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ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION OF DAPAGLIPFLOZIN BY RP-HPLC

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ABSTRACT

A stability indicating RP-HPLC method was developed and validated for analysis of dapaglipflozin in single and dosage form. the separation was achieved by using cosmosil C18 (250mm x 4.6ID, Particle size: 5 µ) as a stationary phase with mobile phase consisting methanol: water (85:15) V/V pH 3. the flow rate 0.9 ml/min and optimum wavelength for detection was 224 nm. the developed method was validated for precision, accuracy, ruggedness, robustness, linearity and range. the developed method showed good linearity range 10-50 µg/ml for dapaglipflozin. the forced degradation studies were performed as per ICH guidelines under acidic, alkali, photolytic, thermolytic conditions. the developed RP-HPLC method was found to be linear over wider concentration range. thus the developed RP-HPLC method can be use for routine quantitative and qualitative analysis of dapaglipflozin in bulk and pharmaceutical formulations like tablets and validated as per ICH guidelines. Hence proposed method could be employed for stability studies on pharmaceutical preparations within pharmaceutical industry.

KEYWORDS: Dapagliflozin, HPLC, Methanol, Reverse Phase Chromatography, Validation.

INTRODUCTION

Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor indicated for managing diabetes mellitus type 2. When combined with diet and exercise in adults, dapagliflozin helps to

improve glycemic control by inhibiting glucose resorption in the proximal tubule of the nephron and causing glycosuria. Dapagliflozin was approved by the FDA on Jan 08, 2014.
This product is available in the following dosage forms:
-Tablet

Dapagliflozin is a C-glycosyl comprising beta-D-glucose in which the anomeric hydroxy group is replaced by a 4-chloro-3-(4-ethoxybenzyl)phenyl group. It has a role as a hypoglycemic agent and a sodium-glucose transport protein subtype 2 inhibitor.

As per our detailed literature review it has been found only few analytical methods for the dapaglipflozin have been reported. no stability indicating assay has been reported. therefore the attempt is made to develop simple, accurate, precise, rapid and economical RP-HPLC method for the determination of dapaglipflozin in dosage form. further generation of degradation profile on RP-HPLC through stress testing.

REVIEW

An extensive survey was carried out for the estimation of Dapaglipflozin in bulk and marketed dosage forms. Some of the methods reported are present below.

1) Gunasekar Manoharan*, Ahmed M Ismaiel, Zeyad Mohammed Ahmed

A sensitive, feasible RP-HPLC method has developed and validated for the analysis of Dapagliflozin in raw and tablet formulation. Successful separation of drugs products is developed on a C(18) 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. Good 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. The tailing factor was 0.970 respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Dapagliflozin in tablet formulation.

2) Jeyabaskaran. M1, Prof. Rambabu. C2 and Dhanalakshmi. B3

The present work is concerned with application of simple, precise, accurate, reproducible and specific RP-HPLC method for estimation of dapagliflozin (DGF) in bulk and pharmaceutical dosage forms using an Hypersil BDS 250mm x 4.6 mm, 5□ column in isocratic mode with 0.1% Ortho phosphoric acid buffer and acetonitrile 50:50% v/v as mobile phase at a flow rate of 1ml/min. The injection volume was 10 µl and the total runtime was set as 5min. The determination of analytes was carried out at 245nm using PDA detector. The retention time for DGF was found to be 2.226min. The proposed method has permitted the quantification of DGZ over linearity in the range of 25 – 150 µg/ml and its percentage recovery was found to be 100.12 %. The % RSD of intraday and inter day precision were found 0.6% and 0.29%.

3) Phani RSCH1, Prasad KRS1, Useni Reddy Mallu2*

A simple, precise and stability-indicating Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for simultaneous quantification of Dapagliflozin (DGFZ) and Saxagliptin (SGPT) in combined dosage form. The developed method has been validated with respect to precision, linearity, accuracy, robustness, ruggedness, sensitivity, solution stability. The method has been developed with ammonium dihydrogen phosphate buffer (pH 6.8) and methanol in a ratio of 65:35 v/v as mobile phase at a flow rate of 1.5 ml/min over Intersil ODS C18 column (250 mm × 4.6 mm × 5 µ). The UV detection wavelength was fixed at 280 nm. The column temperature being maintained at ambient temperature. The method shown good linearity with correlation coefficient values of 0.9992 and 0.999 for DGFZ and SGPT. The percent recoveries of two drugs found within the limits of (98.00-102.0%). The Limit of Quantification (LOQ) concentrations of DGFZ and SGPT are 0.312 µg/ml and 0.156 µg/ml respectively. The Limit of Detection (LOD) concentrations of DGFZ and SGPT are 0.156 µg/ml and 0.078 µg/ml respectively. According to International Conference on Harmonization (ICH) guidelines Forced degradation study was validated.

4) Manasa Sanagapati*1 Dhanalakshmi K1 Nagarjuna Reddy G2 Sreenivasa S3

The present study describes the development and subsequent validation of a stability indicating reverse phase HPLC (RP-HPLC) method for the analysis of Dapagliflozin in its API. The proposed method utilizes BDS column (maintained at ambient temperature), gradient run (using mixture of acetonitrile and ortho phosphoric acid as mobile phase), effluent flow rate (1ml/min) and detection at 245nm using PDA detector. The developed

method was successfully validated for different validation parameters as per ICH guidelines. The stability of the drug was determined by studying the degradation of the drug under acidic, alkaline, peroxide, neutral, heat and UV conditions.

MATERIAL AND INSTRUMENTS

1. Material
2. API: Dapaglipflozin 10 gm.
3. Formulation: Forxiga 100 mg Tablets

Sr.no	Chemical/Solvent/Reagent	Make	Grade
1	Water	MI	HPLC
2	Methanol	Honeywell	HPLC
3	Acetonitrile	Fisher Scientific	HPLC
4	Phosphate buffer pH 3	Fisher Scientific	AR

4. Instrument

Sr.no	Name of Instrument	Company Name
1	HPLC Instrument	System: HPLC Binary Gradient System Model no.: HPLC 3000 Series Software: HPLC Workstation
2	UV-Spectrophotometer	Analytical Technologies Limited Model: UV 2012
3	Pump	P-3000-M Reciprocating (40MPa)
4	Column (C18)	Column: Cosmosil C18 (250mm x 4.6ID, Particle size: 5 µ)
5	pH meter	VSI pH meter (VSI 1-B) Model: DPH-500
6	Balance	WENSARTM High Resolution Balance Model: PGB 100
7	Sonicator	Wenser Ultra Sonicator Model: WUC- 4L

Experimental work

Finally mobile phase containing potasium dihydrogen phosphate buffer (pH 3) Methanol: Buffer (85:15% v/v) was found to give best resolution for drug. The observation observed with different compositions of mobile phase has shown in the table of trail taken.

RP-HPLC method development: Trials taken

Sr.no	Mobile Phase	Column	λmax(nm)	Flow rate	Obs.
1	Methanol: Water (80:20) pH 3	Hypersil BDS C18, 250×4.6 mm,5µ	224nm	0.8ml/min	Peak shape was not good
2	Methanol: Water (80:20) pH 3	Hypersil BDS C18, 250×4.6 mm,5µ	224nm	0.85ml/min	Peak shape was not good
3	Methanol: Water (80:20) pH 3	Hypersil BDS C18, 250×4.6 mm,5µ	224nm	0.95ml/min	Peak shape was not good
4	Methanol: Water (85:15) pH 3	Hypersil BDS C18, 250×4.6 mm,5µ	224nm	0.95ml/min	Peak shape was not good
5	Methanol: Water (85:15) pH 3	Hypersil BDS C18, 250×4.6 mm,5µ	224nm	0.9ml/min	Good Peak Shape Observed

I. Trial no.1

Sample Name: Dapaglipflozin Trial 01

Wavelength: 224nm

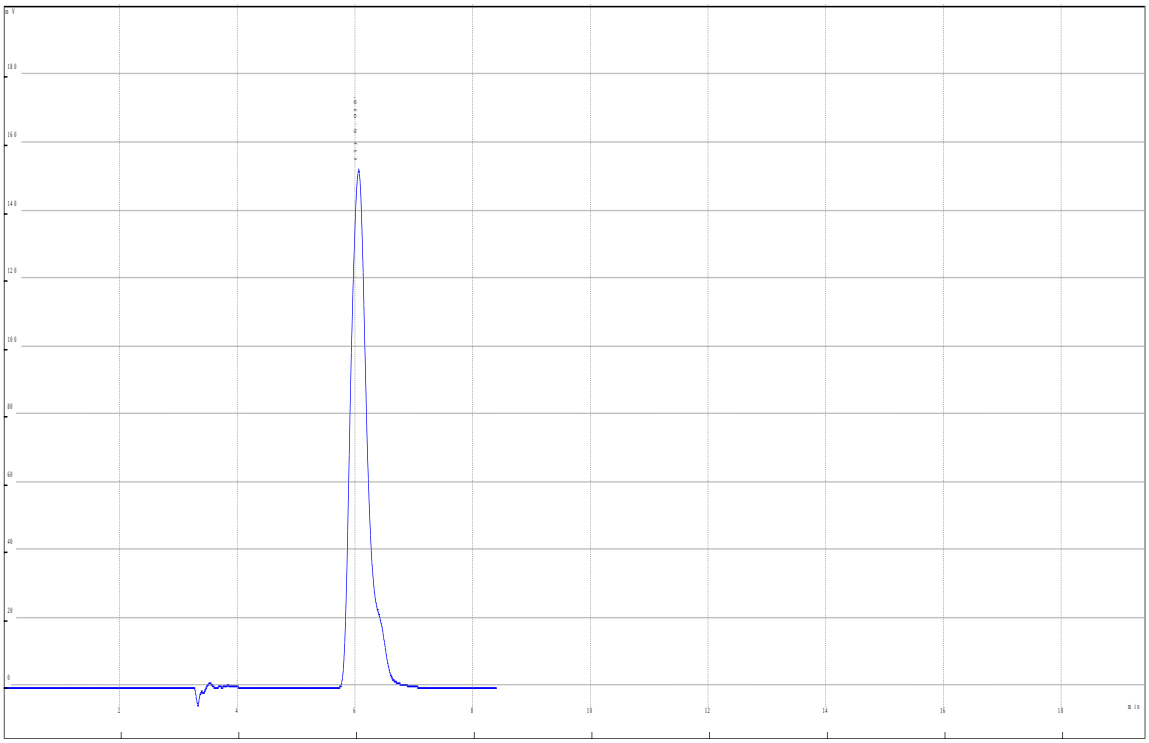
Mobile Phase: Methanol:Water (80:20)

Sample volume: 20µl

Flow rate: 0.8 ml/min

Pressure:9-10MPa

Run time: 8.37min



RT(min)	Area	Resolution	T.plate no	Asymmetry
6.018	3314630	0.00	6854	1.37

II. Trial no.2

Sample Name: Dapaglipflozin Trail 02

Wavelength: 224nm

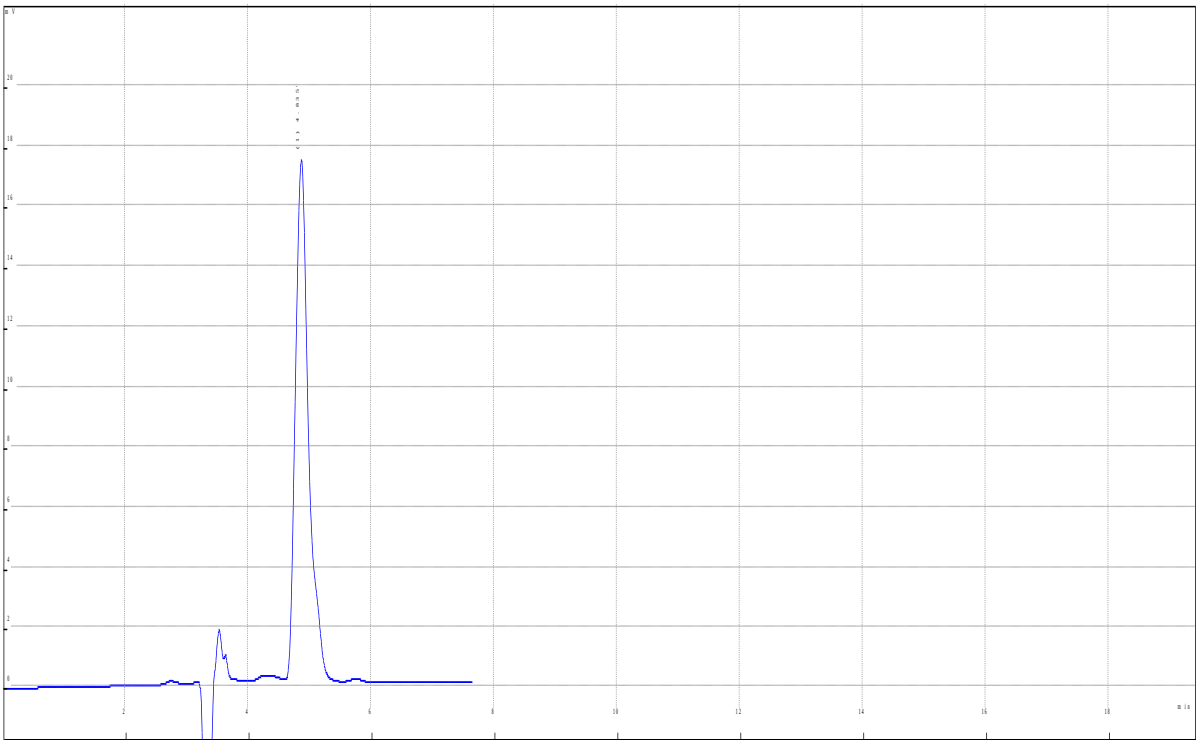
Mobile Phase: Methanol:Water (80:20)

Sample volume: 20µl

Flow rate: 0.85 ml/min

Pressure:9-10MPa

Run time: 7.62min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.835	443310	0.00	7206	1.38

III. trial no 3

Sample Name: Dapaglipflozin Trial 03

Wavelength: 224nm

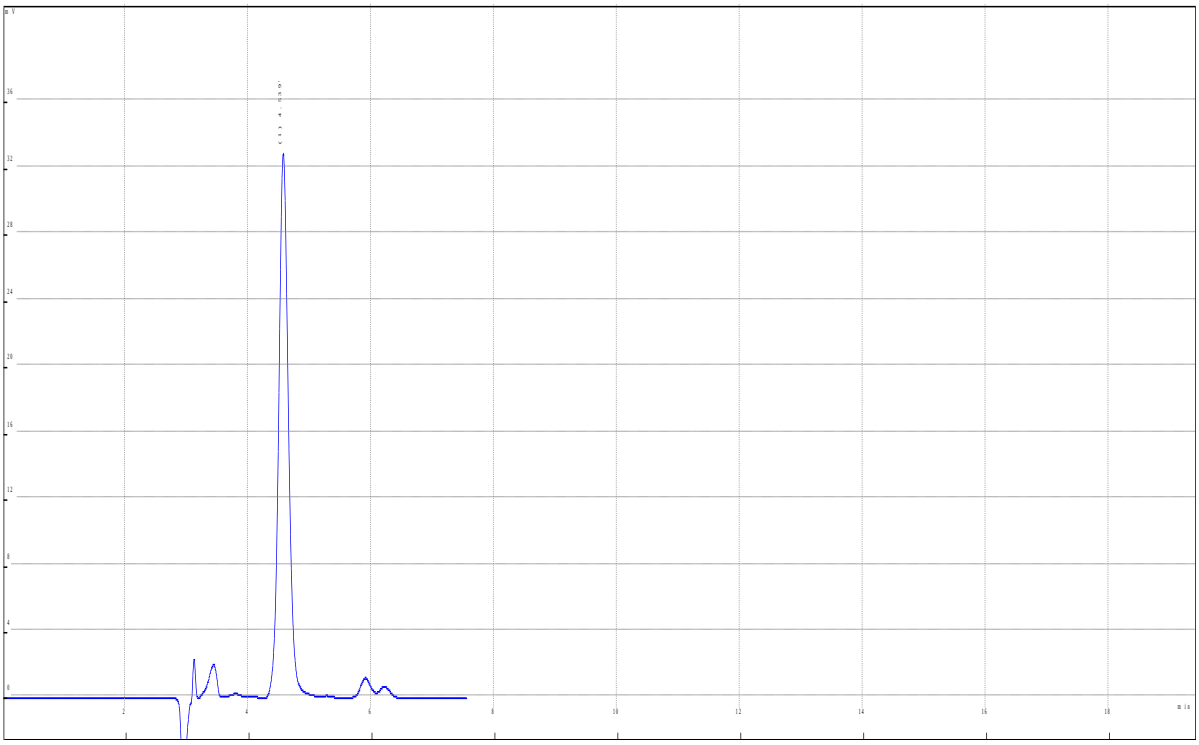
Mobile Phase: Methanol:Water (80:20)

Sample volume: 20µl

Flow rate: 0.95 ml/min

Pressure:9-10MPa

Run time: 7.53min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.539	504378	0.00	7435	1.17

