ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.4.P70-84

International Journal of Life science and Pharma Research

Research Article

Analytical Chemistry For Efficient Drug Analysis

Estimation of Dapagliflozin in Pure and Marketed Formulation by Validated Reverse Phase-High Performance Liquid Chromatographic Method

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Abstract: Dapagliflozin is a highly selective, orally active and reversible inhibitor of the human sodium glucose co- transporter 2 (SGLT2). The dapagliflozin is a diabetic drug which is used with proper diet and exercise in adults with type 2 diabetes to improve the glycemic control. The aim of this study is to develop a rapid, precise and accurate RP- HPLC method for estimation of dapagliflozin in bulk and formulation as per ICH guidelines. The chromatography was carried on Sunsil C18 (150 X4.5mm, 5µ) column HPLC (Water's) using a mixture of methanol and water at different ratios and finally optimized with 85:15 v/v as mobile phase with flow rate 1.0ml/min at 225nm. The correlation regression is found to be 0.999. The retention time was found to be 2.74min. The accuracy of the present method was evaluated at 50%, 100% and 150%. The recoveries of dapagliflozin API and tablet were found to be in the range of 98.5% - 100.6%. Precision studies were carried out and the relative standard deviation values were less than two. The method was found to be robust. The limit of detection and limit of quantification were determined as 0.011µg/ml and 0.034µg/ml. The proposed method was found to be rapid, precise, accurate and robust and can be used for the estimation of dapagliflozin in API and Pharmaceutical dosage form.

Keywords: Dapagliflozin, UV- Spectroscopy, RP-HPLC, Validation.

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Recieved On 16 March 2020
Revised On 26 August 2020
Accepted On 09 September 2020
Published On 05 October 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation V.Navya Sree, Dr.K. Bhavyasri, Dr.M.Sumakanth, R.Swethasri , Estimation of Dapagliflozin in Pure and Marketed Formulation by Validated Reverse Phase-High Performance Liquid Chromatographic Method.(2020).Int. J. Life Sci. Pharma Res.10(4), P70-84 http://dx.doi.org/ 10.22376/ijpbs/lpr.2020.10.4.P70-84

1. INTRODUCTION

Dapagliflozin is used for the treatment of diabetes mellitus type 2 and functions to improve glycemic control in adults when combined with diet and exercise. It is an inhibitor of sodium-glucose co-transporter 2, which prevents glucose reabsorption in the kidney.[1] Using dapagliflozin leads to heavy glycosuria (glucose elimination in urine), which can lead to weight loss and the tiredness. Dapagliflozin was approved by the FDA on 2014, Jan 08. It was not recommended for the patients with diabetes mellitus type 1.[2] The chemical name of Dapagliflozin is (2S,3R,4R5S,6R)-2-{4-chloro-3- [(4-ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol. The molecular formula of dapagliflozin is $C_{21}H_{25}ClO_6$.[3] The main objective of this proposed method is to develop a new rapid, simple, precise, accurate and economical analytical method for the estimation of dapagliflozin.[4]

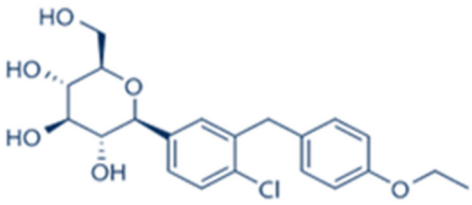

Fig 1: Structure of Dapagliflozin

2. MATERIALS AND METHODS

Pharmaceutical grade dapagliflozin was purchased from Laurus Labs. The solvents used for the procedure are of analytical grade.[5] The HPLC grade chemical used is methanol and double distilled water and they were obtained from SDFCL. All the solutions were filtered through vacuum filters and sonicated. The marketed formulation of dapagliflozin (Forxiga) is obtained from AstraZeneca Pharma India Limited.[6]

2.1 Apparatus

U.V. Visible double beam spectrophotometer shimadzu along with two matched cuvettes were used. Stock solutions were prepared with AR grade methanol and double distilled water by filtering and sonicating them and used for analysis.[7] The HPLC system used for the study is Water's HPLC model 1525. The column used was Sunsil C18 (150mm X 4.6mm, 5µ).[8] Auto sampler 171 Plus and the detector consisting of a water's dual λ absorbance detector operated at 225nm. Software used for HPLC is empower 3.0.[9]

2.2 Chromatographic conditions

As the drug is soluble in methanol, the experimentation was started with the mobile phase methanol: water with 70:30 ratio , and tried at different levels of combination containing these solvents. The optimal composition of mobile phase was determined as methanol: water (85:15v/v). The mobile phase was filtered through 0.45µm nylon filter, and then sonicated for at least 10 min.[10]

2.3 Preparation of sample solution (formulation)

Weighed quantity equivalent to 10mg was taken in to 10ml volumetric flask and few ml quantity of methanol was added and sonicated to dissolve and the volume was made upto the mark with methanol, shaken well and filtered through 0.45µ filter paper (1000µg/ml) solution. From the 1000µg/ml solution, 1ml was pipetted out and taken in to another 10ml volumetric flask and the volume was made up to the mark with methanol (100µg/ml). 1ml was pipetted out from 100µg/ml solution and taken into another 10ml volumetric flask and volume was made up to the mark with methanol (10µg/ml)

2.4 Preparation Of Standard Stock Solution

Accurately weighed 10mg of dapagliflozin was taken into a 10ml volumetric flask and was dissolved with methanol and made up to the mark (1000µg/ml solution A). 1ml was pipetted out from solution A and taken into another 10ml volumetric flask and volume was made up to the mark with methanol (100µg/ml solution B). 1ml was pipetted out from solution B and taken into another 10ml volumetric flask and the volume was made up to the mark with methanol (10µg/ml). 10µl of 10µg/ml solution was injected into HPLC system[11] Corresponding peak area was measured and respective concentration was calculated from the calibration curve.[12]

2.5 Preparation of mobile phase

Methanol and water of HPLC grade were taken and filtered through 0.45µ filter paper and sonicated for 10min separately.

2.6 Method development trials

Trial 1:

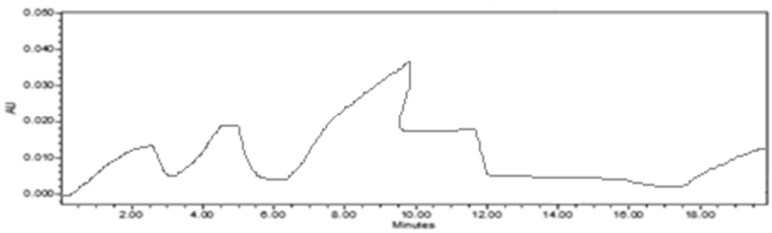

Fig 2. Chromatogram of Dapagliflozin trial-1

Mobile phase used for trial 1 phosphate buffer: methanol (50:50) by using column Sunsil C18 (150mm X 4.6mm, 5µ) with flow rate 0.5ml/min at 225nm Observation: improper baseline was observed. No peak is seen. No parameters are satisfying.

Trial 2:

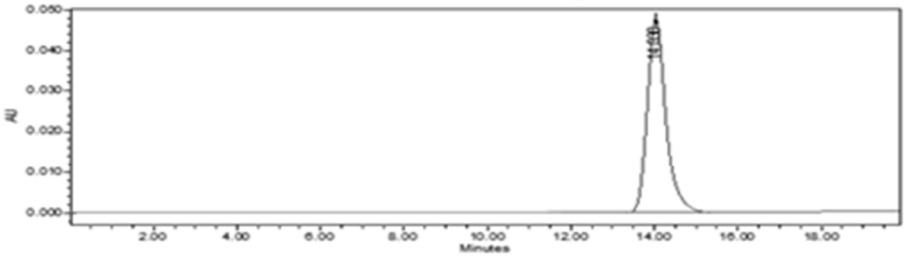

Fig 3. Chromatogram of Dapagliflozin trial-2

Mobile phase used for trial 2 is methanol: water (50:50) by using column sunsilC18 (150mm X4.6mm, 5µ) with flow rate 0.5ml/min at 225nm Observation: peak shape is good, no fronting or tailing was observed, retention time was found to be 14.00min which is more when compared to literature review. So retention time have to be changed.

