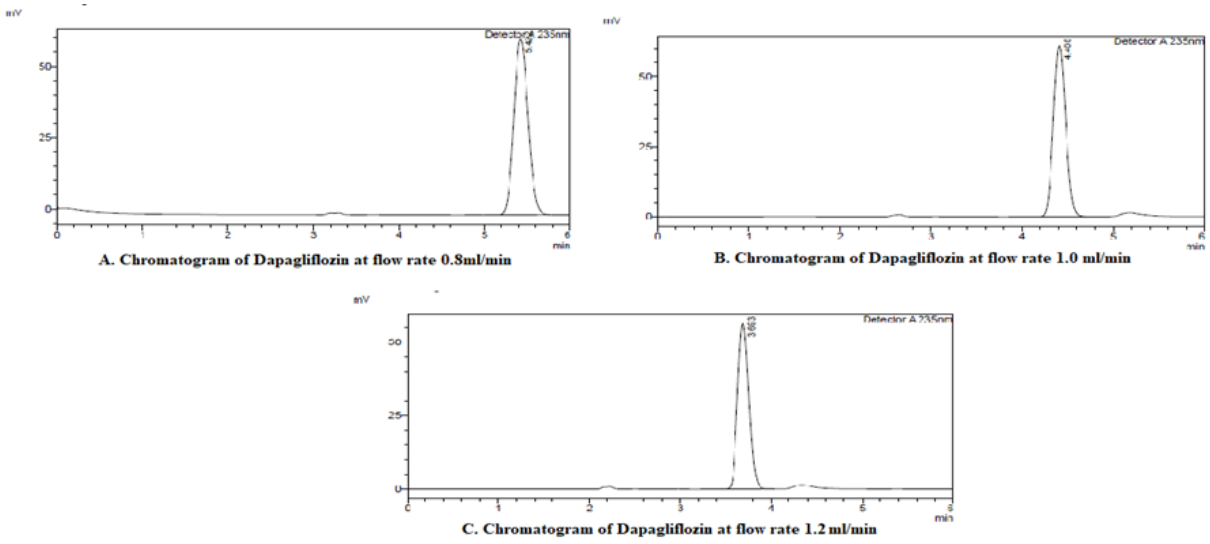
The standard for measurement of the method's capacity to remain unaffected by menial deliberately made changes in chromatographic condition and indication of its reliability during normal usage is called robustness. The

robustness of the developed RP-HPLC method is analysed on the basis of %RSD values and the system's efficient suitability parameters gained by introduction of deliberate changes in factors like flow rate(±0.2 mL min^{-1}), column temperature (±5°C), mobile phase (±5) and wavelength (±5nm). The outcome of the robustness data is illustrated in Table No. 11. It was inferred that the %RSD values less than 2%. The developed method of robustness was within the acceptance criteria, which thus validates the robustness of the developed method.

Table 11: Data for Robustness study of Dapagliflozin by HPLC method

Sr. No.	Factors	Variable	Conc. (µg/mL)	Mean Peak Area	SD	% RSD
1.	Flow rates (mg/mL)	0.8	6	709660.7	839.4143	0.118284
2.		1.0	6	573095.3	363.8301	0.063485
3.		1.2	6	478805	411.253	0.085892
4.	Wavelength(nm)	230	6	916186.7	354.5029	0.038693
5.		235	6	573422.3	101.1599	0.017641
6.		240	6	297532.3	181.1141	0.060872
7.	Temperature	30	6	571860	243.875	0.042646
8.		35	6	573095.3	363.8301	0.063485
9.		40	6	569679	212.3511	0.037276
10.	Mobile phase ratio	75:25	6	584377.3	882.7867	0.151064
11.		80:20	6	573095.3	363.8301	0.063485
12.		85:15	6	573507.7	270.245	0.047121