IV. Trial no.4

Sample Name: Dapaglipflozin Trial 04

Wavelength: 224nm

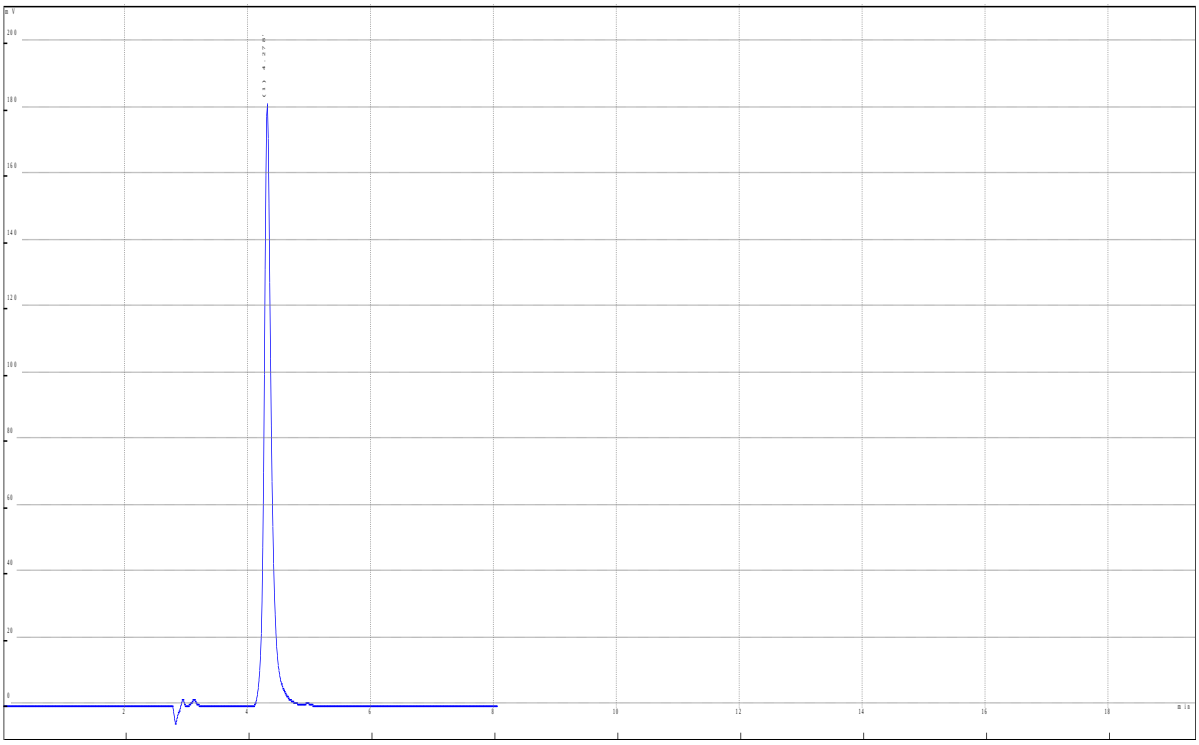
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.95 ml/min

Pressure:9-10MPa

Run time: 8.03min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.278	1866335	0.00	7283	1.28

V. Trial no.5

Sample Name: Dapaglipflozin Trail 05

Wavelength: 224nm

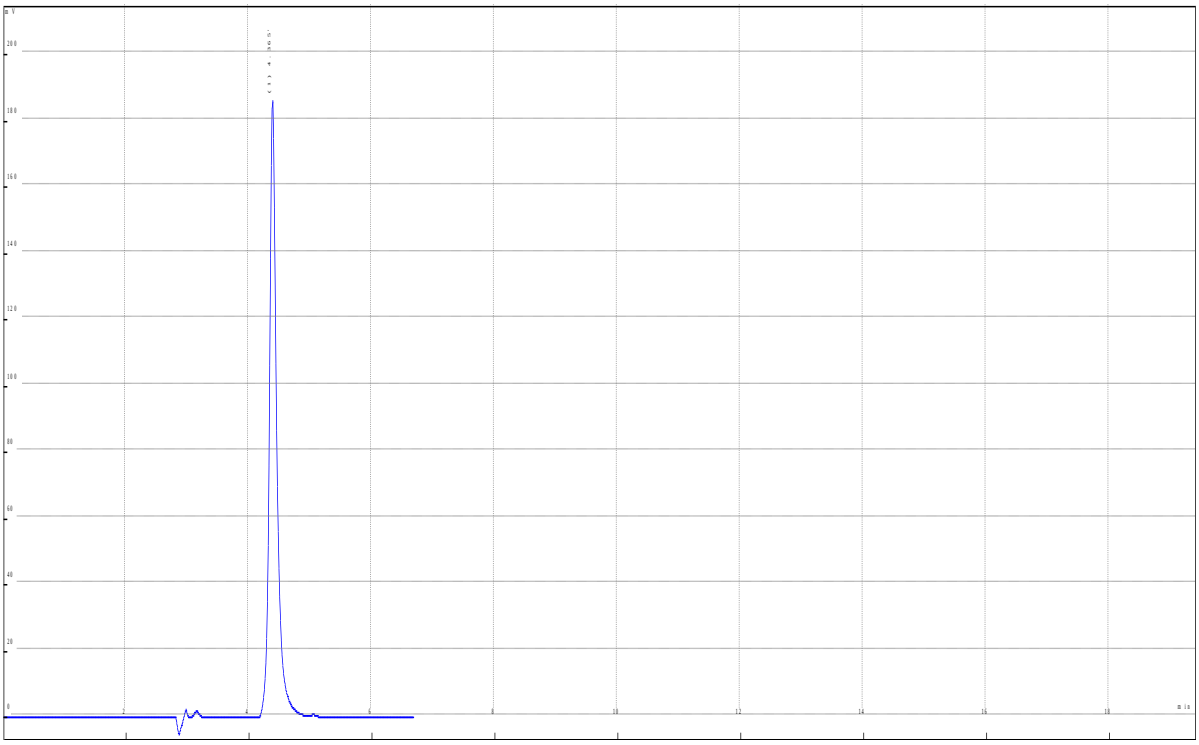
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 6.67min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.365	1571864	0.00	7343	1.28

Application of proposed method for analysis of marketed formulation

i. Standard Stock Solution

• Procedure

Accurately weighed quantity of Dapagliflozin 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with diluent. The resultant solution is used as standard stock solution of Dapagliflozin (Conc. 100 µg/ml).

ii. Sample solution preparation

• Procedure

Weigh accurately tablet content which is equivalent to 10mg (Dapagliflozin) transfer into 100ml volumetric flask and dilute with mobile phase, sonicate it for 10 min to get Dissolve and filter through 0.45µ filter paper to get 100ml stock solution.

Equal volume (20µL) of standard and sample solutions injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of Dapagliflozin was calculated by comparing a sample peak with that of standard. Amount of drug in tablet was calculated using following formula For Assay of Dapagliflozin

$$Mg/ml = \frac{AT \times WS}{AS \times WT} \times 100$$

Where,

AT -Average area of Dapagliflozin peak in test chromatograms

AS -Average area of Dapagliflozin peak in standard chromatograms

WS -Weight of Dapagliflozin working standard taken in mg

WT -Weight of sample taken in mg

Further calculate the amount Dapagliflozin present in % of Label claim using the following formula:

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/ml)}}{\text{Label claim in mg per ml Dapagliflozin}} \times 100$$

iii. System Suitability Test

System suitability is a Pharmacopeial requirement and is used to verify, whether the resolution (here not apply) and reproducibility of chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from six replicate injection of standard drug solution.

• Acceptance Criteria

RSD should not be more than 2.0 % for five replicate injections of standard USP Tailing Factor is not more than 2.0. The column efficiency as determined for Plate Count should be more than 2000.

iv. Validation of method for analysis of Dapagliflozin

A. Linearity

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range.

• Determination

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

• Acceptance Criteria

The plot should be linear.

Correlation Coefficient should not be less than 0.999.

I. Preparation of standard stock solution

10.0 mg of Dapagliflozin working standard was weighed accurately and transferred into 10.0 ml volumetric flask, 7 ml of diluents was added and sonicated to dissolve and finally the volume was made with diluents and mixed. This solution was used to prepare linearity solution. This solution was filtered through 0.45µ whatman filter paper.

II. Preparation of linearity solution

Linearity was performed by diluting standard stock solution. From stock solution aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 ml diluted to 10ml with diluent such that the final concentration of Dapagliflozin in the range of 10 to 50 µg/ml.

Sample	**Linearity stock solutionTransfer (ml)**	**Final volume (ml)**
Linearity – 10%	0.1	10
Linearity – 20%	0.2	10
Linearity – 30%	0.3	10
Linearity – 40%	0.4	10
Linearity – 50%	0.5	10

B. Accuracy (recovery)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte.

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

• Acceptance Criteria

Mean recovery should be in the range of 98-102%.

The Relative Standard Deviation should not be more than 2.0%.

1) Preparation of standard stock solution

10.0 mg of Dapagliflozin working standard was weighed accurately and transferred into 10 ml volumetric flask, 7 ml of diluent was added and sonicated to dissolve and finally the volume was made with diluents and mixed. The working standard concentration is 1000 µg/ml.

2) Procedure for Preparation of sample Solution

Prepare the standard solution by taking stock solution equivalent to 50%, 100%, and 150%, each in triplicate. Inject each preparation into the HPLC system.

Table no- 6: Concentration table for accuracy.

Sample	Concentration of Std. (ppm)	Concentration of tablet. (ppm)	Total Concentration (ppm)
Accuracy 50%	10	20	30
Accuracy 50%	10	20	30
Accuracy 100%	20	20	40
Accuracy 100%	20	20	40
Accuracy 150%	30	20	50
Accuracy 150%	30	20	50

3. Calculation

$$\text{AMOUNT FOUND (X)} = \text{AREA} + \text{C} \div \text{M}$$

Where,

X = Amount found

C = Intercept

M = Slope

C. 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.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

• Determination

Prepare six different test solution of 100% test concentration from same sample matrix,

Inject 3 duplicate injections in morning and 3 at Evening.

• Acceptance criteria

% RSD not more than 2% for test results.

a. Preparation of standard stock solution

10 mg of Dapagliflozin working standard was weighed accurately and transferred into 10 ml volumetric flask, 7 ml of diluent(mobile phase) was added and sonicated to dissolve and finally the volume was made with diluents and mixed. The working standard concentration is 1000 µg/ml.

D. Limit of Detection (LOD)

The lowest conc. of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental conditions simply indicates that the sample is below or above certain level. Limit test prescribed as percentage or as parts per million. The limit of detection will not only depend on the procedure of analysis but also on type of instrument.

S/N= 2/1 or 3/1

Where,

S= SignalN=Noise

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD= 3.3 (SD)/S

Where, SD= Standard deviation

S= Slope

E. Robustness

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay.

The sample along with standard was injected under different chromatographic conditions as shown below.

Changes in flow rate. (± 1 ml/min),

Change in Methanol % in Mobile phase

Carry out the following procedure individually by changing following variation in chromatographic conditions.

Change in flow rate of mobile phase by ± 1 ml/min.

Change in Acetonitrile % in Mobile phase ± 10 %

F. Limit of Quantitation

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample. The S/N ratio should not less than 10 and RSD ≤ 3%

S/N= 10/1

Where,

S= Signal

N= Noise

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOQ= 10 (SD)/S

Where,

SD= Standard deviation

S= Slope

1. RESULT AND DISCUSSION

i. Preformulation Study of Drug

Dapaglipflozin was studied for organoleptic character such as Colour, odour, taste, appearance.

ii. Colour, Odour and Appearance

Table no. 7: Colour, Odour and Appearance of Drug.

Sr.No	Parameter	Result
1	Colour	White
2	Odour	Odourless
3	Taste	unpleasant
4	Appearance	Amorphous

iii. Determination of Physical Constant of Drug

Table no. 8: Determination of Physical Constant of Drug.

Sample	Observed melting point	Standard melting point
Dapaglipflozin	56^0c	$55\text{-}58^0$c

iv. Solubility

Solubility was done in different solvents like Water, Methanol, Acetonitrile etc.

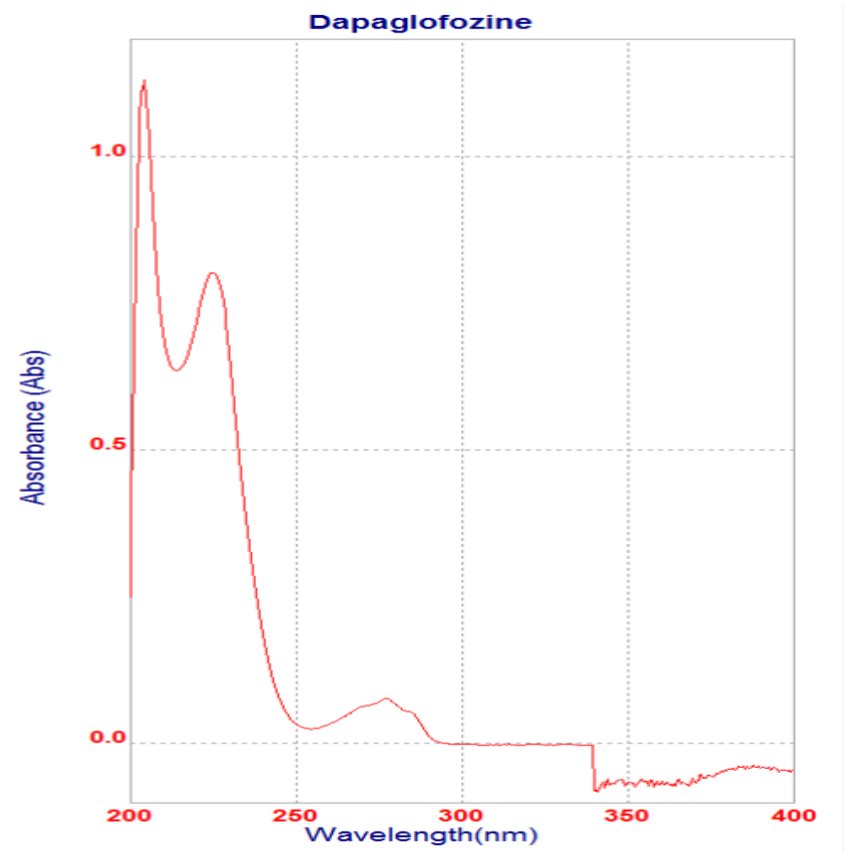
Table no. 9: determination of solubility of drug.

Solvent	Water	Methanol
Solubility of drug(mg/ml)	2.66	17.12

v. determination of wavelength maxima

I. determination of wavelength maxima for dapaglipflozin

Calibration curve of dapaglipflozin was done in phosphate buffer pH 3 at *



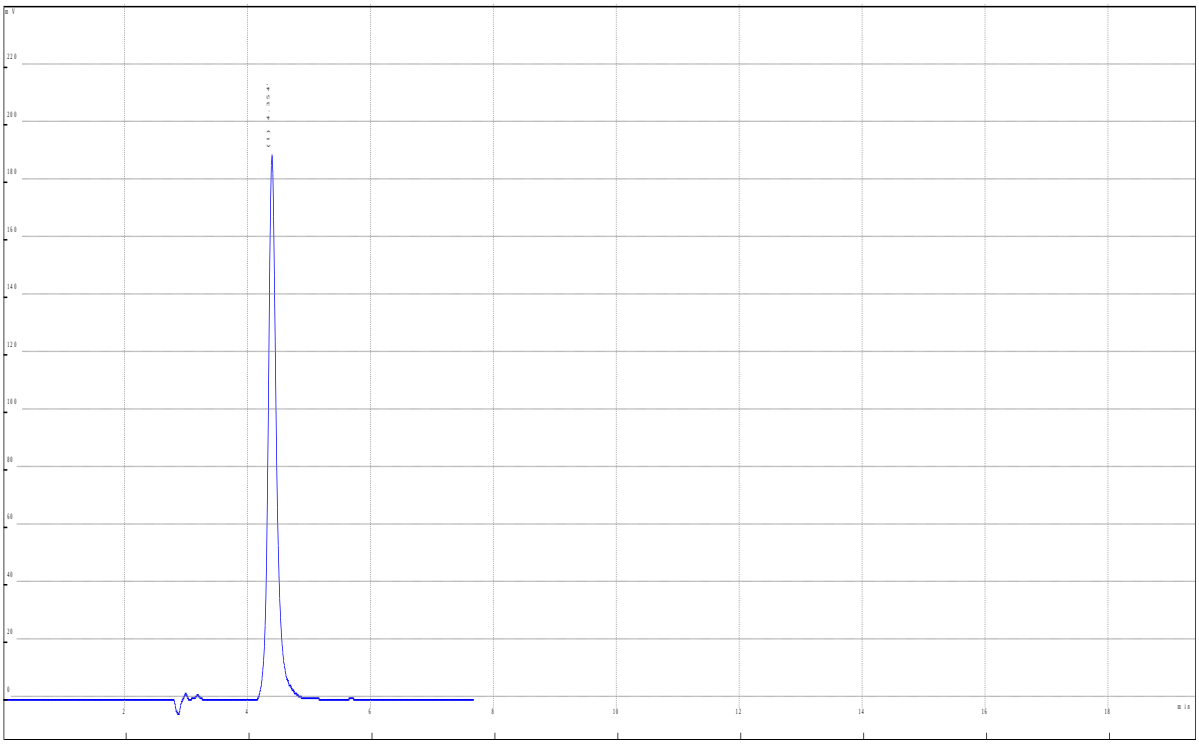
High performance liquid chromatography (HPLC) Method for analysis of dapaglipflozin

a. optimized method

Table 10-optimized method.