Trial 3:

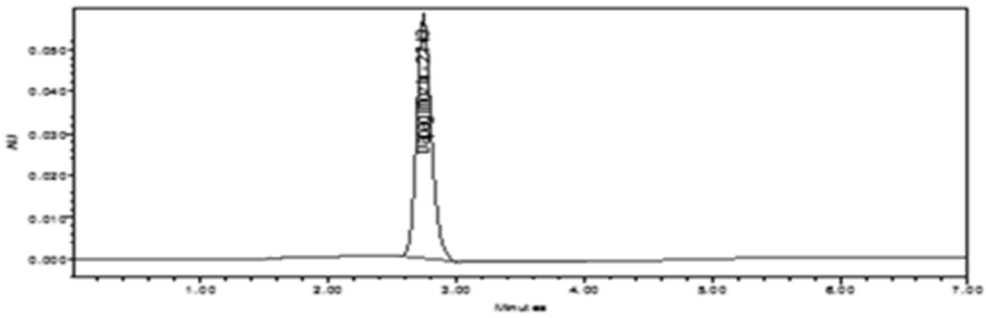

Fig 4. Chromatogram of Dapagliflozin trial -3

Mobile phase used for trial 3 methanol: water (85:15) by using column sunsilC18 (150mmX 4.6mm, 5µ) with flow rate 1ml/min at 225nm. Observation: There is good retention time, no tailing and fronting meeting all the system suitability parameters. Hence this trail was considered to be the optimized method. Conclusion of trials: By above performed trials, the trial 3 was optimized so further validation parameters were performed based on the optimized method.

2.7 *Validation parameters*

1. Linearity
2. Precision
3. Accuracy
4. Robustness
5. LOD & LOQ

2.8 Preparation of solutions

2.8.1 Preparation of 3µg/ml solution

From the standard stock solution-B 0.3µl were pipetted out and transferred into 10ml volumetric flask and the volume was made up to the mark with methanol (3µg/ml).

2.8.2 Preparation of 6µg/ml solution

From the standard stock solution-B 0.6µl were pipetted out and was transferred into 10ml volumetric flask and the volume was made up to the mark with methanol (6µg/ml).

2.8.3 Preparation of 9µg/ml solution

From the standard stock solution-B 0.9µl were pipetted out and transfered into a 10ml volumetric flask and the volume was made up to the mark with methanol (9µg/ml).

2.8.4 Preparation of 12µg/ml solution

From the standard stock solution-B 1.2µl were pippeted out and transfered into 10ml volumetric flask and the volume was made up to the mark with methanol (12µg/ml).

2.8.5 Preparation of 15µg/ml solution

From the standard stock solution-B 1.5µl were pipetted out and transfered in to 10ml volumetric flask and the volume was made up to the mark with methanol (15µg/ml).

3. Validation Of Rp-Hplc Method For Dapagliflozin In Bulk Drug:

3.1 Precision

Inter and intraday precision of method was determines by analyzing drug in triplicate at three different levels/day for consecutive six days and the results were expressed as %RSD.[14] Results of precision interday were shown in fig 7- 12 and table no 2 , and the results of intraday precision were shown in fig 13-18 .and table no 3 . The %RSD was found to be within the limits i.e., less than 2.

3.2 Calibration curve (linearity)

The standard solutions were prepared by dilution of stock solution with methanol in concentration range of 3µg/ml, 6µg/ml, 9µg/ml, 12µg/ml, 15µg/ml and 18µg/ml with

concentration on X- axis and absorbance on Y- axis at 225nm. The correlation coefficient for dapagliflozin was found to be 0.999.[13] The results of linearity were shown in fig 20 - 25 and table no 4. And the co relation coefficient was found within the limits i.e., 0.999 shown in fig no 19

3.3 Accuracy

Recovery studies were carried out by using bulk drug samples at three levels of 50%, 100% and 150%. At each level six determinations were performed and the results were obtained were compared with expected results.[15] Results of accuracy 50%, 100% and 150% were shown in fig 26 -34 and table no 5. The %RSD was found to be within the limits i.e., less than 2.

3.4 Robustness

It is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[16] The results of robustness with flow rate 0.9ml were shown in fig 35- 40 and table no 6 and the results of robustness with flow rate 1.1ml were shown in fig 41- 46 and table no 7 . The %RSD was found within the limits i.e., less than 2.

3.5 Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were separately determined and calculated based on the calibration curve of standard solution.[17] The LOD and LOQ were calculated and the results were within limits i.e., less than 2.

4. RESULTS AND DISCUSSION

The present study was performed to develop a rapid, precise and accurate method of dapagliflozin using RP-HPLC in bulk drugs[18]. The optimized chromatographic conditions were maintained using Sunsil C18 column (250 X 4.6mm, 5µm) and mobile phase methanol: water in the ratio of 85:15 with a flow rate of 1ml/min at UV detection 225nm. The retention time of dapagliflozin was found to be 2.74min.[19]

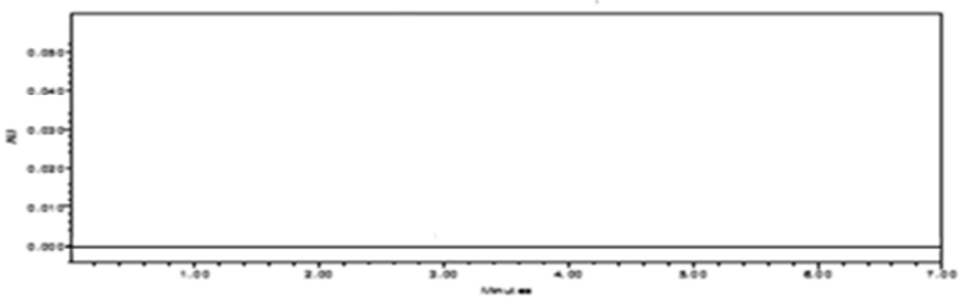

Fig 5: Blank Chromatogram

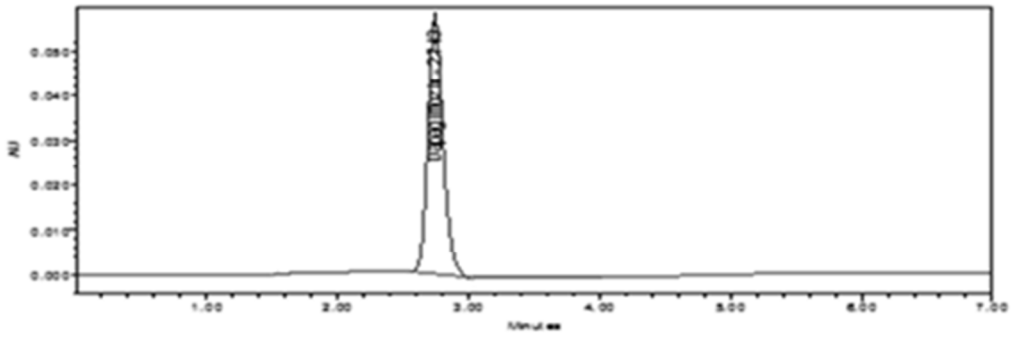

Fig 6: Chromatogram of Dapagliflozin sample

Table 1: result showing sample injection of dapagliflozin

S.No	Name	Rt	Peak area	Theoritical plate count	USP tailing
1	Dapagliflozin	2.76	456154	2776	1.5

4.1 Precision

The method was found to be precise after six replicates for the qualification of Dapagliflozin %RSD was found to be less than 2.0%.[20]