Fig. 18: Chromatogram of Dapagliflozin at different flow rate



A. Chromatogram of Dapagliflozin at flow rate 0.8ml/min

B. Chromatogram of Dapagliflozin at flow rate 1.0 ml/min

C. Chromatogram of Dapagliflozin at flow rate 1.2 ml/min

Fig. 19: Chromatogram of Dapagliflozin at different Wavelength

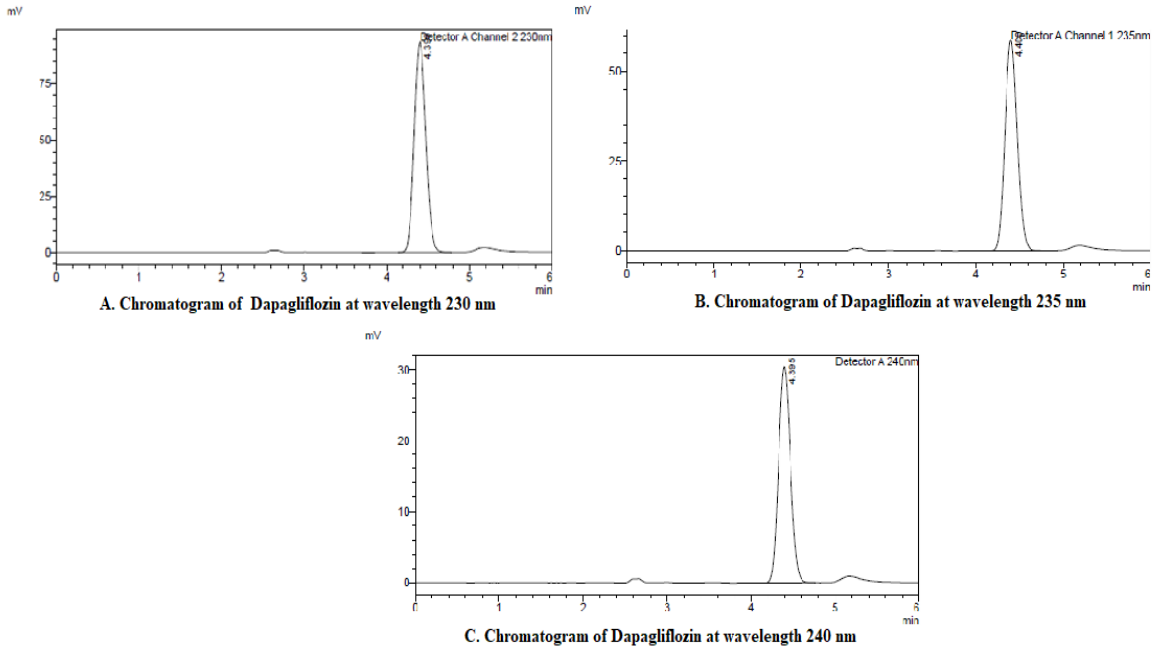


Fig. 20: Chromatogram of Dapagliflozin at different Temperature

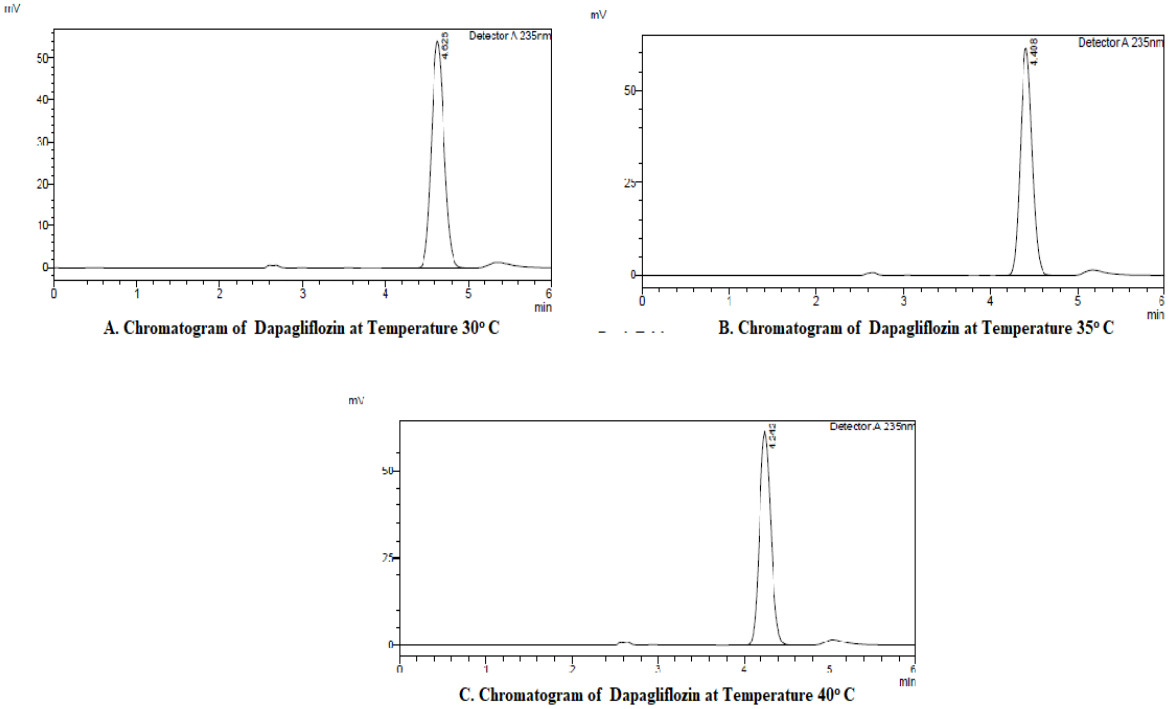
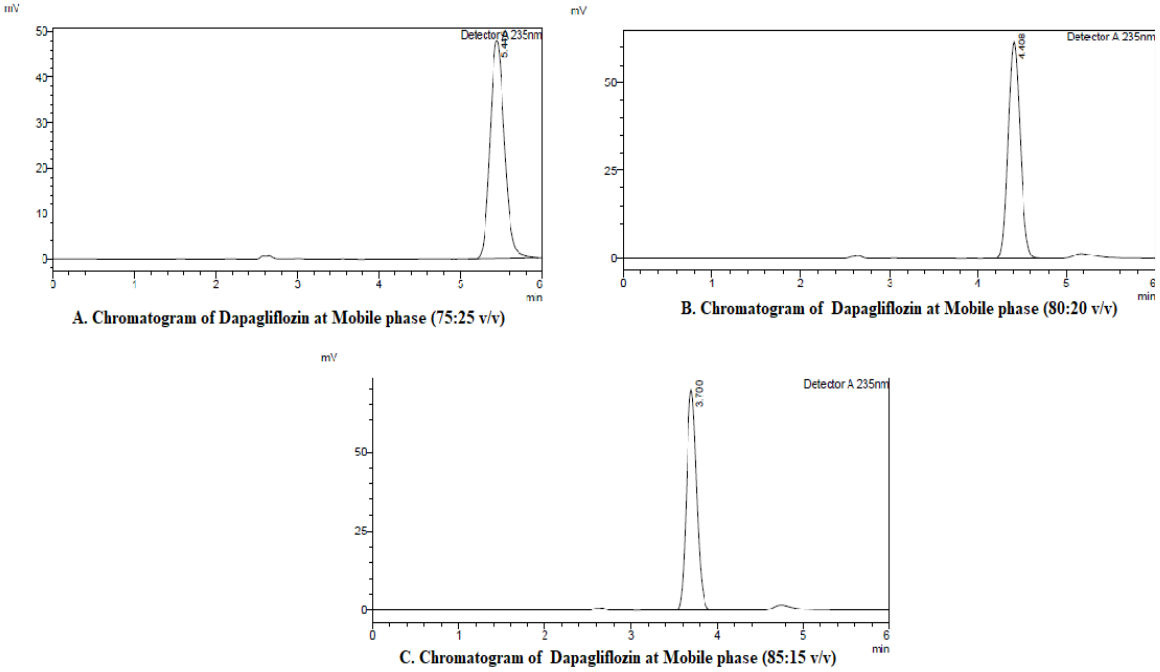


Fig. 21 Chromatogram of Dapagliflozin at different Mobile phase



Ruggedness

The terms ruggedness is measure the capacity to remain unaffected by small, but deliberate variations in methods parameters and provides an indication of its reliability during normal usage.

Table 12: Data for Robustness study of Dapagliflozin by HPLC method

Sr.No.	Analyst	Conc.	Peak Area	Mean	SD	%RSD
1	Analyst 1	50	571707 572101 572235	572014.3	274.4619	0.047982
2	Analyst 2	50	571988 572681 572111	572260	369.7472	0.064612

Fig. 22: Chromatogram of Ruggedness of Dapagliflozin by analyst 1 (50 µg/ml)