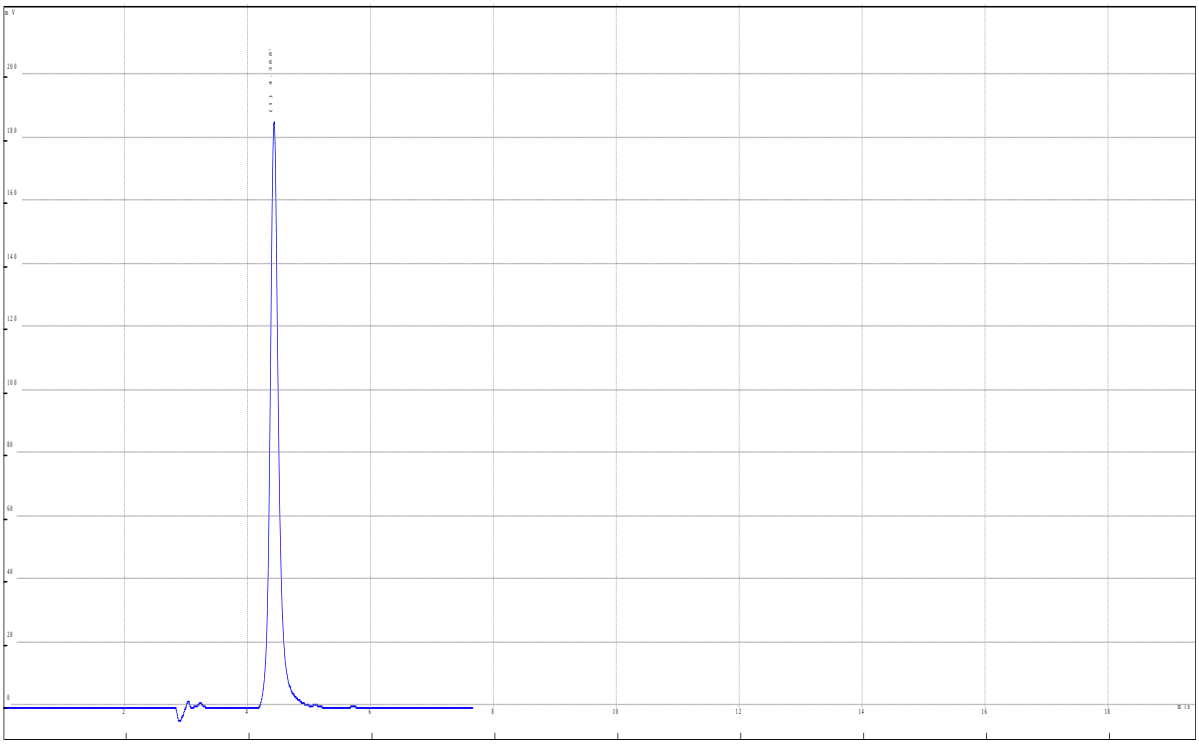
Parameter/conditions	Description/values
Column name	Cosmosil C18,250x4.6mm.5µ
Wavelength	224 nm
Flow rate	0.9 ml/min
Programme	Isocratic
Retention time	4.365
Mobile phase	Methanol:buffer (85:15) with adjusted pH 3 by potassium dihydrogen phosphate

b. Assay chromatogram of sample



Name	RT(min)	Area	Resolution	T.plate no	Asymmetry
Dapaglipflozin	4.354	2119701	0.00	8047	1.22

Chromatogram of standard



Name	RT(min)	Area	Resolution	T.plate no	Asymmetry
Dapaglipflozin	4.388	2122944	0.00	8976	1.19

Table no. 11: data for assay of the developed RP-HPLC method.

Sr.no	% composition	Area of standard	Area of sample	Tablet lable claim	Amount found	% assay
1	% Assay	2122944	2119701	5 mg	4.992	99.84

Validation of developed method

- **Precision**

Interday

DAY 1

❖ **30ppm set1**

Sample Name: Dapaglipflozin 30ppm 01

Wavelength: 224nm

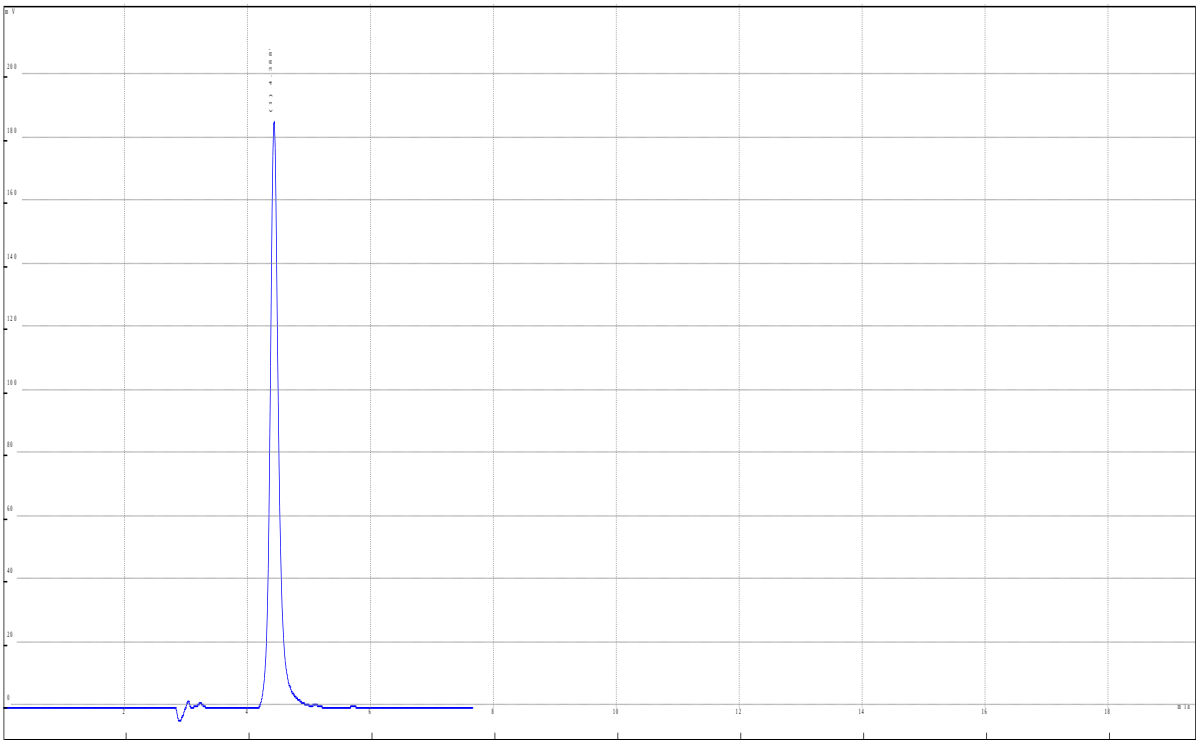
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.63min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.388	2122944	0.00	8976	1.19

❖ 30ppm set 2

Sample Name: Dapaglipflozin30ppm 02

Wavelength: 224nm

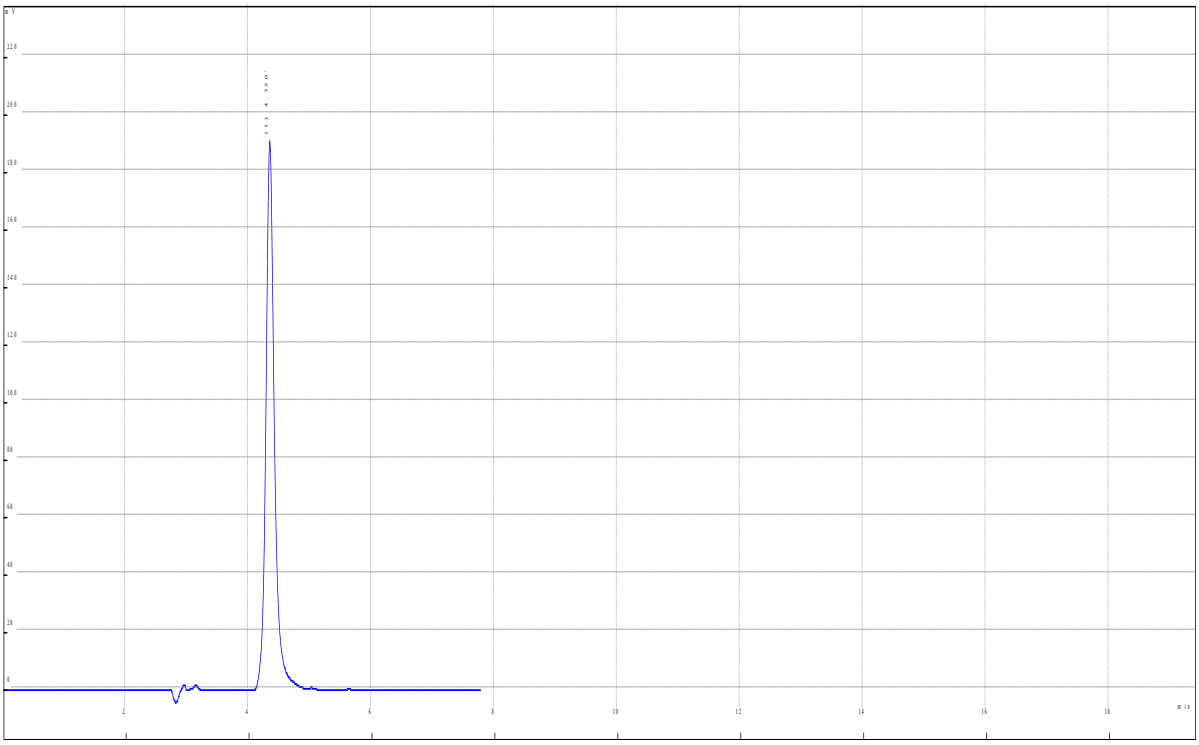
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.75min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.320	2121076	0.00	8731	1.19

❖ 30 ppm set 3

Sample Name: Dapaglipflozin30ppm 03

Wavelength: 224nm

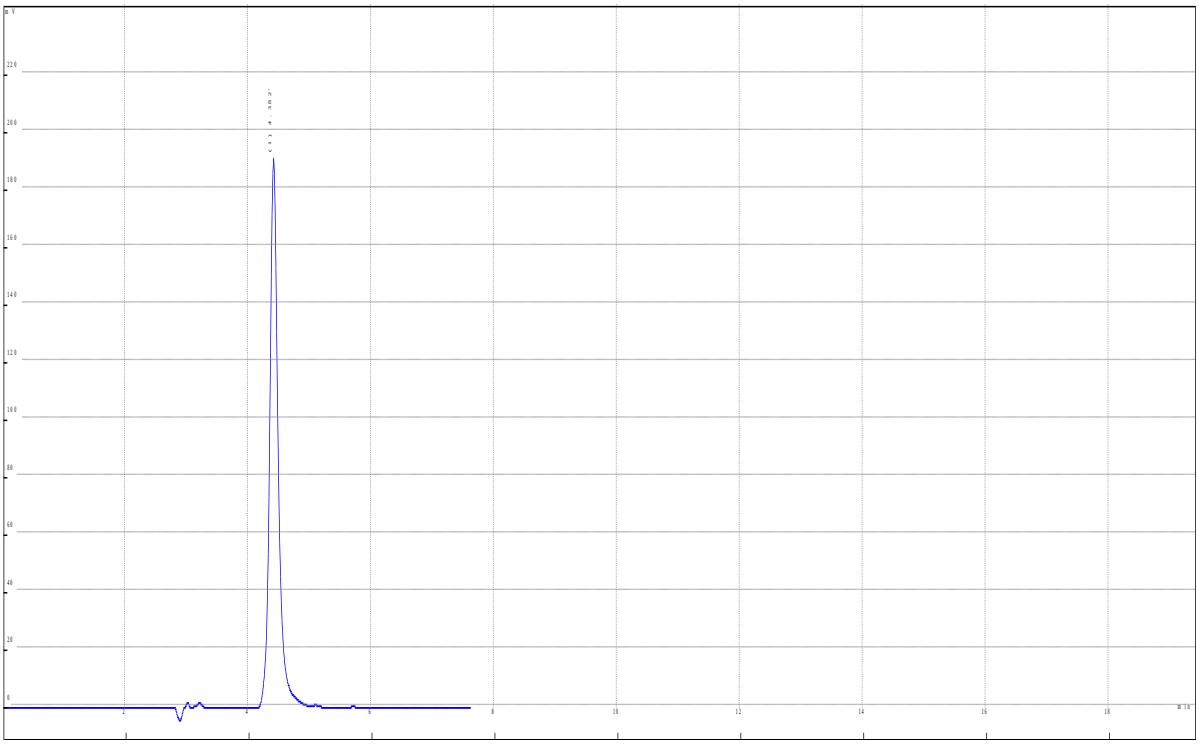
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.60min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.382	2124419	0.00	8026	1.20

DAY 2

30ppm set 1

Sample Name: Dapaglipflozin30ppm Day2 01

Wavelength: 224nm

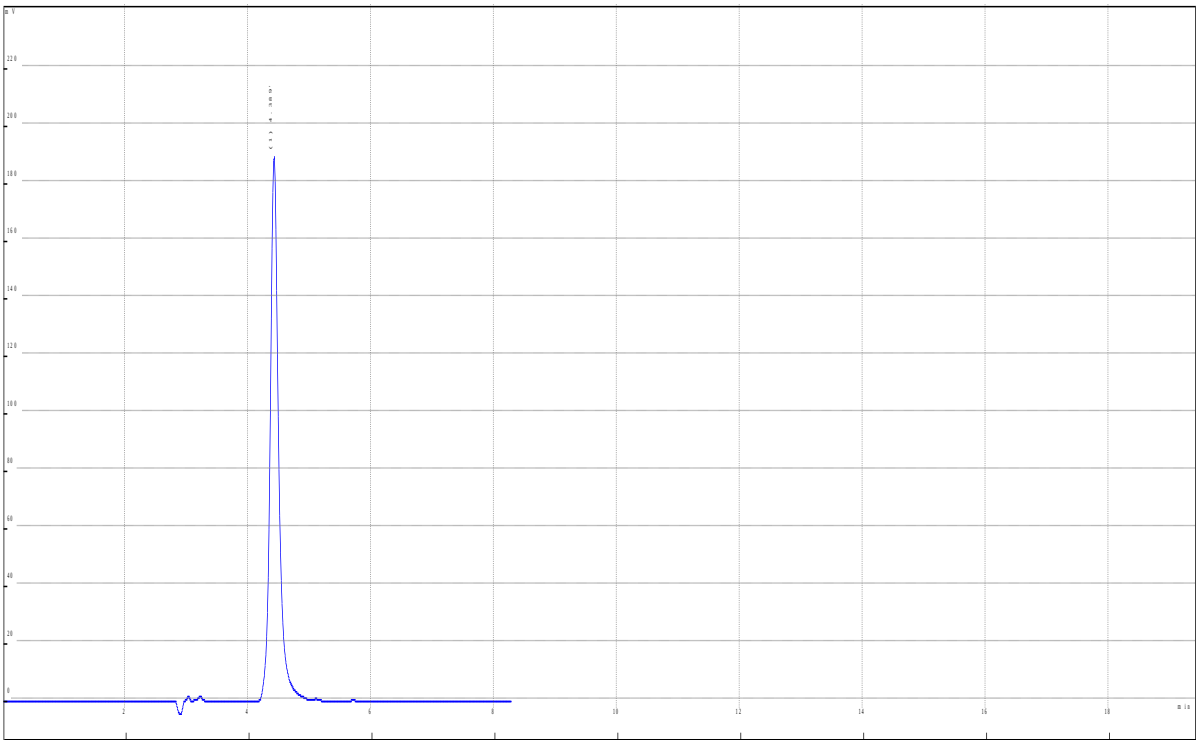
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.26min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.389	2123508	0.00	8084	1.20