4.1.1 Precision Interday

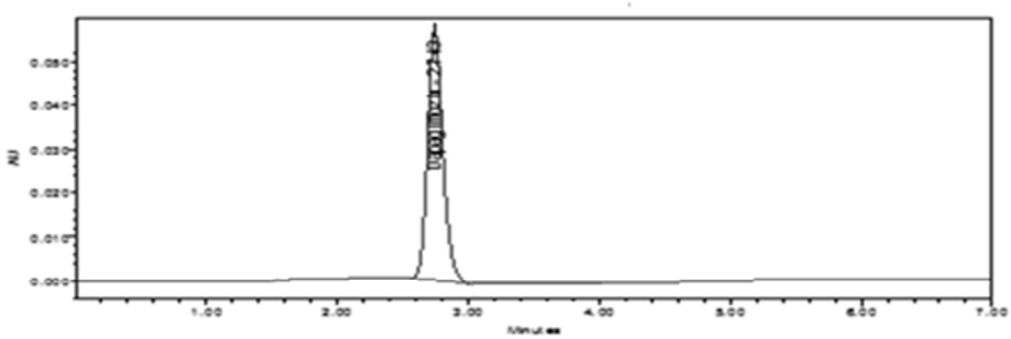

Fig 7: Interday precision chromatogram (10µg/ml) - injection 1

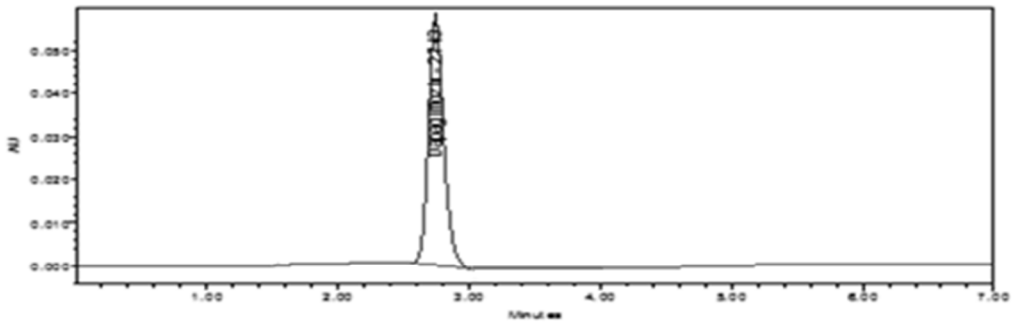

Fig 8: Interday precision chromatogram (10µg/ml) - injection 2

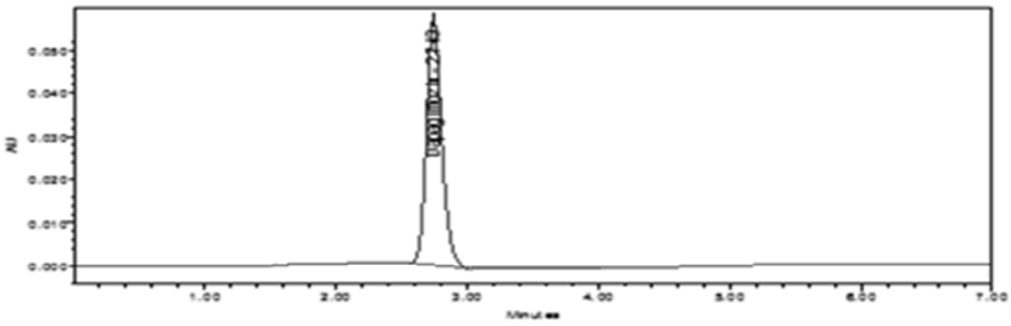

Fig 9: Interday precision chromatogram (10µg/ml) - injection 3

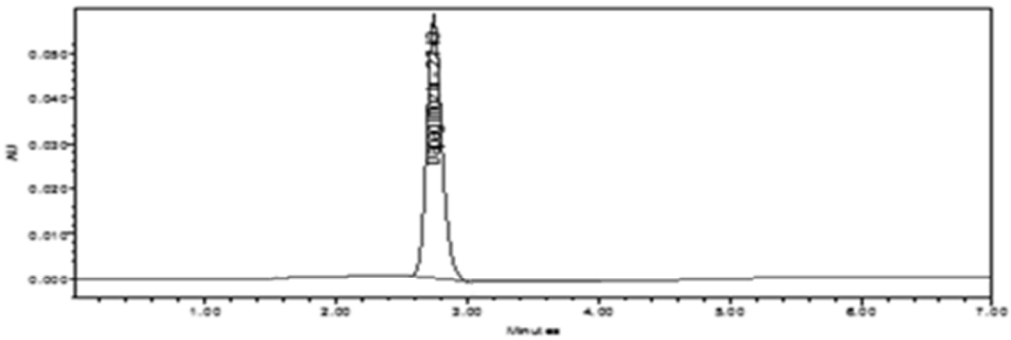

Fig 10: Interday precision chromatogram (10µg/ml)- injection 4

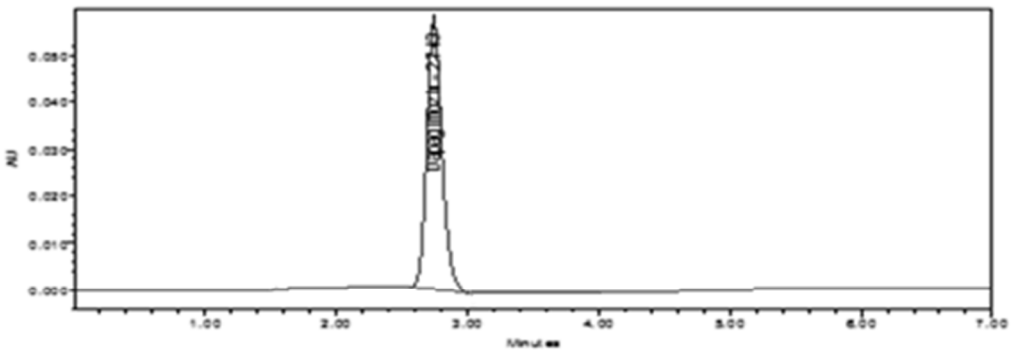

Fig 11 : Interday precision chromatogram (10µg/ml)- injection 5

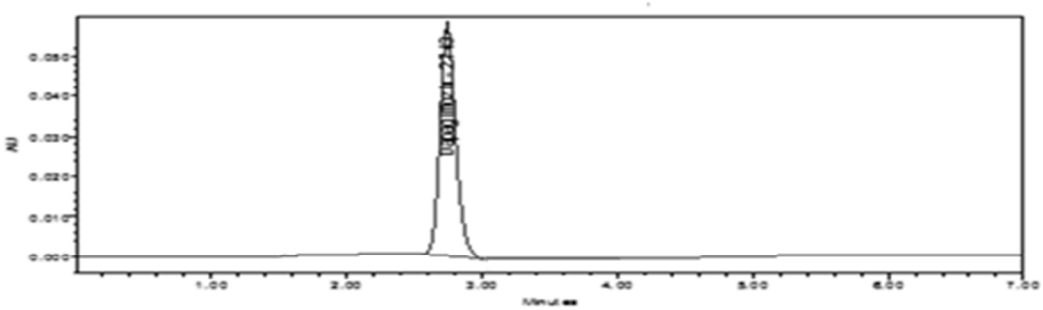

Fig 12 : Interday precision chromatogram (10µg/ml)- injection 6

Table 2: Interday precision results

S.No	Name	Rt	Peak area	Theoretical plate count	USP tailing
1	Dapagliflozin(10µg/ml)	2.79	456154	2777	1.2
2	Dapagliflozin(10µg/ml))	2.77	456236	2776	1.2
3	Dapagliflozin(10µg/ml))	2.77	456462	2778	1.5
4	Dapagliflozin(10µg/ml)	2.79	456098	2777	1.4
5	Dapagliflozin(10µg/ml)	2.78	456288	2777	1.6
6	Dapagliflozin(10µg/ml)	2.79	456344	2777	1.5
	Mean		**456263.7**		
	SD		**131.62**		
	%RSD		**0.02**		

10µg/ml solution was prepared and it was injected into the system with six relicate injections. The data obtained were analyzed by using water's HPLC with software empower 3.0, and the column Sunsil (150mm X 4.5mm, 5µ) with flow rate 1ml/min and it was detected by using the waters 2487 dual absorbance detector and waters 717 plus auto sampler and the drug was eluted at 2.79min, data were presented as SD and the %RSD were found within the limits i.e., less than 2.