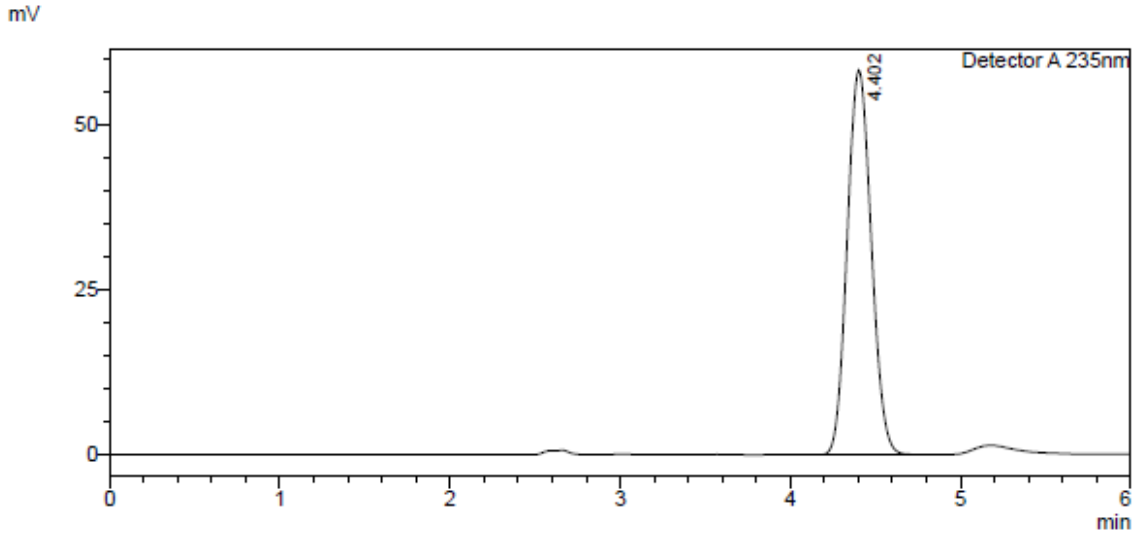
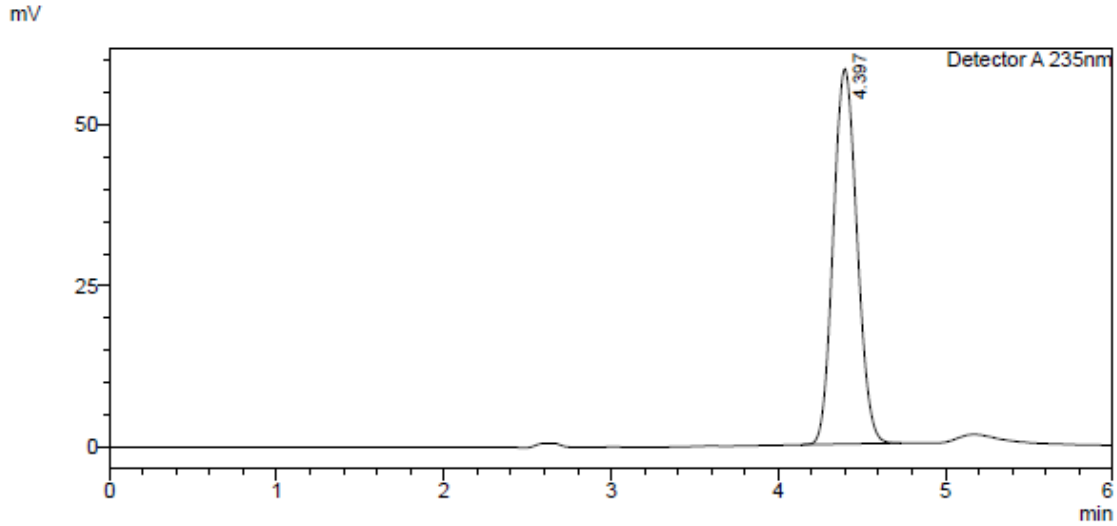


Fig. 23: Chromatogram of Ruggedness of Dapagliflozin by analyst 2 (50 µg/ml)



Conclusion:

The RP-HPLC method developed for estimation of Dapagliflozin was validated as per ICH Q2 (R1) guidelines using various parameters.

In this project, as per our objective RP-HPLC method was developed and validated on analytical column reversed phase Shim-pack GIST C18 (250mm×4.6mm×5µm), with mobile phase Methanol: Water (80:20 v/v). The flow rate was kept at 1.0 ml /min and UV detection was carried out at 235 nm. The retention time for Dapagliflozin was found to be 4.422 min.

RPHPLC method has been developed for estimation of Dapagliflozin. The proposed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of Dapagliflozin. All result are in acceptable limits such as %RSD is less than 2%, Tailing Factor less than 2, theoretical plates less than 2000.

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Conflicts of Interest:

There are no conflicts of interest.

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