30ppm set 2

Sample Name: Dapaglipflozin30ppm Day2 02

Wavelength: 224nm

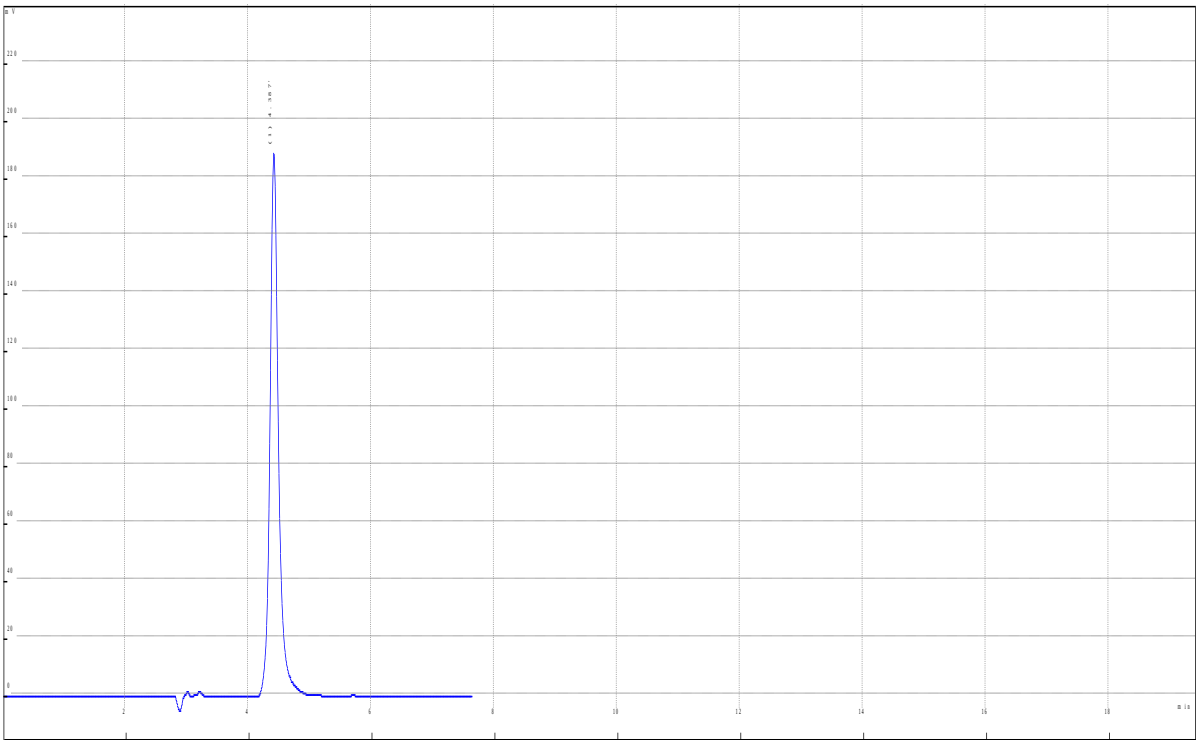
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.62min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.387	2122162	0.00	8921	1.19

30ppm set 3

Sample Name: Dapaglipflozin30ppm Day2 03

Wavelength: 224nm

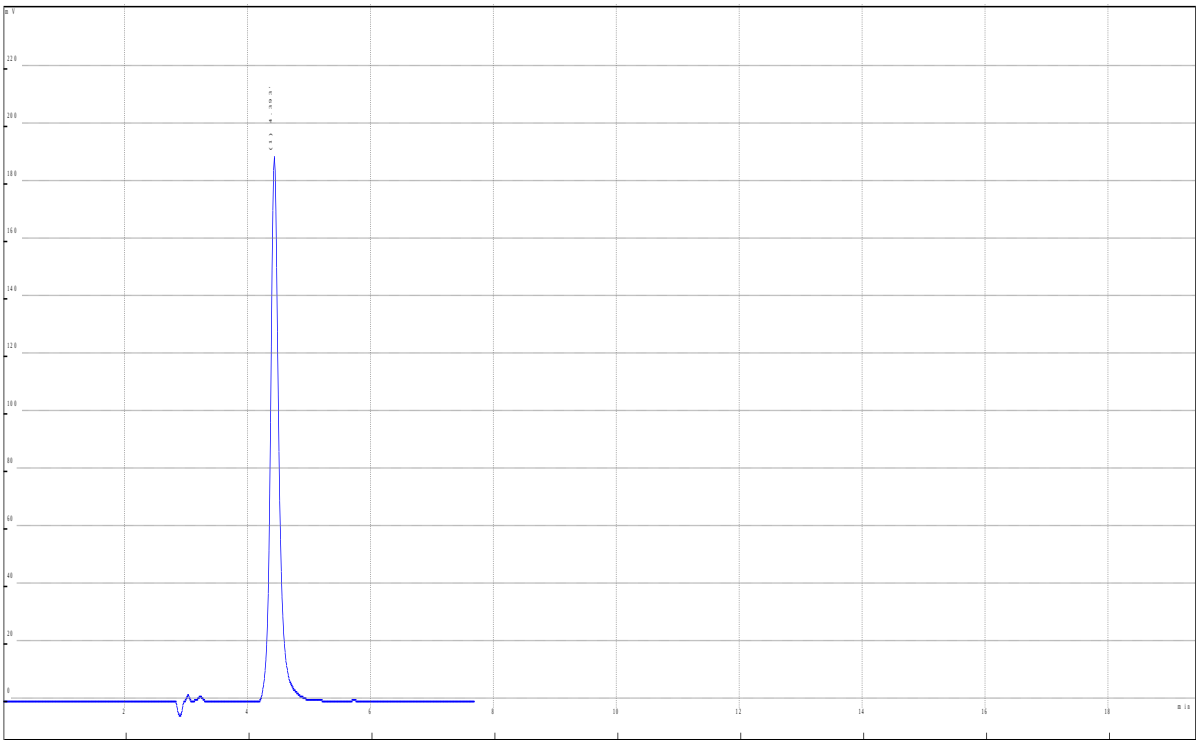
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.66min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.393	2119836	0.00	8815	1.21

Table no. 12: interday precision of dapaglipflozin.

Concentration	Area	
	Day 1	Day 2
30 ppm	2122944	2123508
	2121076	2122162
	2124419	2119836
	Mean	2122324
	%RSD	0.08%

Intraday

Evening

30ppm set 1

Sample Name: Dapaglipflozin30ppm Evening 01

Wavelength: 224nm

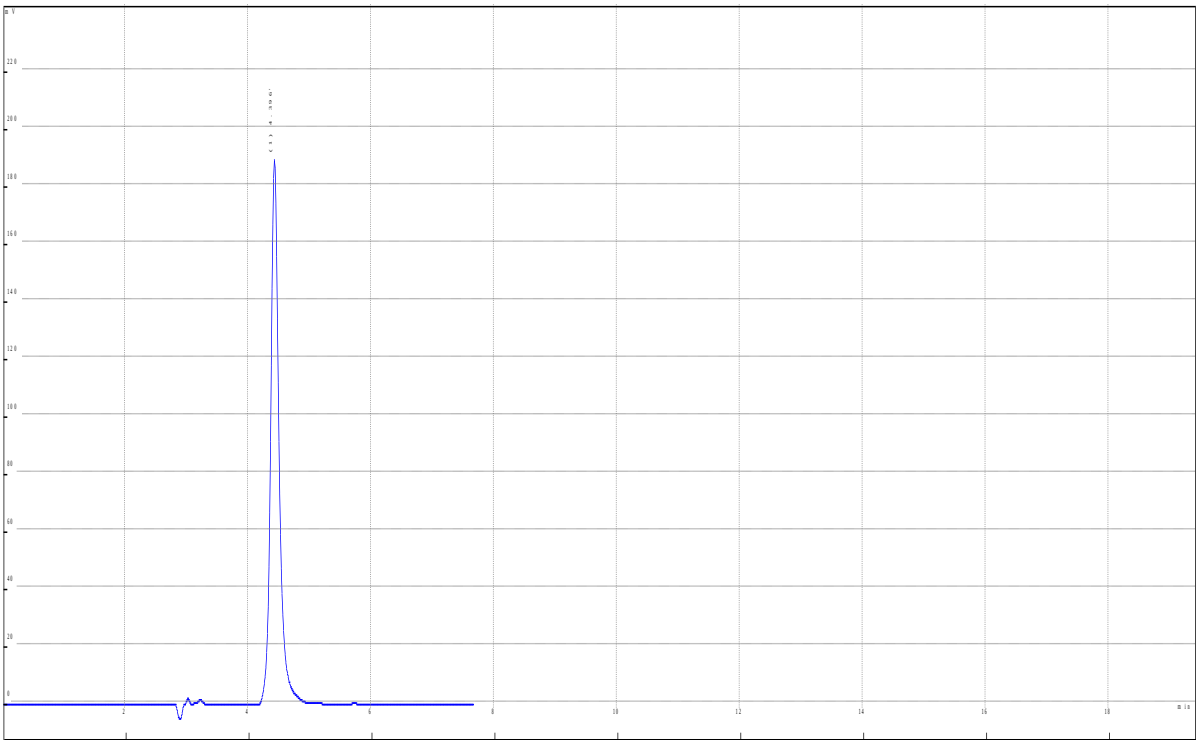
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.64min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.396	2113917	0.00	8980	1.22

30ppm set 2

Sample Name: Dapaglipflozin30ppm Evening 02

Wavelength: 224nm

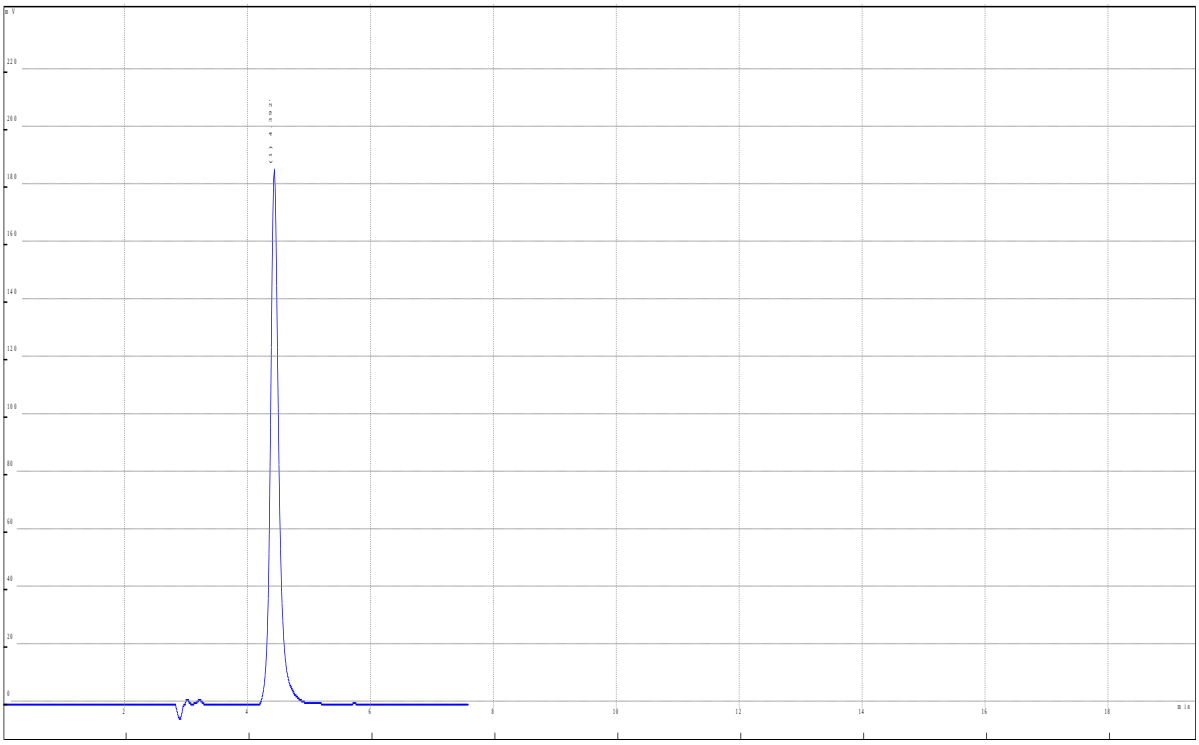
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.56min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.392	2118652	0.00	8037	1.22

30ppm set 3

Sample Name: Dapaglipflozin30ppm Evening 03

Wavelength: 224nm

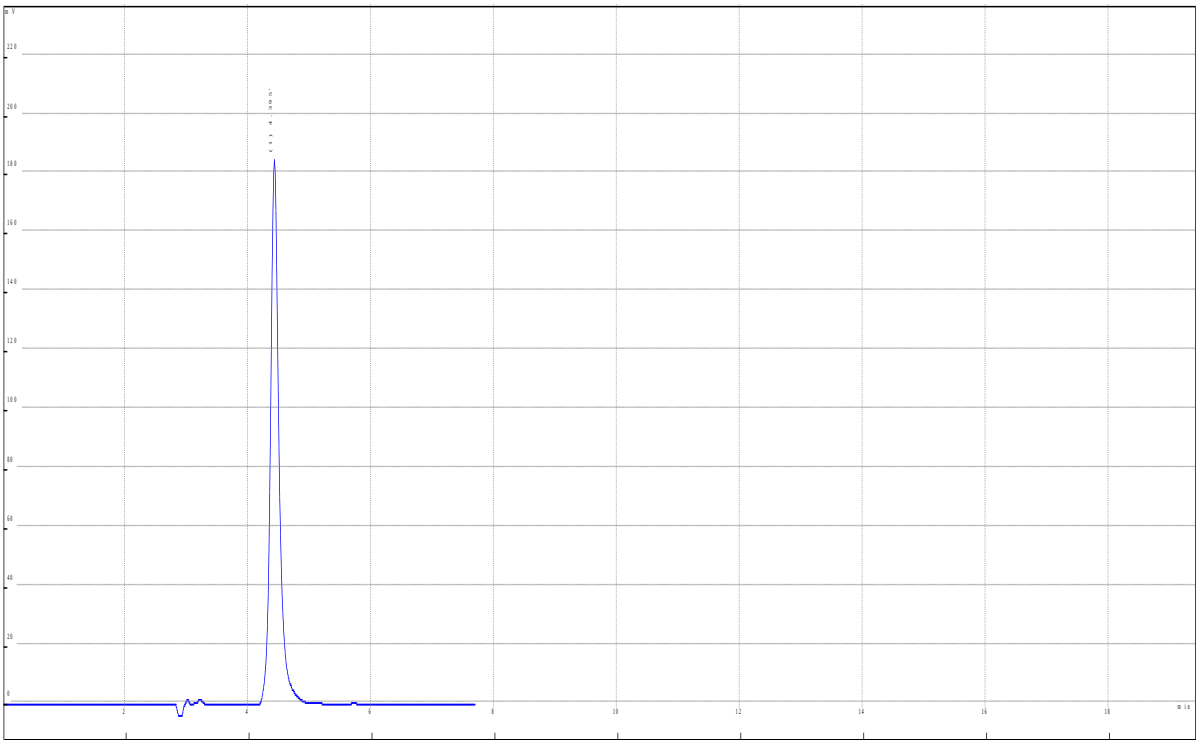
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.67min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.395	2117587	0.00	8039	1.21

Morning

30 ppm set 1

Sample Name: Dapaglipflozin 30ppm 01

Wavelength: 224nm

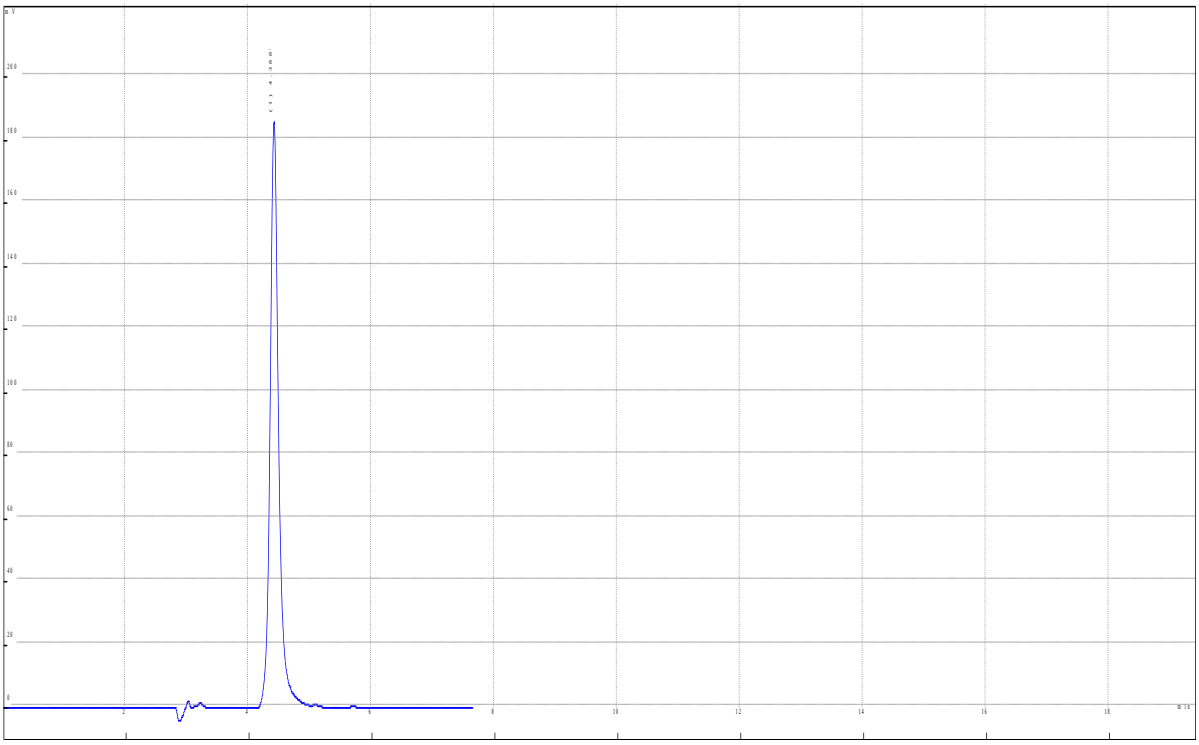
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.63min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	8.388	2122944	0.00	8976	1.19