4.1.2 Precision intraday

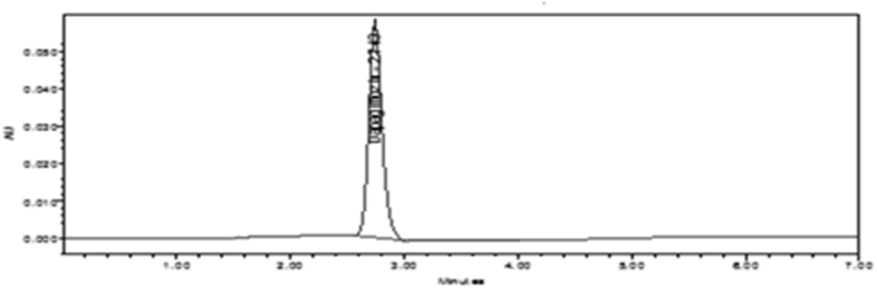

Fig 13 : Intraday precision chromatogram (10µg/ml) - injection 1

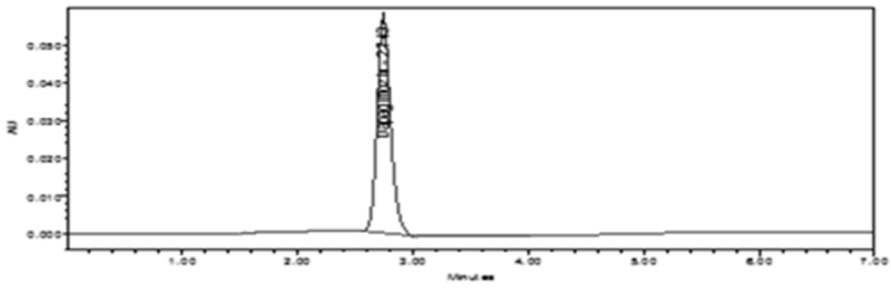

Fig 14 : Intraday precision chromatogram (10µg/ml) - injection 2

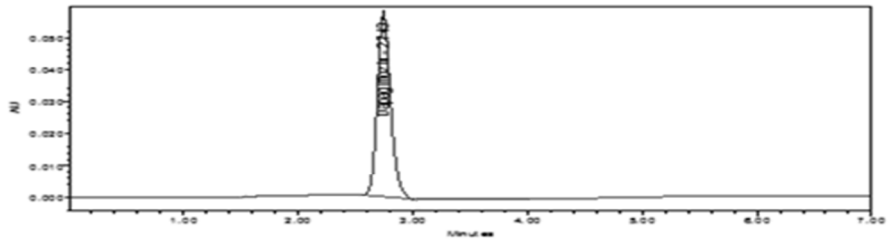

Fig 15 : Intraday precision chromatogram (10µg/ml) - injection 3

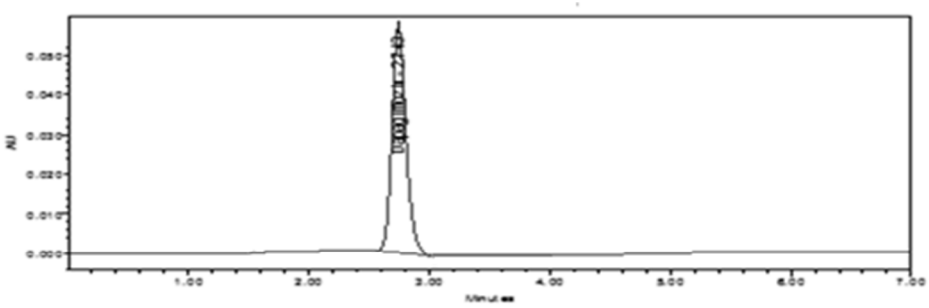

Fig 16 : Chromatogram of Dapagliflozin showing intraday precision injection 4

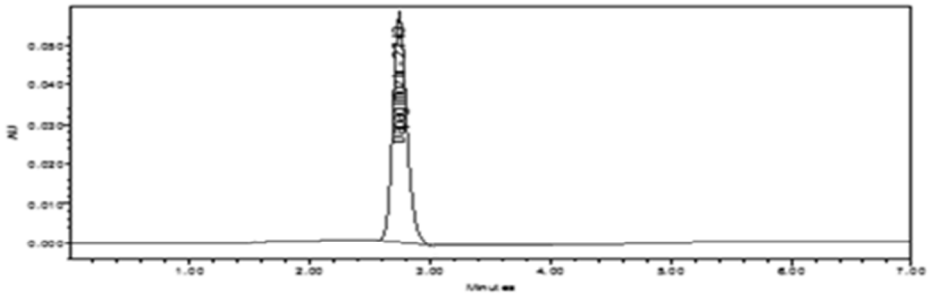

Fig 17 : Intraday precision chromatogram (10µg/ml) - injection 5

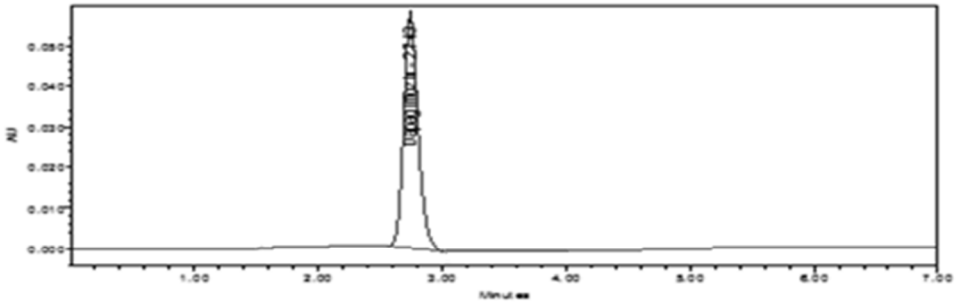

Fig 18 : Intraday precision chromatogram (10µg/ml) - injection 6

Table 3 : precision intraday results

S.No	Name	Rt	Peak area	Theoretical plate count	USP tailing
1	Dapagliflozin(10µg/ml)	2.79	456254	2777	1.2
2	Dapagliflozin(10µg/ml))	2.77	456271	2776	1.2
3	Dapagliflozin(10µg/ml))	2.77	456462	2778	1.5
4	Dapagliflozin(10µg/ml)	2.79	456095	2777	1.4
5	Dapagliflozin(10µg/ml)	2.78	456286	2777	1.6
6	Dapagliflozin(10µg/ml)	2.79	456344	2777	1.5
	Mean		**456285.3**		
	SD		**125.05**		
	%RSD		**0.02**		

In intraday precision, 10µg/ml solution of dapagliflozin was prepared and it was injected into HPLC system for six relicate injections. The data obtained were analyzed by using water's HPLC with software empower 3.0, and the column Sunsil (150mm X 4.5mm, 5µ) with flow rate 1ml/min and it was detected by using the waters 2487 dual absorbance detector and waters 717 plus auto sampler and the drug was eluted at 2.79min and the data were presented as SD and the %RSD were found within the limits i.e., less than 2.