30ppm set 2

Sample Name: Dapaglipflozin30ppm 02

Wavelength: 224nm

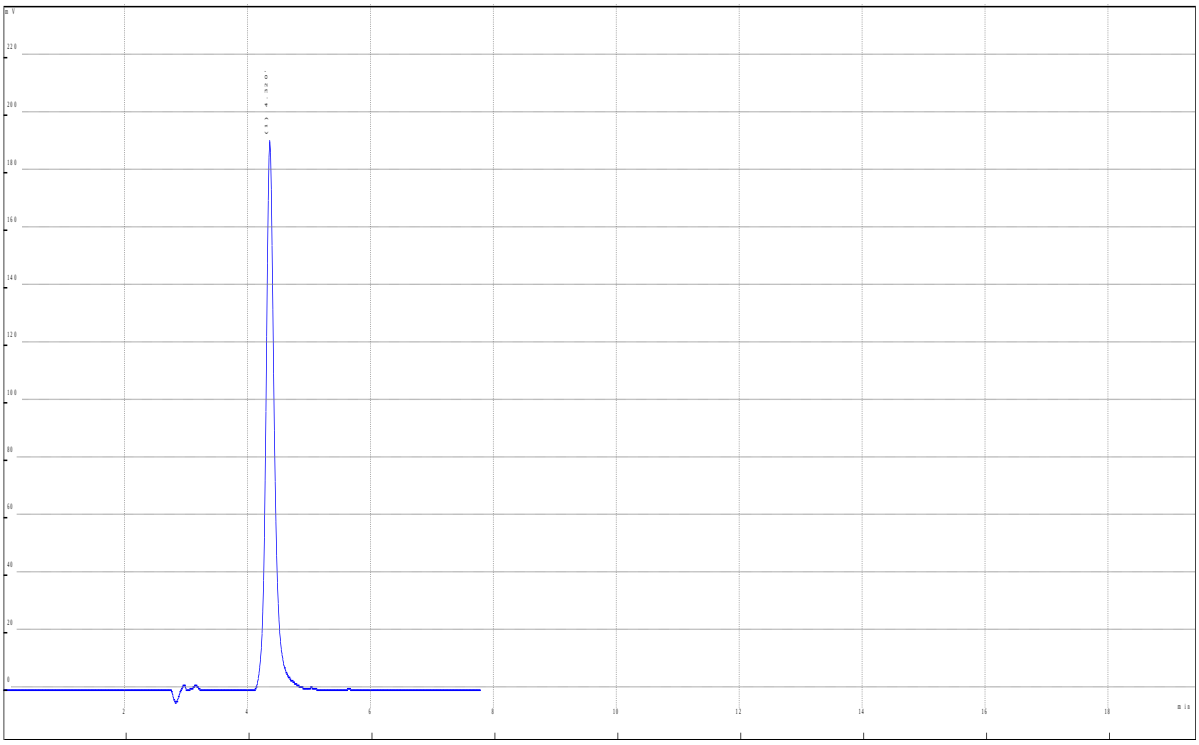
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.75min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.320	2121076	0.00	8731	1.19

30ppm set 3

Sample Name: Dapaglipflozin30ppm 03

Wavelength: 224nm

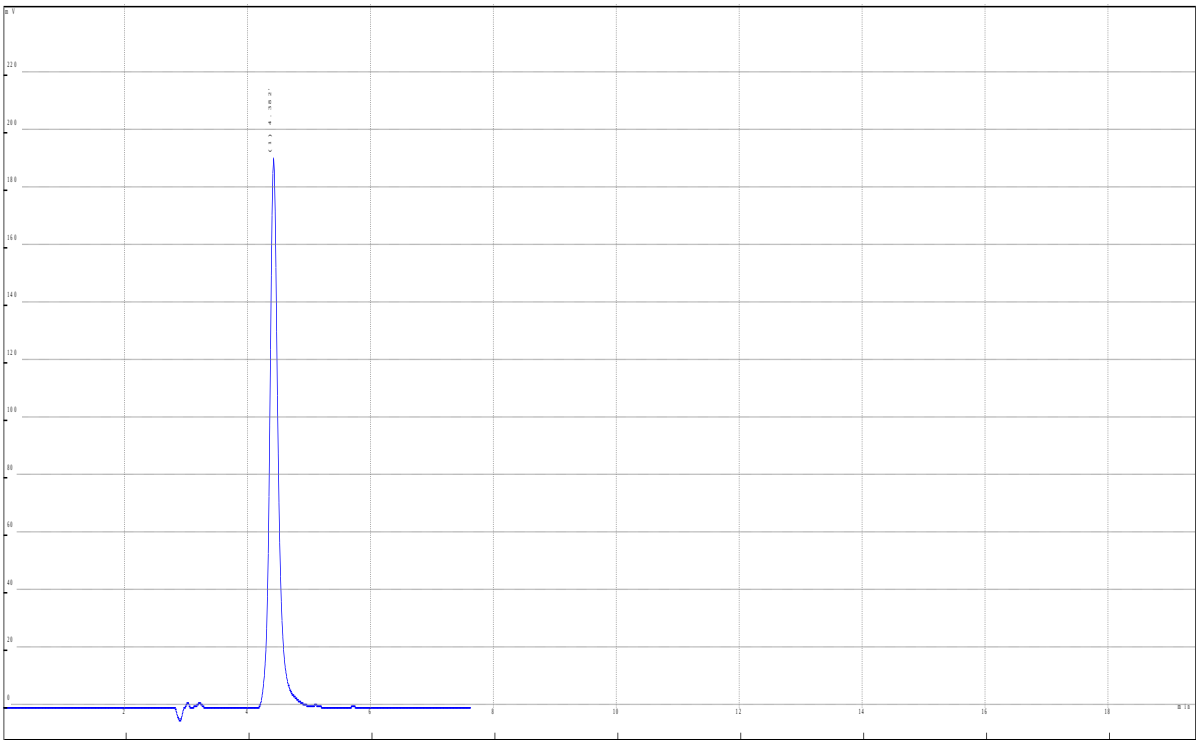
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.60min



Rank	RT(min)	Area	Resolution	T.plate no	Asymmetry
1	4.382	2124419	0.00	8.26	1.20

Table no. 13: intraday precision of dapaglipflozin.

Concentration	Area	
	Evening	Morning
30 ppm	2113917	2122944
	2118652	2121076
	2117587	2124419
	Mean	2119766
	%RSD	0.18%

- **LINEARITY**
- **10ppm**

Sample Name: Dapaglipflozin10ppm 01

Wavelength: 224nm

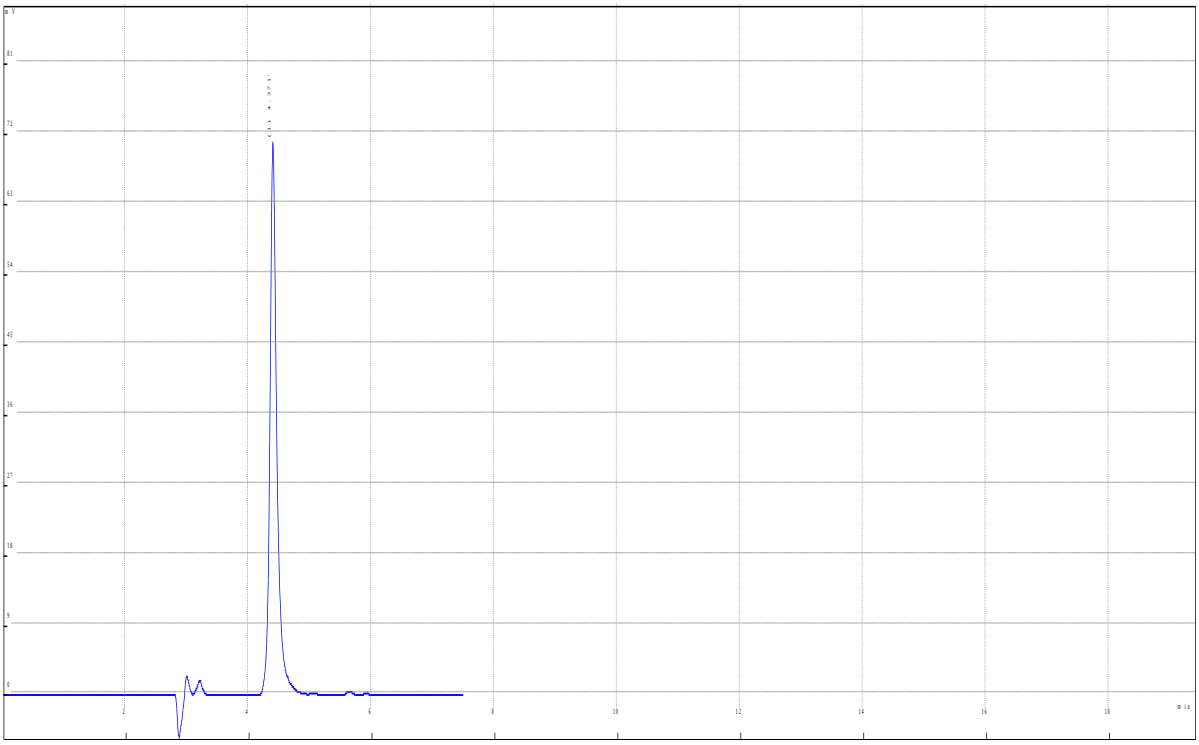
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.47min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.371	816101	0.00	8775	1.29

- **20ppm**

Sample Name: Dapaglipflozin 20ppm 01

Wavelength: 224nm

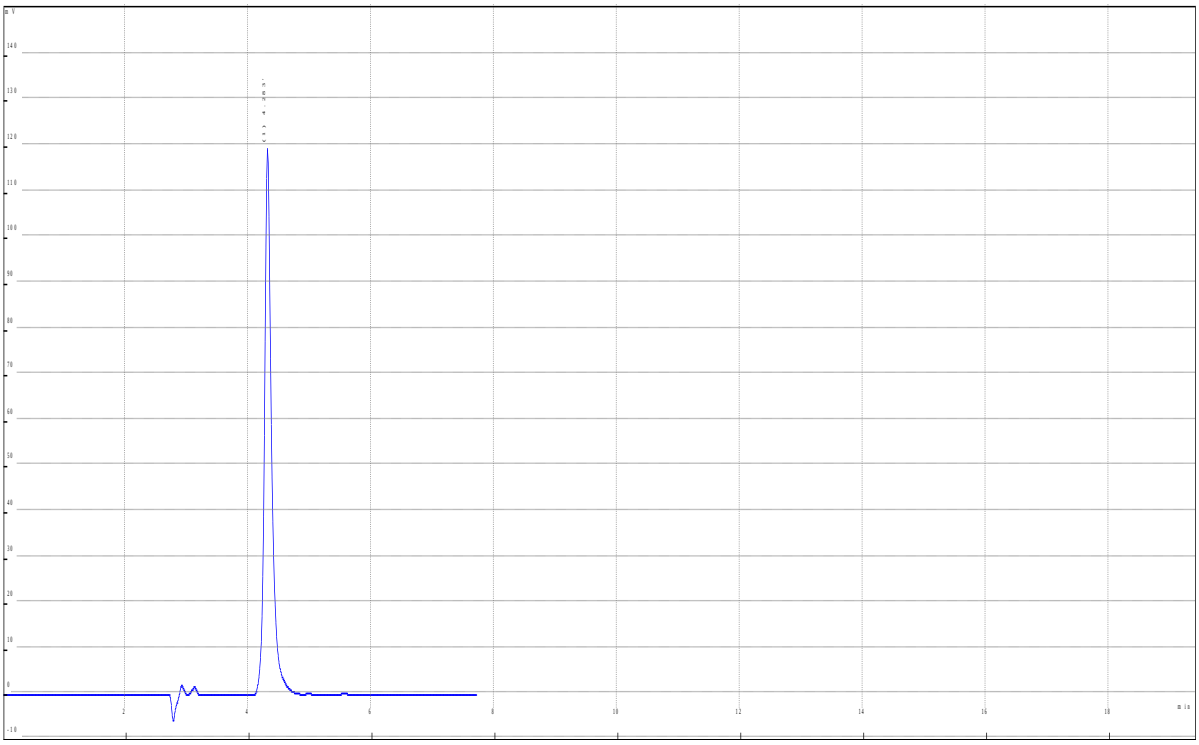
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.69min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.283	126331	0.00	8149	1.29

- **30ppm**

Sample Name: Dapaglipflozin 30ppm 01

Wavelength: 224nm

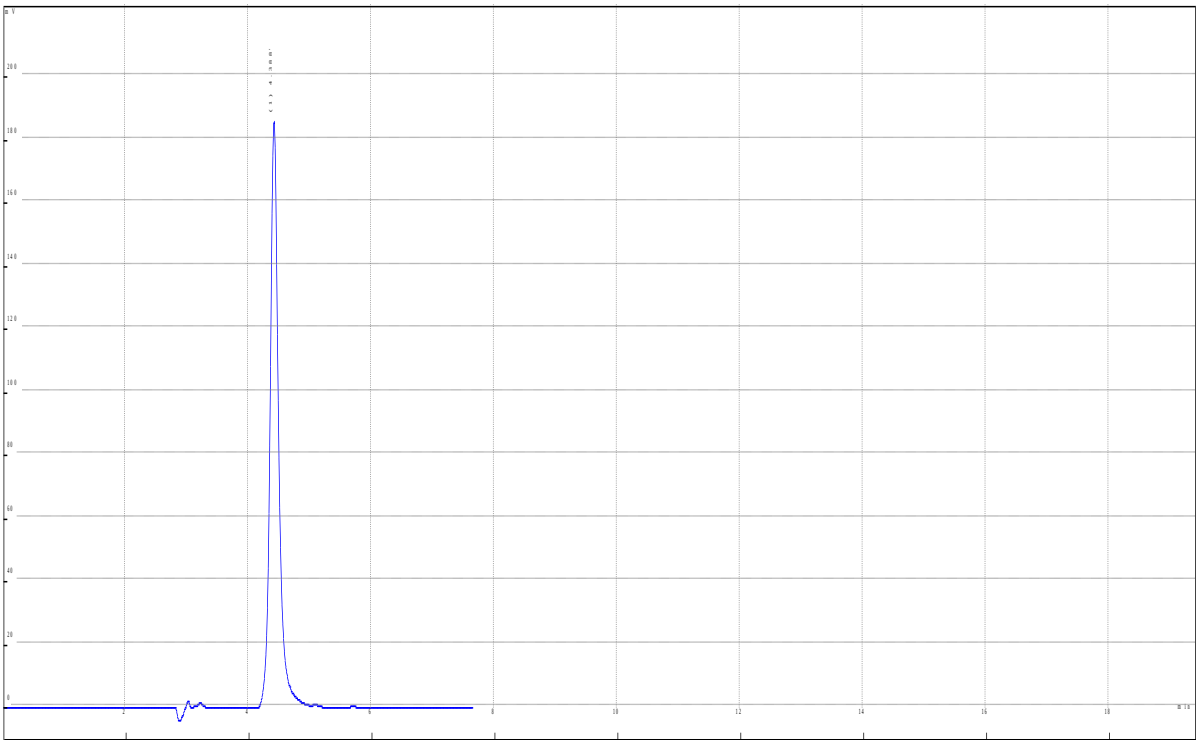
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.63min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.388	2122944	0.00	8976	1.19

- **40ppm**

Sample Name: Dapaglipflozin40ppm 01

Wavelength: 224nm

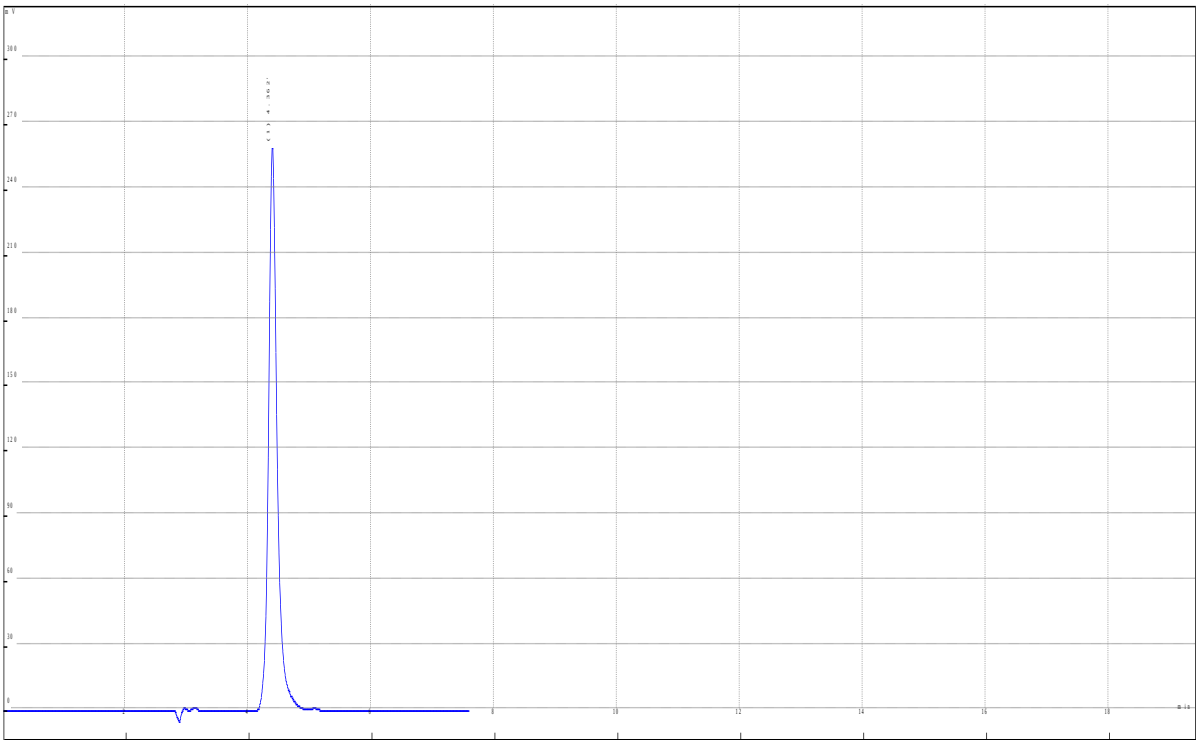
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.57min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.362	2806995	0.00	8914	1.23

- **50ppm**

Sample Name: Dapaglipflozin 50ppm 01

Wavelength: 224nm

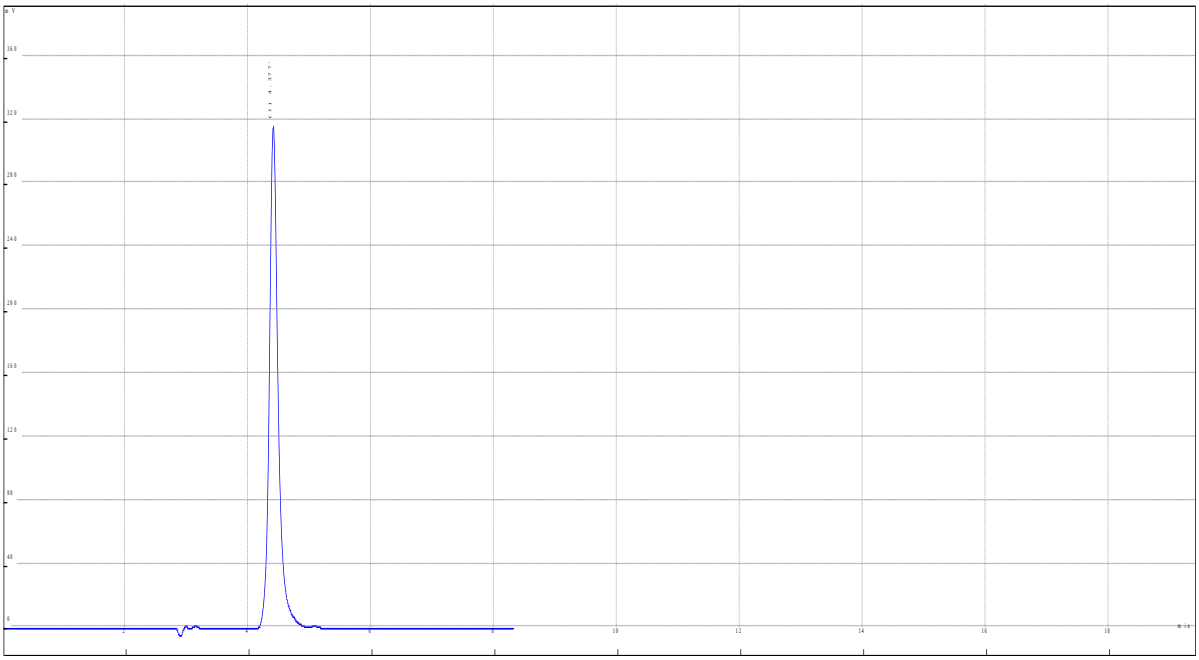
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.30min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.377	3467127	0.00	8848	1.23

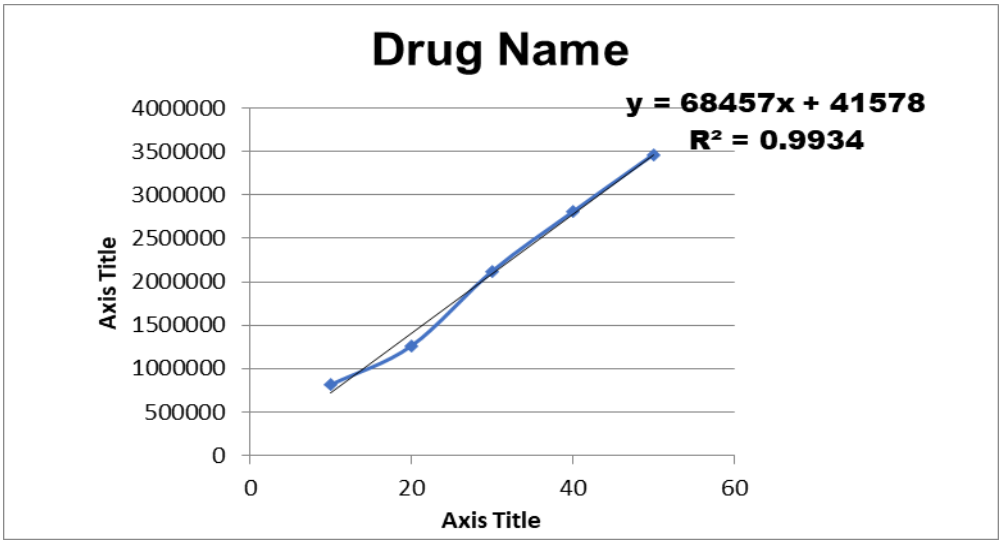


Figure. Graph of linearity.

Table no. 17: linearity of dapaglipflozin.

Z	Concentration(µg/ml)	Area
1	10	816101
2	20	1263317
3	30	2122944
4	40	2806995
5	50	3467127
Correlation coefficient	1	N/A
Slope(m)	68457	
Intercept(c)	41578	

- **Accuracy**
- **10ppm**

Sample Name: Dapaglipflozin10ppm 01

Wavelength: 224nm

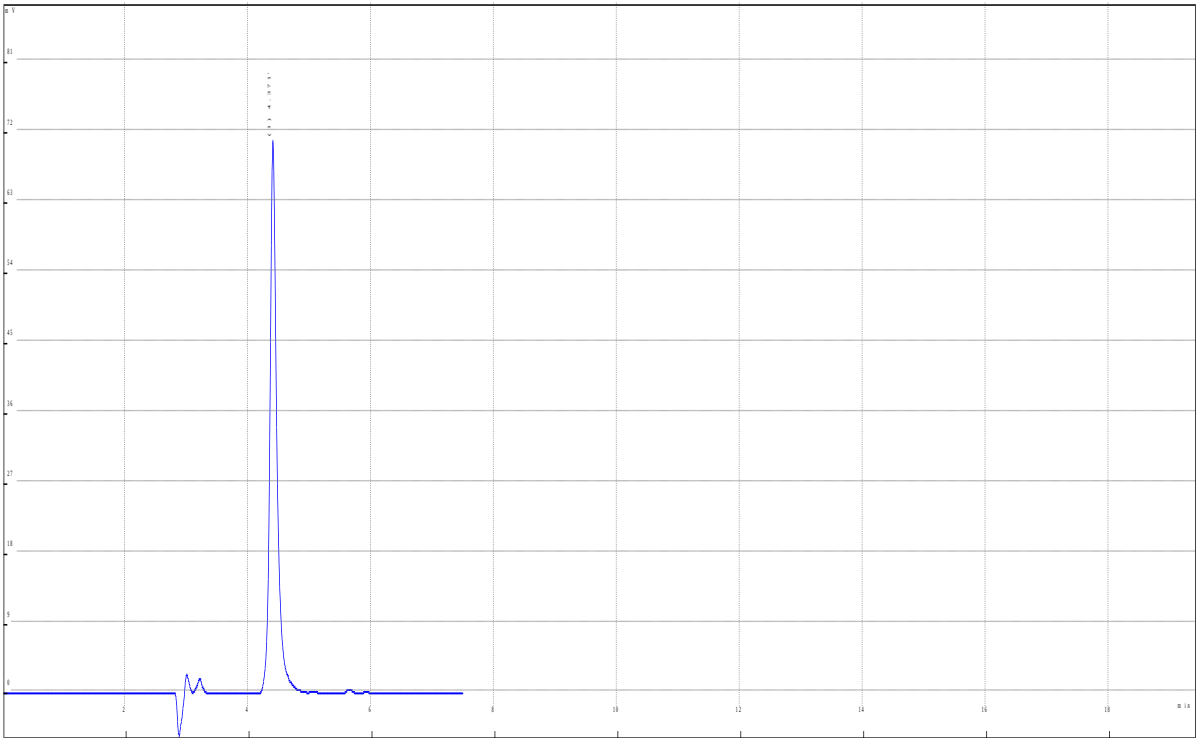
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.47min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.371	816101	0.00	8775	1.29

10ppm

Sample Name: Dapaglipflozin10ppm 02

Wavelength: 224nm

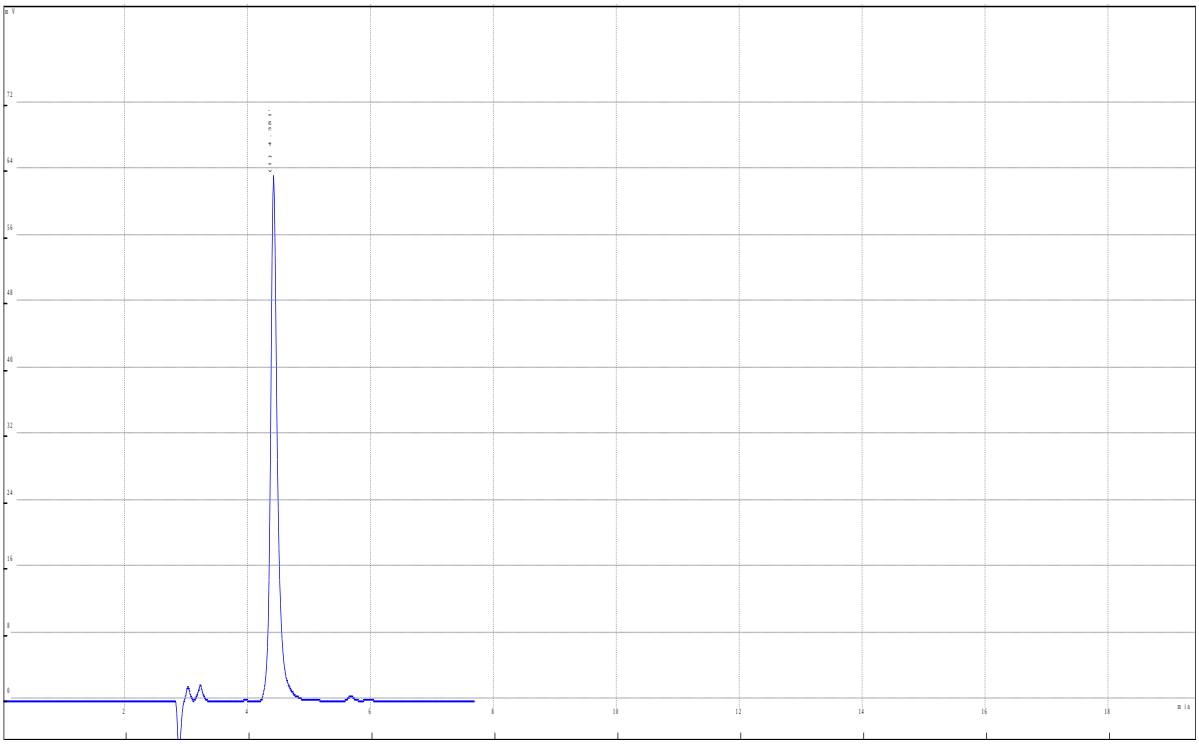
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.66min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.381	816205	0.00	8676	1.28

10ppm

Sample Name: Dapaglipflozin10ppm 03

Wavelength: 224nm

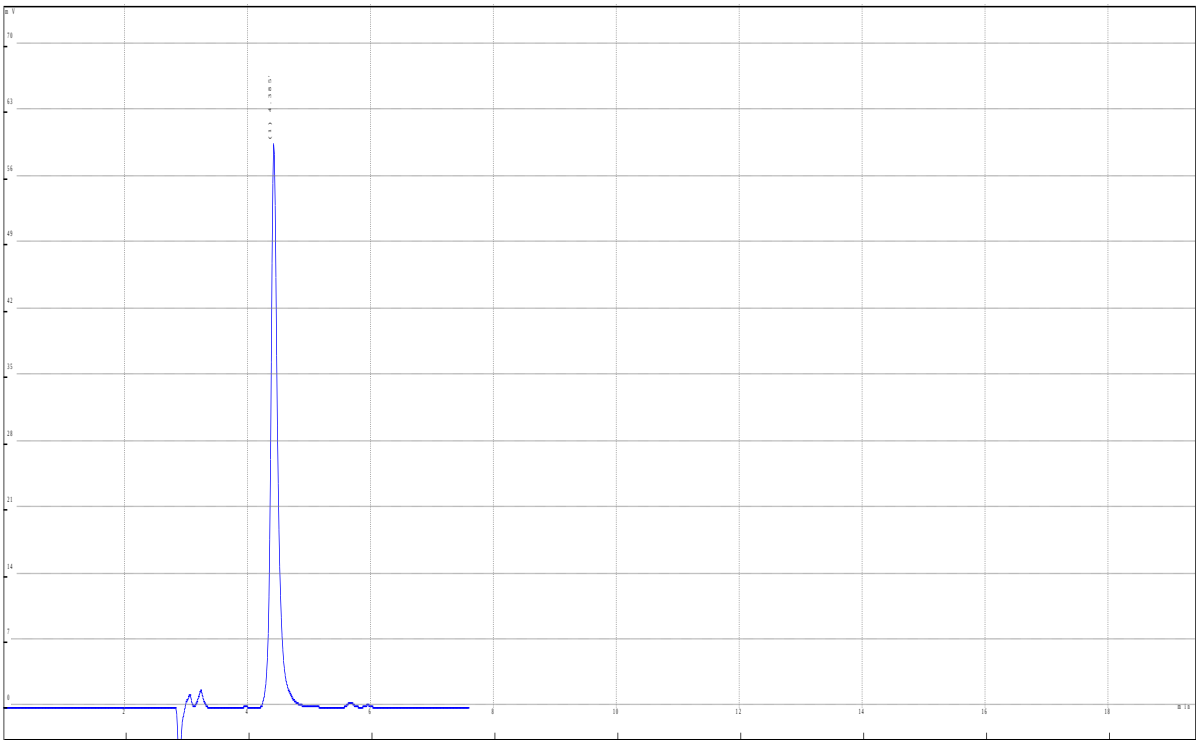
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.57min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.385	816047	0.00	8172	1.28

30ppm

Sample Name: Dapaglipflozin 30ppm 01

Wavelength: 224nm

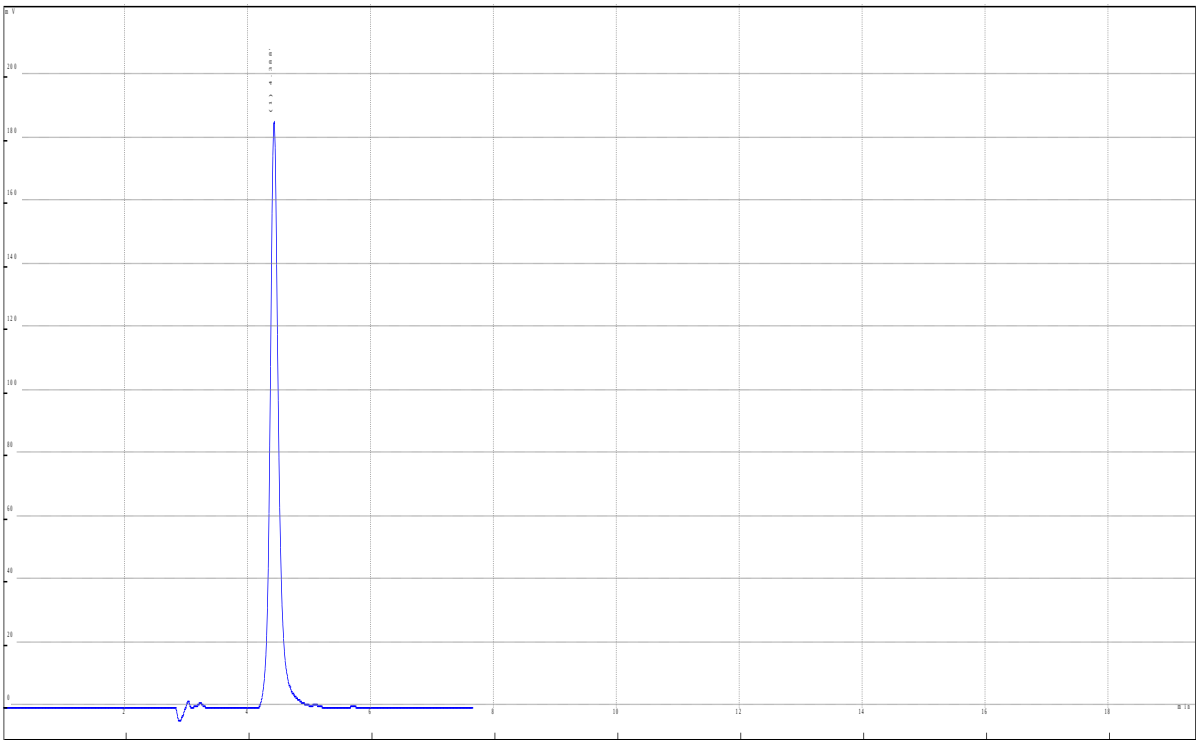
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.63min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.388	2122944	0.00	8976	1.19