4.2. Linearity

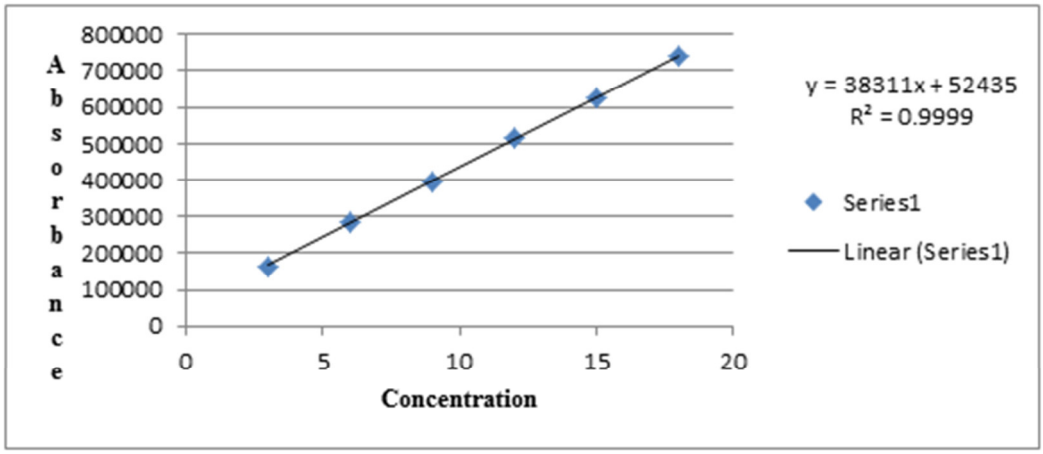

Fig 19 : Linearity plot of dapagliflozin

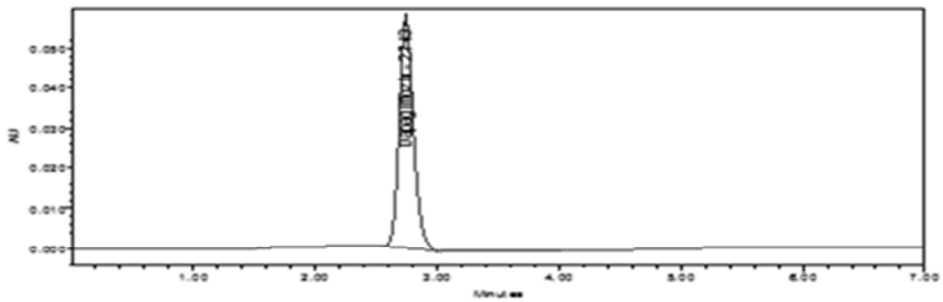

Fig 20 : Dapagliflozin linearity chromatogram of 3µg/ml solution

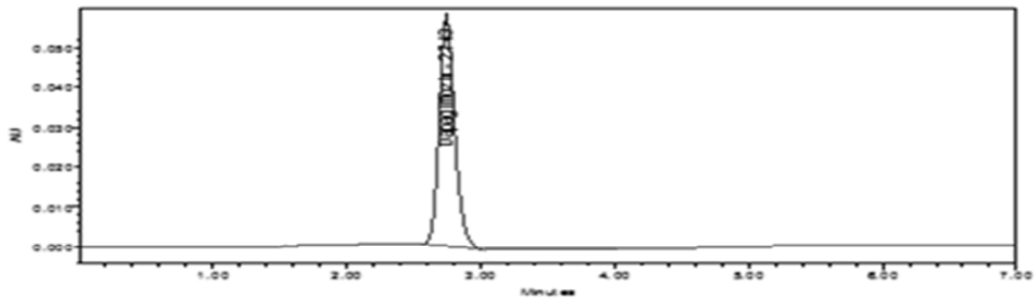

Fig 21 : Dapagliflozin linearity chromatogram of 6µg/ml solution

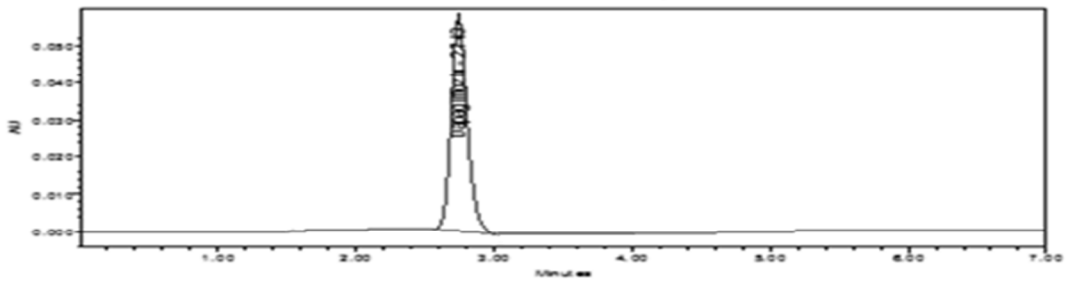

Fig 22 : Chromatogram of Dapagliflozin showing linearity 9µg/ml

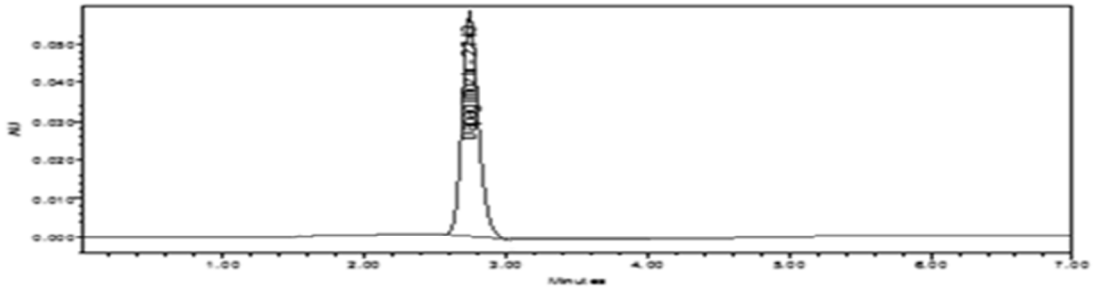

Fig 23 : Dapagliflozin linearity chromatogram of 12µg/ml solution

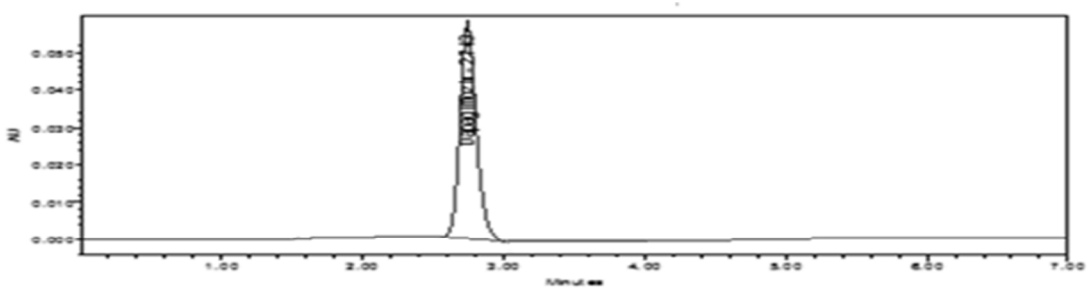

Fig 24 : Dapagliflozin linearity chromatogram of 15µg/ml solution

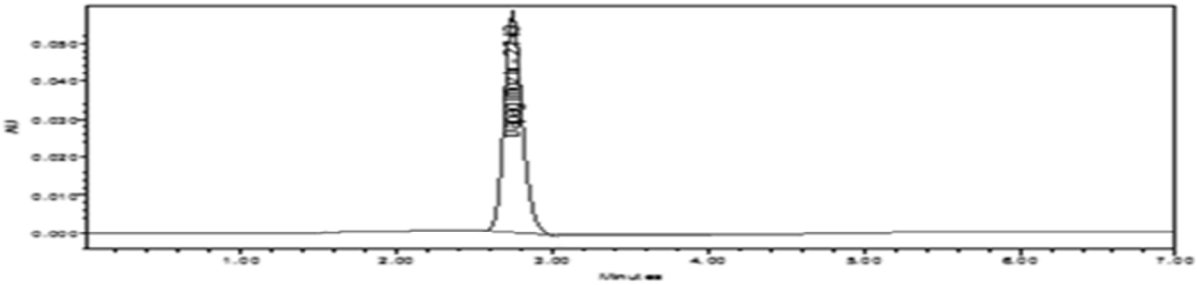

Fig 25 : Dapagliflozin linearity chromatogram of 18µg/ml solution

Table 4: linearity results

S.No	Name	Rt	Area	Height	Theoretical plate count	USP tailing
1	Dapagliflozin(3µgml)	2.74	166077	22521	2813	1.2
2	Dapagliflozin(6µg/ml)	2.79	294626	27551	2900	1.2
3	Dapagliflozin(9µg/ml)	2.75	355611	49126	2926	1.5
4	Dapagliflozin(12µg/ml)	2.75	514259	73957	2939	1.4
5	Dapagliflozin(15µg/ml)	2.76	624952	77947	2977	1.6
6	Dapagliflozin(18µg/ml)	2.77	754562	815421	2975	1.5

For linearity six different concentrations like 3µg/ml, 6µg/ml, 9µg/ml, 12g/ml, 15µg/ml and 18µg/ml of dapagliflozin were prepared and injected into HPLC system. And the data obtained were analyzed by using water's HPLC with software empower 3.0, and the column Sunsil (150mm X 4.5mm, 5µ) with flow rate 1ml/min and it was detected by using the waters 2487 dual absorbance detector and waters 717 plus auto sampler, Rt was found to be 2.76. The correlation co efficient was found within limits i.e., r^2 0.999

4.3. Accuracy

The recovery of drugs was determined by spiking drugs at three levels ranging from 50- 150% of label claim. The recovery range was found to be between 98-103%.[21]