30ppm

Sample Name: Dapaglipflozin30ppm 02

Wavelength: 224nm

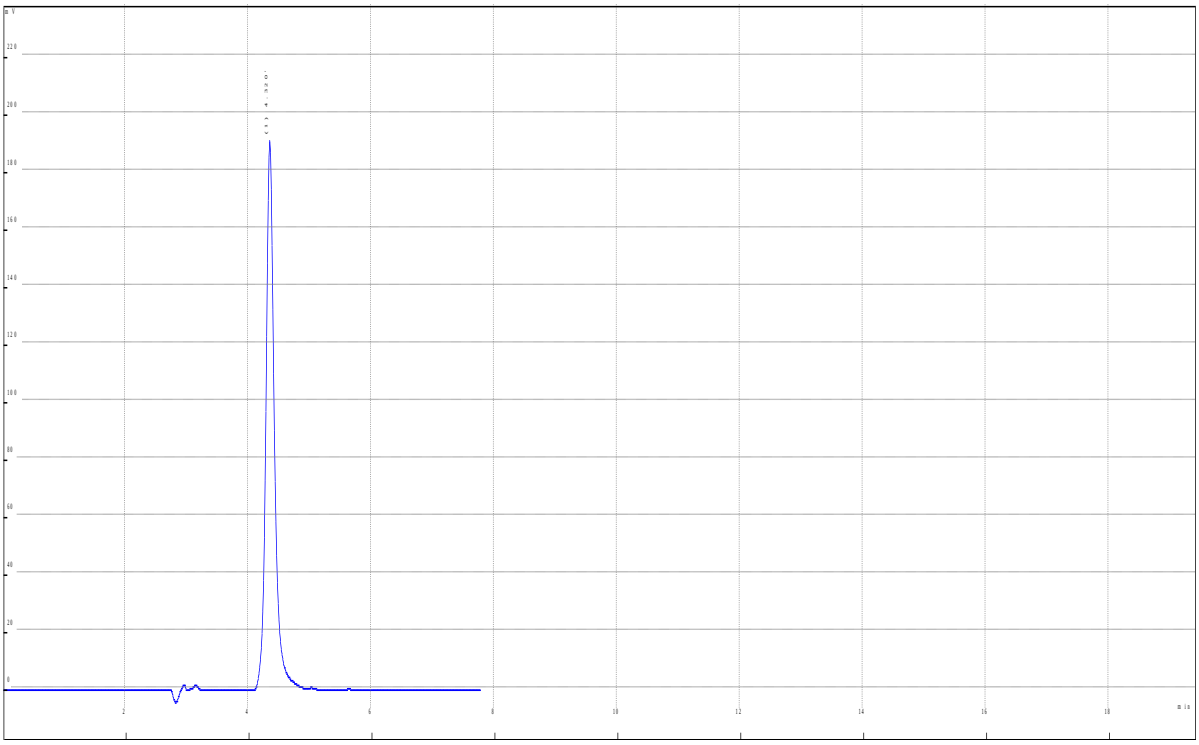
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.75min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.320	2121076	0.00	8731	1.19

30ppm

Sample Name: Dapaglipflozin30ppm 03

Wavelength: 224nm

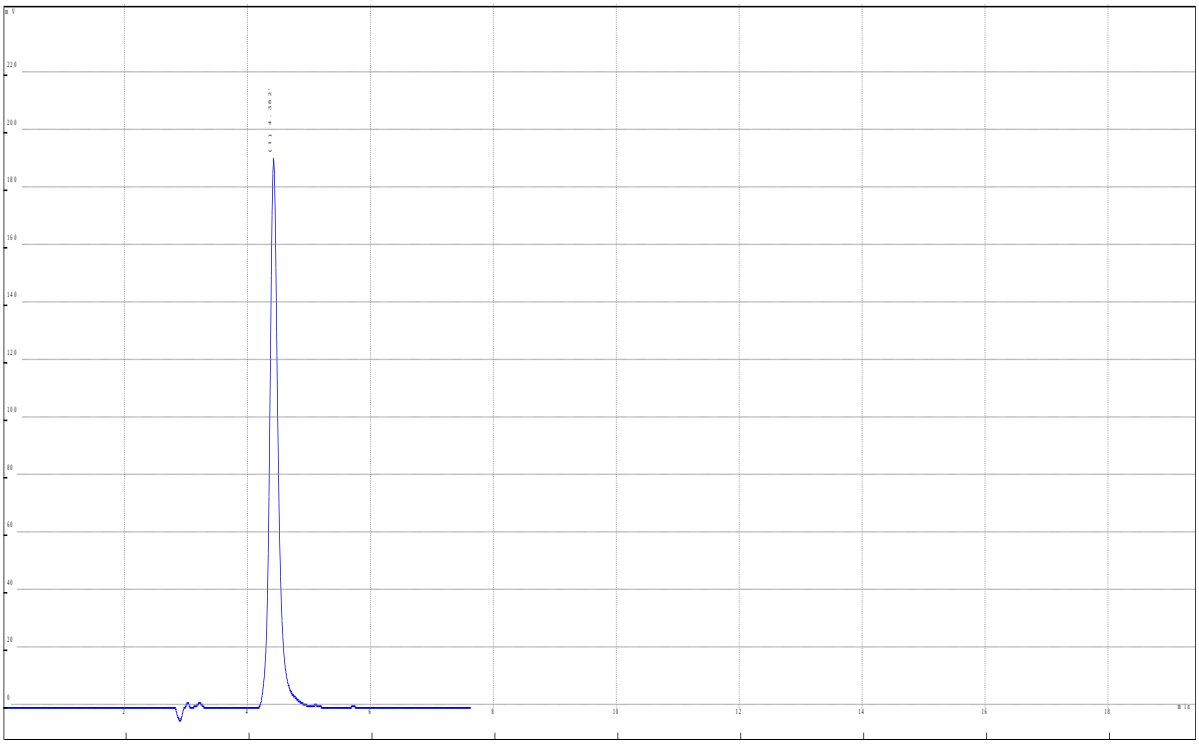
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.60min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.382	2124419	0.00	8026	1.20

50ppm

Sample Name: Dapaglipflozin 50ppm 01

Wavelength: 224nm

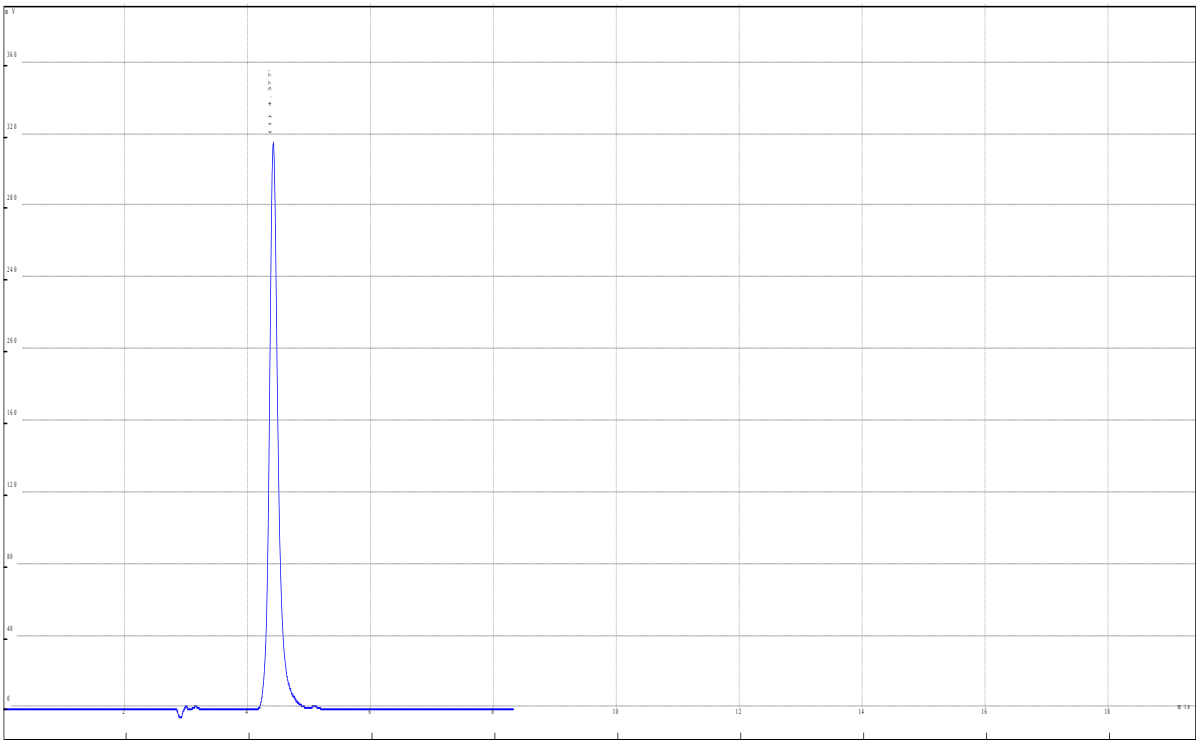
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.30min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.377	3467127	0.00	8848	1.23

50ppm

Sample Name: Dapaglipflozin 50ppm 02

Wavelength: 224nm

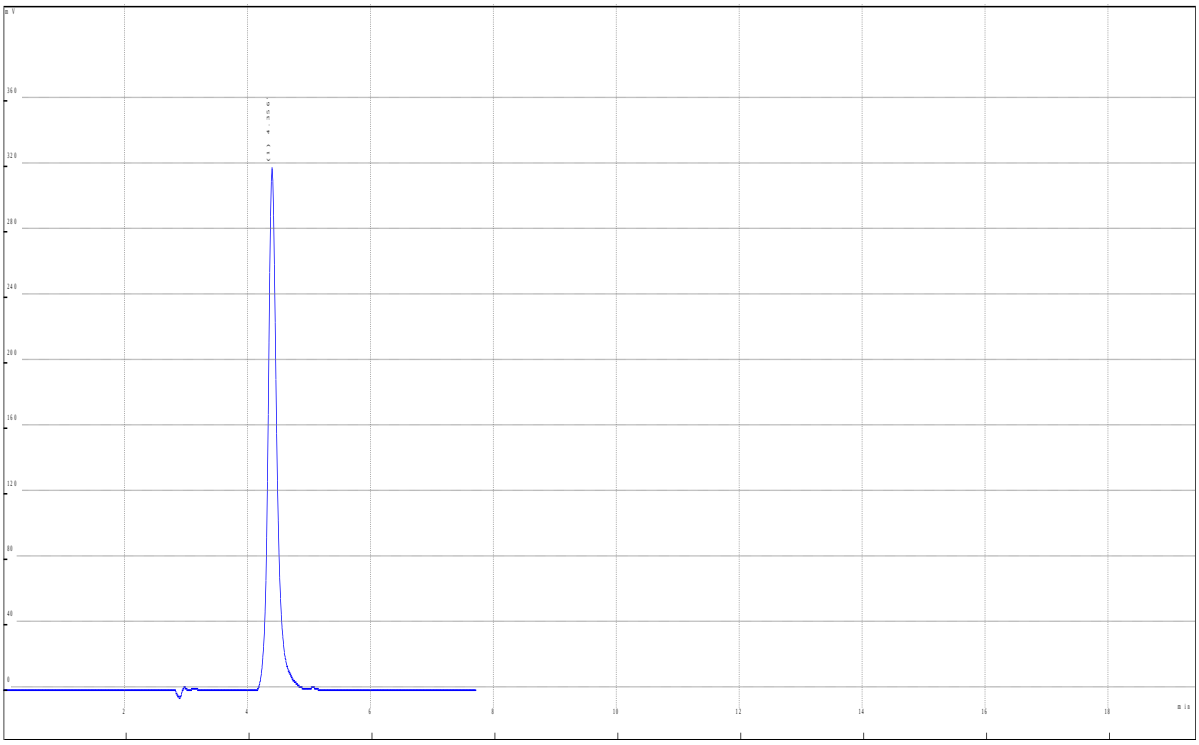
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.68min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.356	3464141	0.00	8890	1.23

50ppm

Sample Name: Dapaglipflozin 50ppm 03

Wavelength: 224nm

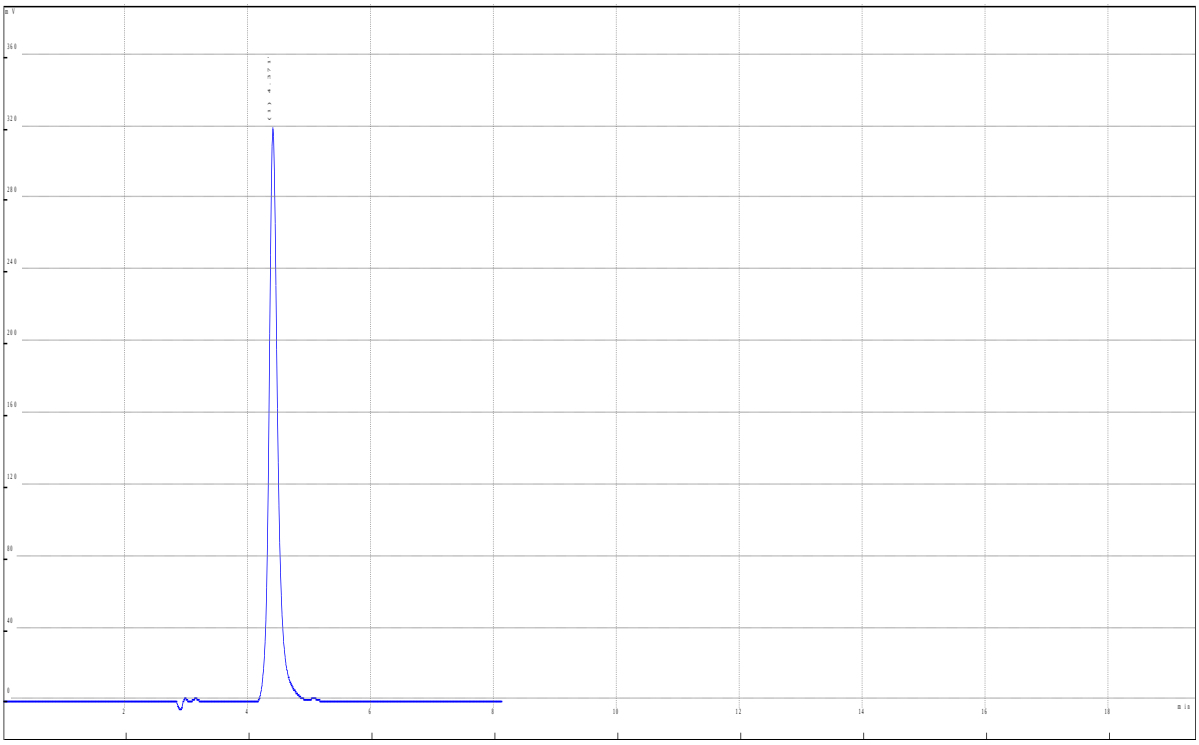
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.11min



Area	Resolution	T.plate no	Asymmetry
3463578	0.00	7906	1.24

Table no. 18 for accuracy of dapaglipflozin.