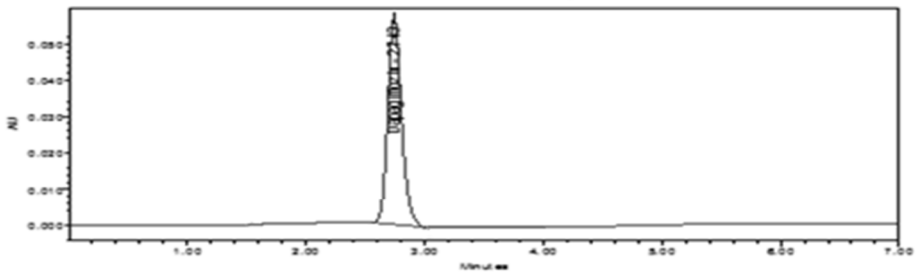

Fig 26 : Chromatogram of accuracy 50% solution- injection 1

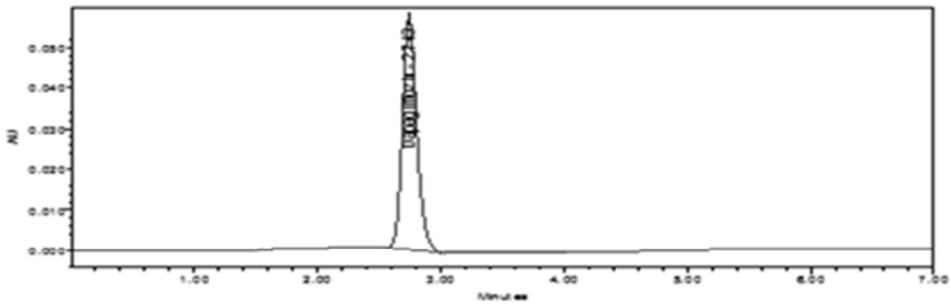

Fig 27 : Chromatogram of accuracy 50% solution- injection 2

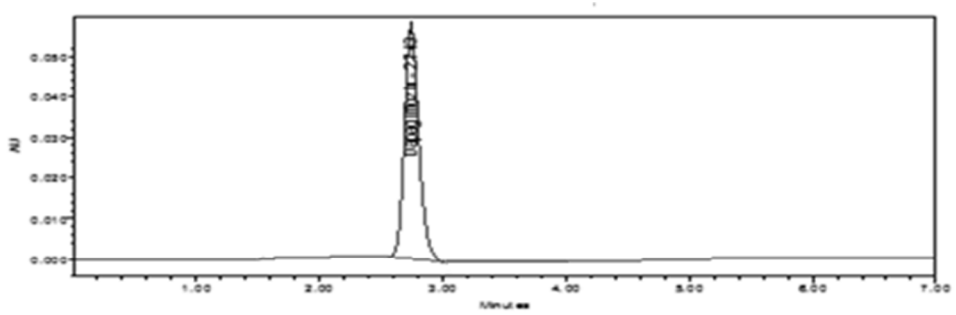

Fig 28 : Chromatogram of accuracy 50% solution- injection 3

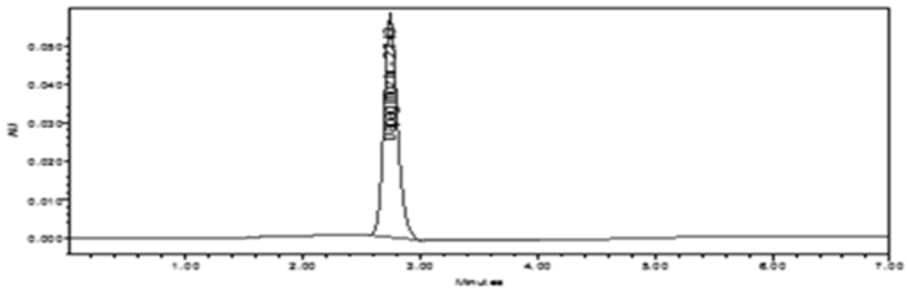

Fig 29 : Chromatogram of accuracy 100% solution- injection 1

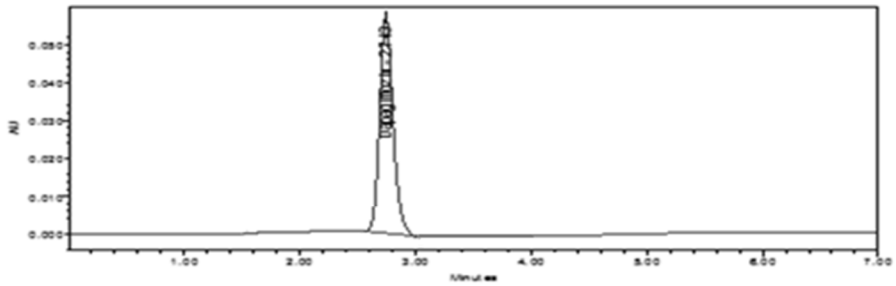

Fig 30 : Chromatogram of accuracy 100% solution- injection 2

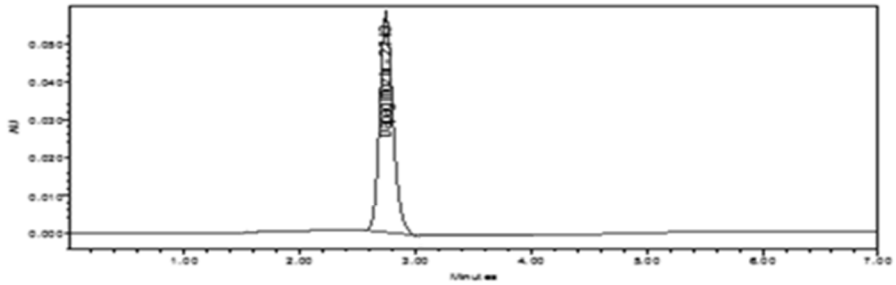

Fig 31 : Chromatogram of accuracy 100% solution- injection 3

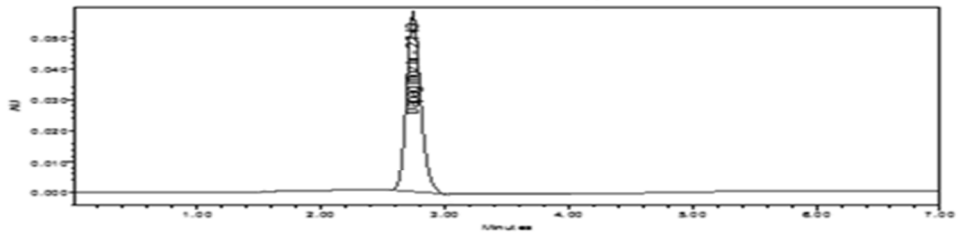

Fig 32 : Chromatogram of accuracy 150% solution- injection 1

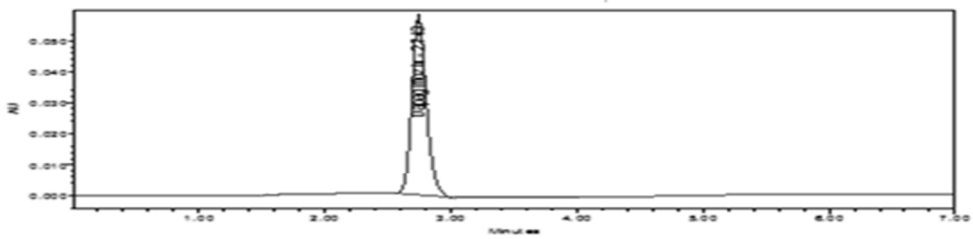

Fig 33 : Chromatogram of accuracy 150% solution- injection 2

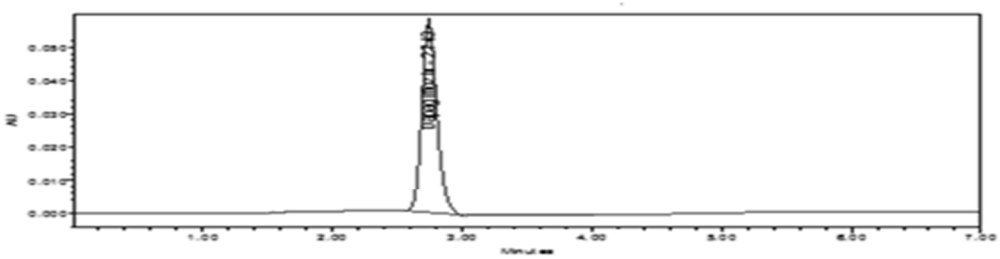

Fig 34 : Chromatogram of accuracy 150% solution- injection 3

Table 5 : accuracy results

S.No	Name	Sample + Standard	Rt	Peak area	%Recovery	SD	%RSD
1	Dapagliflozin 50%	6+3	2.75	355611 355624 355626	98.5 98.5 98.6	**0.0577**	**0.02%**
2	Dapagliflozin 100%	6+6	2.79	504652 504647 504654	98.1 98.0 98.1	**0.0577**	**0.01%**
3	Dapagliflozin 150%	6+9	2.76	614982 614980 614985	100.1 100.5 100.6	**0.0264**	**0.04%**

For accuracy studies three different levels of solution were prepared like 50%, 100% and 150% and each level were injected three times into HPLC system. and the data obtained were analyzed by using water's HPLC with software empower 3.0, and the column Sunsil (150mm X 4.5mm, 5µ) with flow rate 1ml/min and it was detected by using the waters 2487 dual absorbance detector and waters 717 plus auto sampler and the drug was eluted at 2.79min and data were presented as SD and the %RSD were found within the limits i.e., less than 2.

4.4 *Robustness*

The flow rate was changed (±1) and the 10µg/ml solution was injected into HPLC system for six replicates[22]

4.4.1 Robustness with flow rate 0.9ml

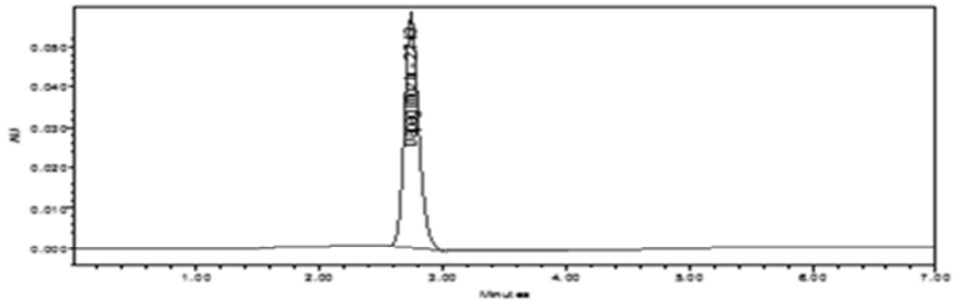

Fig 35 : Chromatogram of robustness at 0.9ml/min (10µg/ml) - injection 1

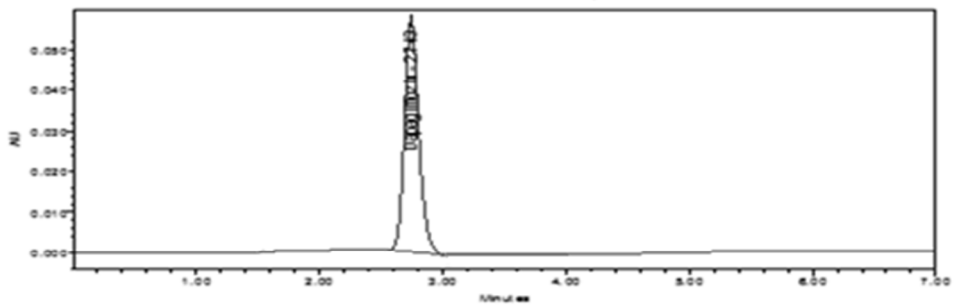

Fig 36 : Chromatogram of robustness at 0.9ml/min (10µg/ml) - injection 2

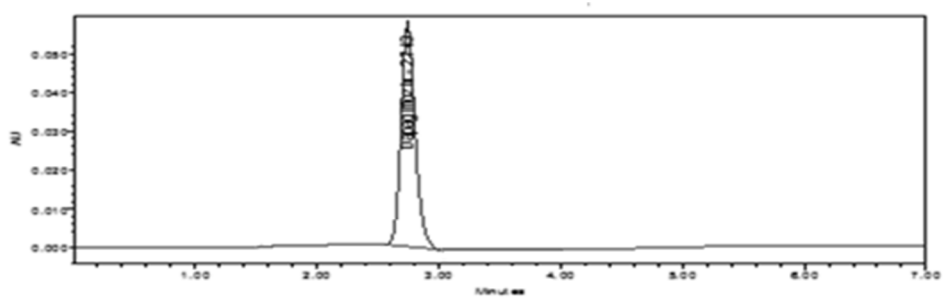

Fig 37 : Chromatogram of robustness at 0.9ml/min (10µg/ml) - injection 3

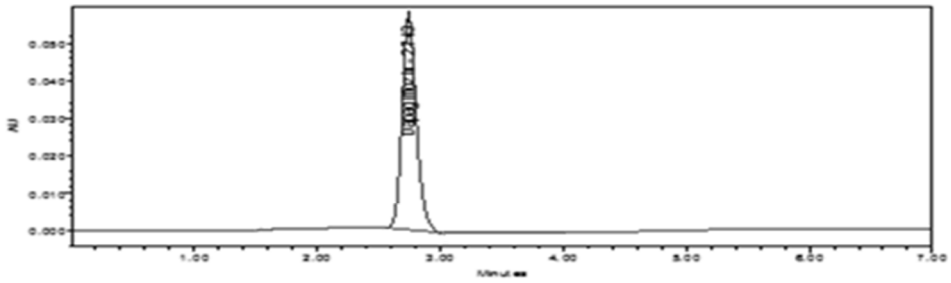

Fig 38 : Chromatogram of robustness at 0.9ml/min (10µg/ml) - injection 4

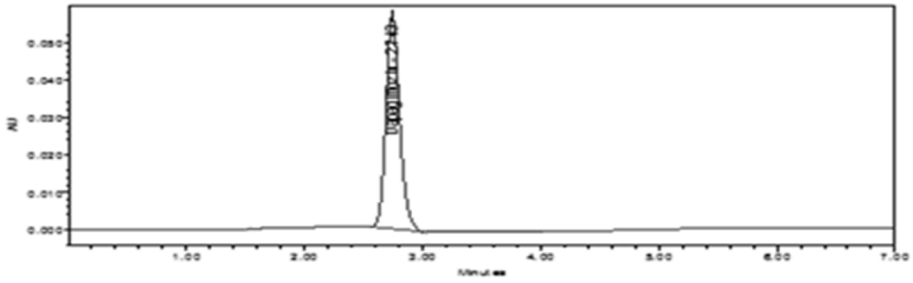

Fig 39 : Chromatogram of robustness at 0.9ml/min (10µg/ml) - injection 5

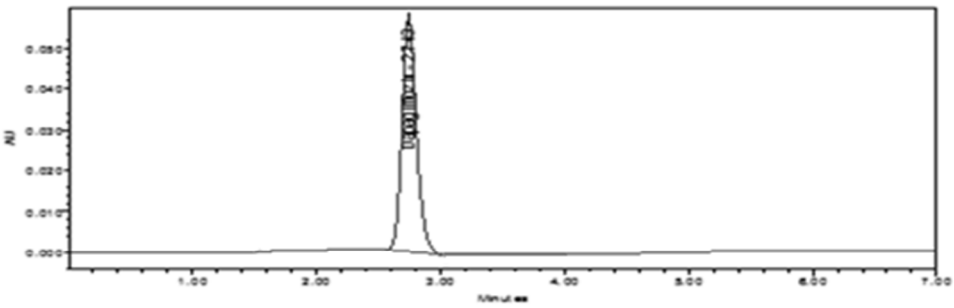

Fig 40 : Chromatogram of robustness at 0.9ml/min (10µg/ml) - injection 6

Table 6: Robustness flow rate 0.9ml/min results

S.No	Name	Rt	Peak area	Theoretical plate count	USP tailing factor
1	Dapagliflozin(10µg/ml)	2.76	456156	2777	1.2
2	Dapagliflozin(10µg/ml)	2.78	456279	2777	1.1
3	Dapagliflozin(10µg/ml)	2.78	456321	2776	1.5
4	Dapagliflozin(10µg/ml)	2.79	456243	2776	1.3
5	Dapagliflozin(10µg/ml)	2.79	456078	2777	1.1
6	Dapagliflozin(10µg/ml)	2.79	456212	2777	1.4
	Mean		456214		
	SD		87.60		
	%RSD		**0.01%**		

For the study of robustness 10µg/ml solution was prepared and six replicate injections were given into the system .The data obtained were analyzed by using water's HPLC with software empower 3.0, and the column Sunsil (150mm X 4.5mm, 5µ) with flow rate 0.9ml/min and it was detected by using the waters 2487 dual absorbance detector and waters 717 plus auto sampler and the drug was eluted 2.79 and data were presented as SD and the %RSD were found within the limits i.e., less than 2,

5.4.2 *Robustness with flow rate 1.1ml/min*

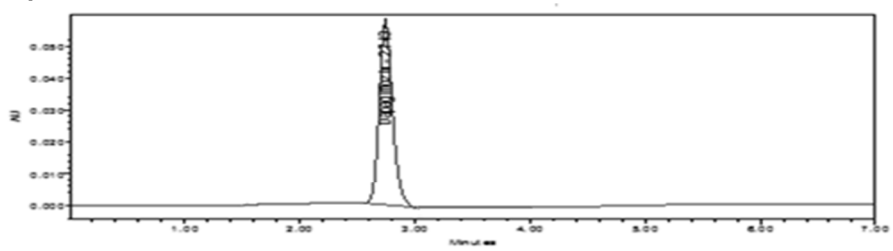

Fig 41 : Chromatogram of robustness at 1.1ml/min (10µg/ml) - injection 1

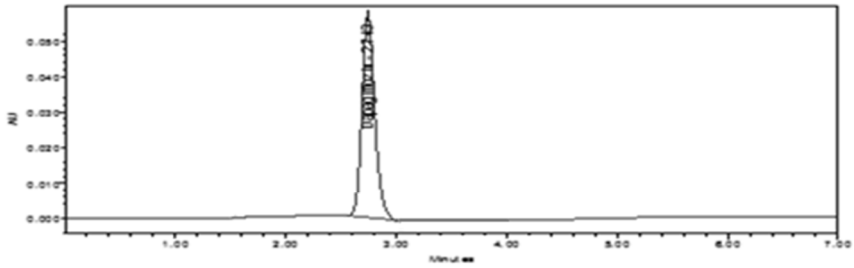

Fig 42 : Chromatogram of robustness at 1.1ml/min (10µg/ml) - injection 2

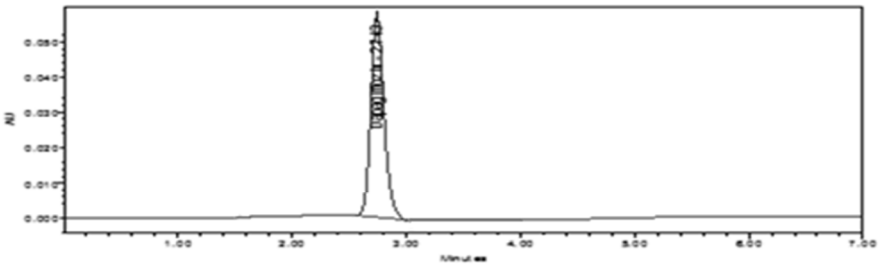

Fig 43 : Chromatogram of robustness at 1.1ml/min (10µg/ml) - injection 3

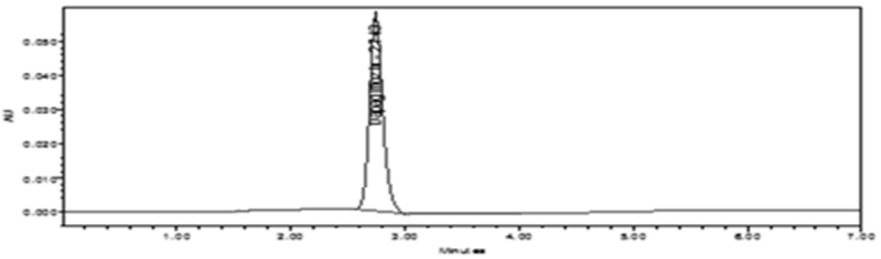

Fig 44 : Chromatogram of robustness at 1.1ml/min (10µg/ml) - injection 4

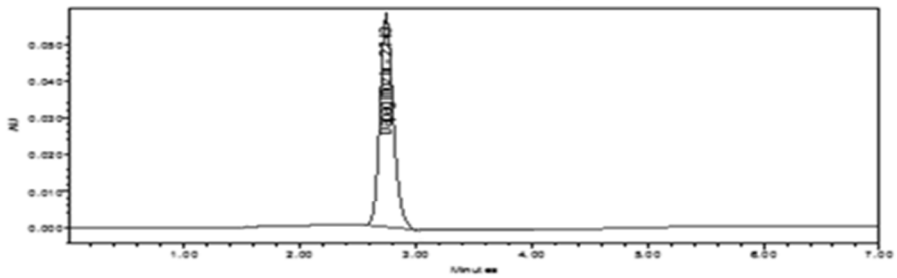

Fig 45 : Chromatogram of robustness at 1.1ml/min (10µg/ml) - injection 5

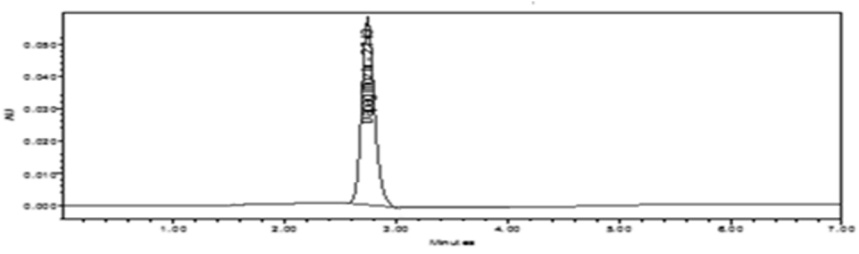

Fig 46 : Chromatogram of robustness at 1.1ml/min (10µg/ml) - injection 6

Table 7 : Robustness with flow rate 1.1ml/min results

S.No	Name	Rt	Peak area	Theoretical plate count	USP tailing factor
1	Dapagliflozin(10µg/ml)	2.77	456146	2777	1.2
2	Dapagliflozin(10µg/ml)	2.74	456269	2777	1.1
3	Dapagliflozin(10µg/ml)	2.79	456225	2776	1.5
4	Dapagliflozin(10µg/ml)	2.76	456343	2776	1.3
5	Dapagliflozin(10µg/ml)	2.76	456078	2777	1.1
6	Dapagliflozin(10µg/ml)	2.77	456312	2777	1.4
	Mean		456223		
	SD		110.4		
	%RSD		**0.01%**		

In this study 10µg/ml solution of dapagliflozin was prepared and six replicate injections were given into the HPLC system. The data obtained were analyzed by using water's HPLC with software empower 3.0, and the column Sunsil (150mm X 4.5mm, 5µ) with flow rate 1.1ml/min and it was detected by using the waters 2487 dual absorbance detector and waters 717 plus auto sampler and the drug was eluted at 2.77min and the data were presented as SD and the %RSD were found within the limits i.e., less than 2.

5.5 Limit of detection (LOD)

$$LOD = 3.3 \times \sigma / s$$
$$= 3.3 \times 131.6 / 38246 \quad (1)$$
$$= 0.011 \mu g/ml$$

σ = standard deviation, s =slope of calibration curve

5.6 *Limit of quantification (LOQ)*

$$LOQ = 10 \times \sigma / s$$
$$= 10 \times 131.6 / 38246 \quad (2)$$
$$= 0.034 \mu g/ml$$

σ = standard deviation, s = slope of calibration curve

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and dug products. The goal of this study was to develop and validate a RP-HPLC method for the estimation of dapagliflozin in bulk and pharmaceutical products. The main objective of method development was to determine the drug content present in the formulation and its percentage purity[23-26]. The chromatographic conditions like mobile phase composition, flow rate was optimized and the method was developed and validated successfully. In initial development of the method various mobile phases were tried to get a sharp peak, finally methanol: water in the ratio of 85:15(v/v) was selected which gave a single sharp peak with retention of 2.74min. Commercial marketed formulation of dapagliflozin was analyzed for its contents and Percentage of content was calculated. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis. The method was validated statistically for various parameters like standard deviation, % relative standard deviation, slope and intercept.

5.7 Scope of work

Extended study for the drug may include degradation studies by HPLC and characterization studies by various hyphenated techniques using bioanalytical methods

6. CONCLUSION

In our study, proposed analytical method was found to be rapid, precise, accurate and linear in range of 3- 15µg/ml, the correlation regression (R^2) being 0.999, when injected into Sunsil C_{18} column (150mm X 4.6mm, 5µ) where detection wavelength was monitored at 225nm. LOD and LOQ values were found to be 0.011µg/ml and 0.034µg/ml respectively. The percentage recovery of the spiked sample at various concentration levels, (50%, 100% and 150%) was found to be between 98.5% and 100.6% . A good sharp peak was eluted at 2.74min using methanol: water (85: 15) as eluting solvents. All the system suitability parameters were found to be within limits. Therefore this method can be employed for routine laboratory analysis.

7. ACKNOWLEDGEMENT

We are thankful to RBVRR women's college of pharmacy for providing us with resources to carry out this research and for giving us the opportunity to write this research article.

8. AUTHORS CONTRIBUTION STATEMENT

The research work, manuscript preparation and grammar check was done by Miss V. Navya Sree. The research work was guided by Dr. K. Bhavya Sree, Dr. M Sumakanth and R. Swetha Sree and the critical revision and final proof reading of manuscript were done by Dr. K. Bhavya Sree.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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