			Standard Deviation		Accuracy	Precision
Conc.	Conc.	Area	Mean	SD	%SD	%RSD
	10	816101				
1	10	816205	816117.6667	80.30774143	0.0098402	
	10	816047				
	30	2122944				
2	30	2121076	2122813	1675.345636	0.078921	0.035085169
	30	2124419				
	50	3467127				
3	50	3464141	3464948.667	1907.378917	0.0550478	
	50	3463578				

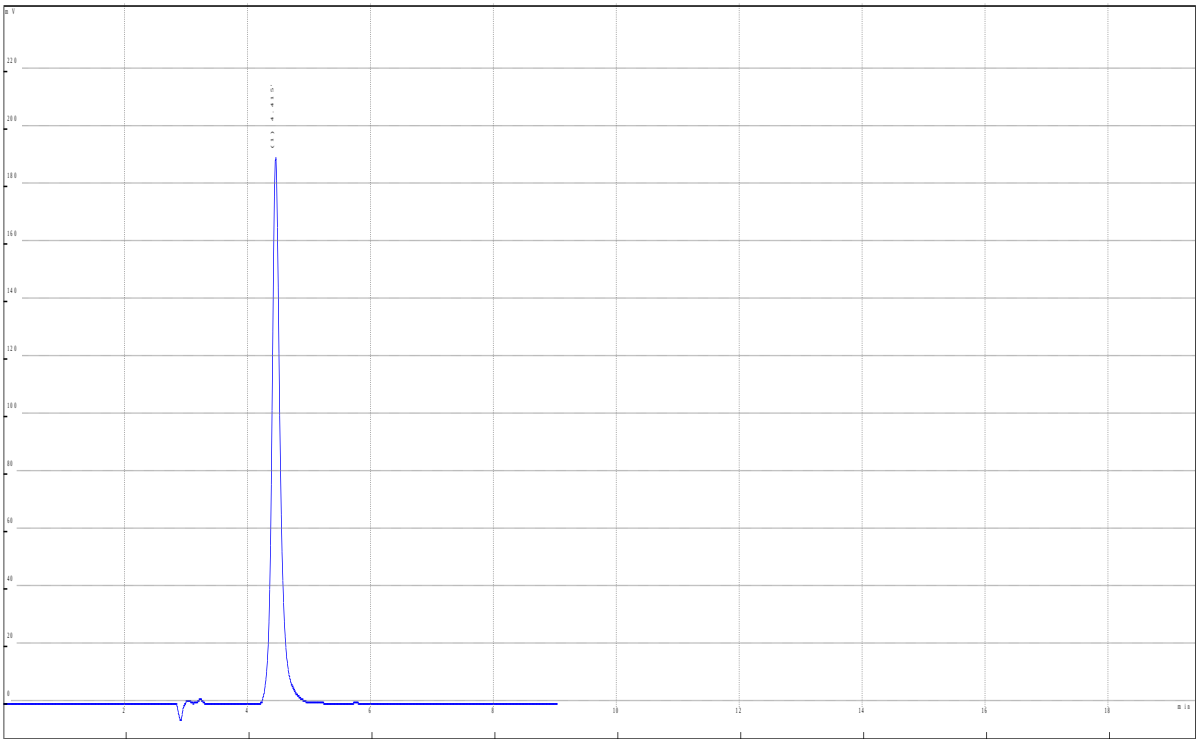
- **Limit: %SD and %RSD value should be less than 2%**

% recovery

Sample

- **50% recovery**
- Sample Name: Dapaglipflozin30ppm 50% Recovery
- Wavelength: 224nm
- Mobile Phase: Methanol:Water (85:15)
- Sample volume: 20µl

- Flow rate: 0.9 ml/min
- Pressure:9-10MPa
- Run time: 9.01min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.415	2117290	0.00	8676	1.21

100% recovery

Sample Name: Dapaglipflozin 40ppm 100% Recovery

Wavelength: 224nm

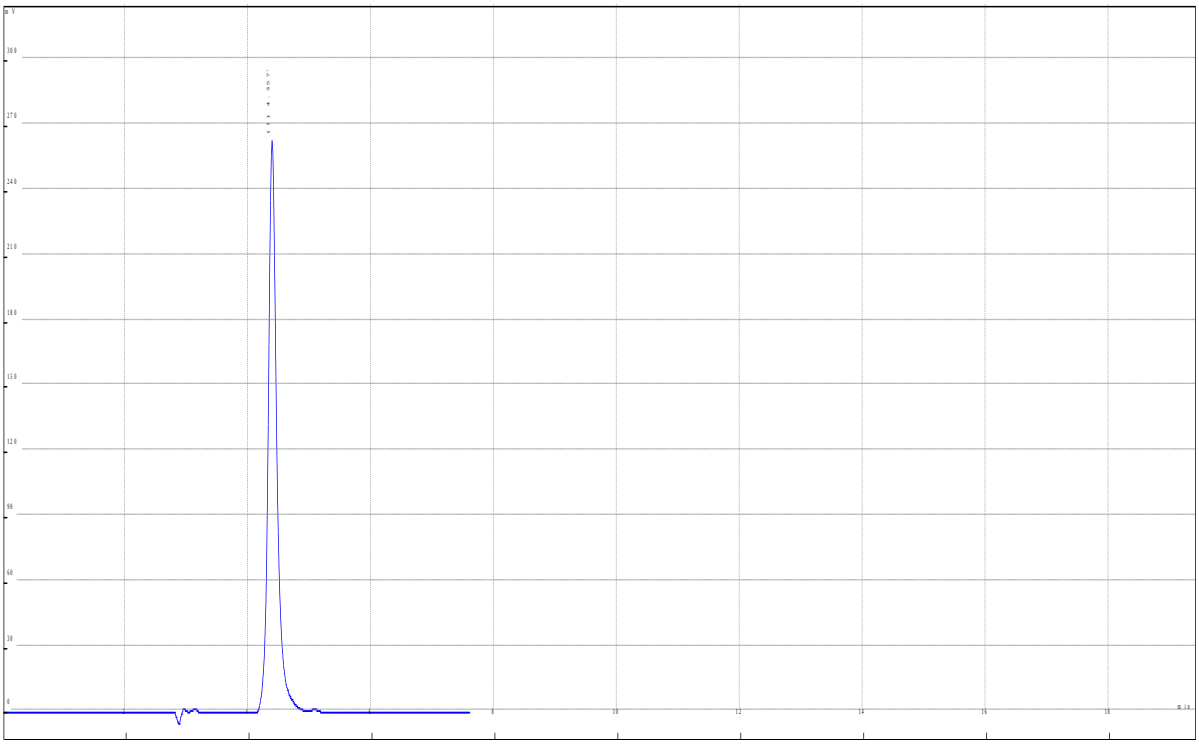
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.58min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.357	2807939	0.00	8694	1.22

150% recovery

Sample Name: Dapaglipflozin 50ppm 150% Recovery

Wavelength: 224nm

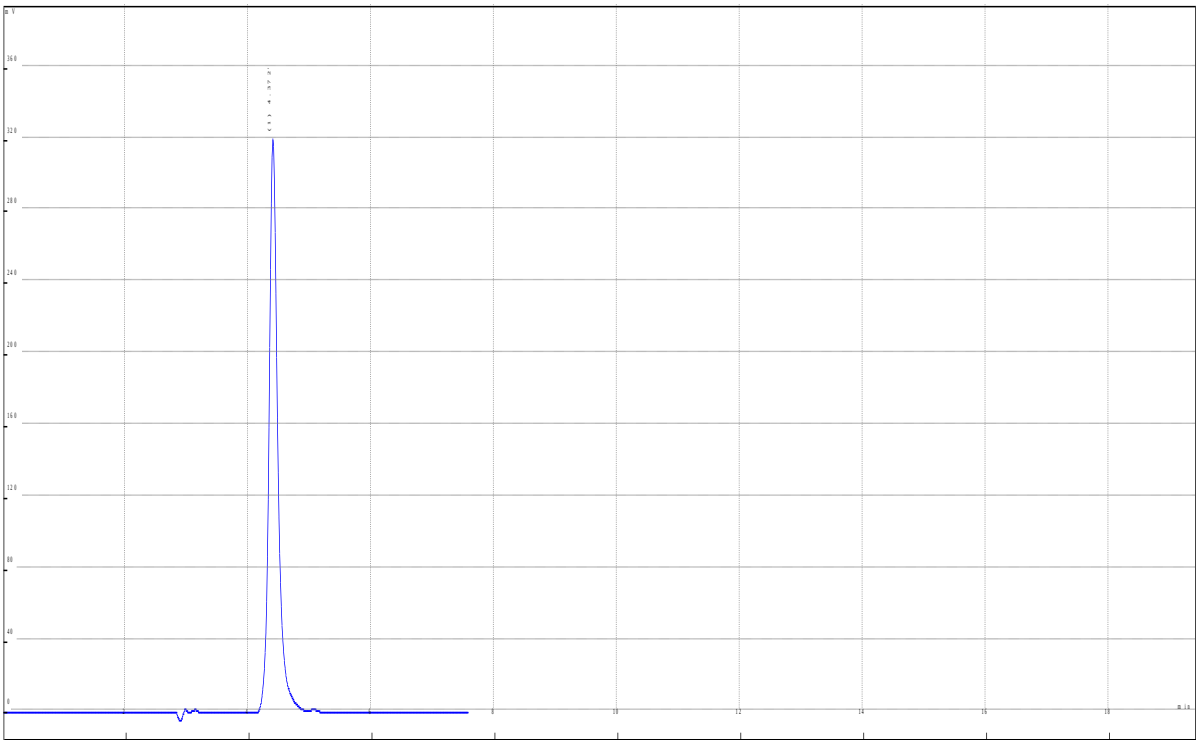
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.56min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.372	3463150	0.00	8439	1.23

Standard

30ppm

Sample Name: Dapaglipflozin 30ppm 01

Wavelength: 224nm

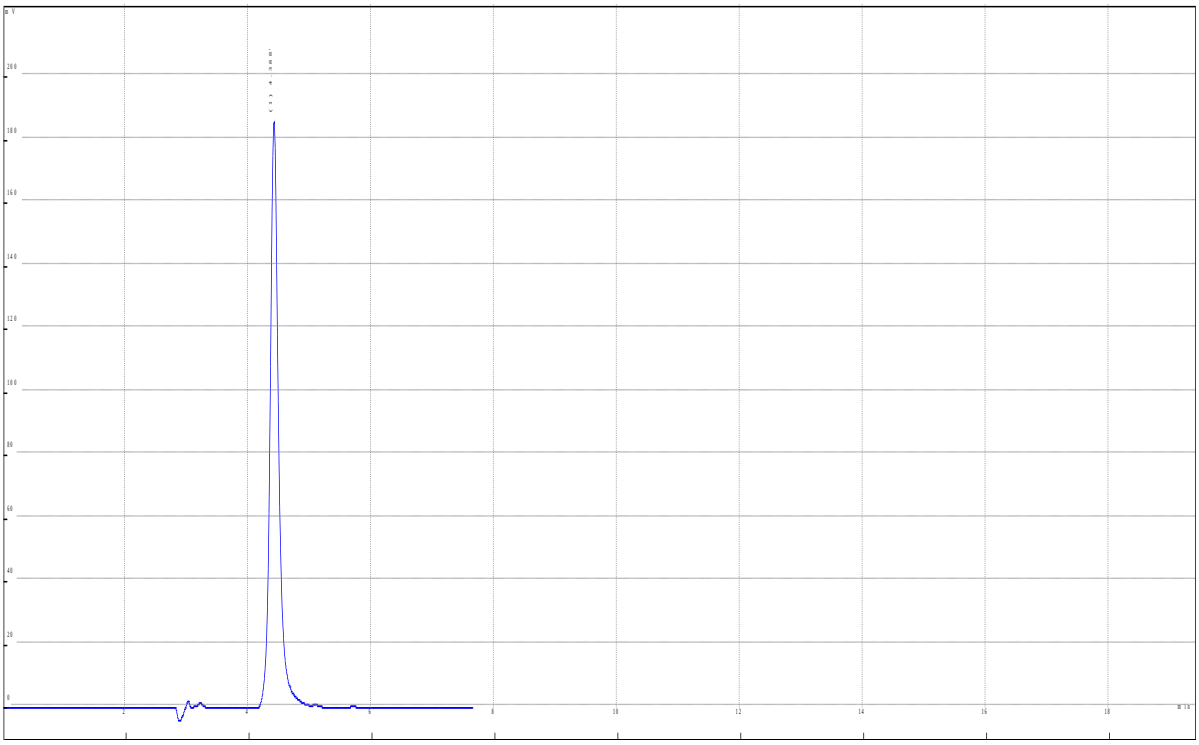
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.63min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.388	2122944	0.00	8976	1.19

40ppm

Sample Name: Dapaglipflozin40ppm 01

Wavelength: 224nm

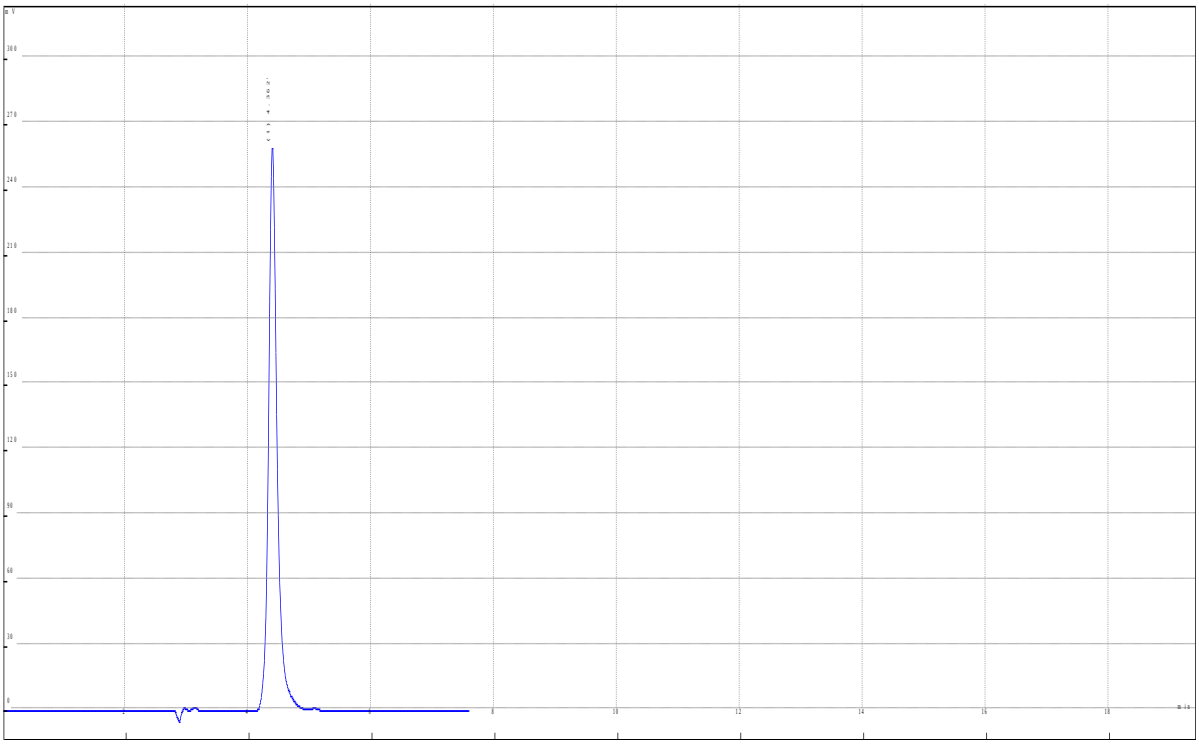
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.57min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.362	2806995	0.00	8914	1.23

50ppm

Sample Name: Dapaglipflozin 50ppm 01

Wavelength: 224nm

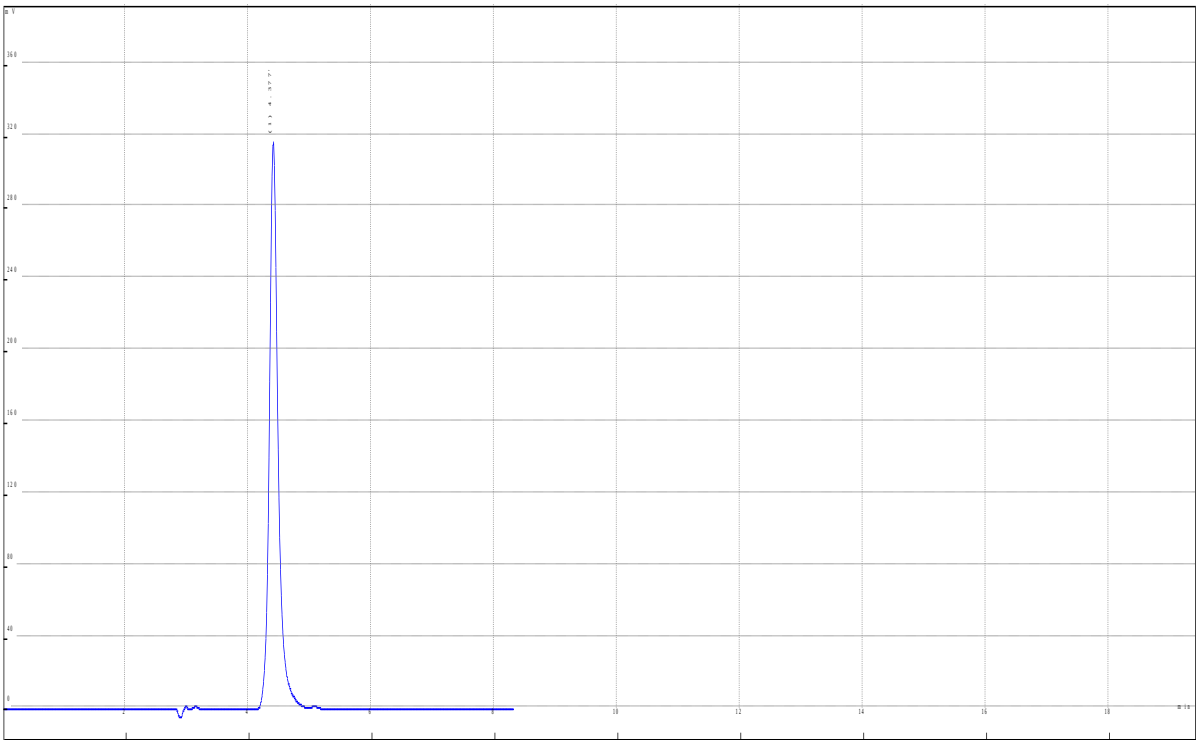
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.30min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.377	3467127	0.00	8848	1.23

Accuracy results obtained by % recovery

Table no. 19 for % recovery of dapaglipflozin.

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	2122944	2117290	99.73367173
2	100% Recovery	2806995	2807939	100.0336303
3	150% Recovery	3467127	3463150	99.88529408

- **Robustness**

A. Change in wavelength

1) 224 nm

Sample Name: Dapaglipflozin 20ppm 01

Wavelength: 224nm

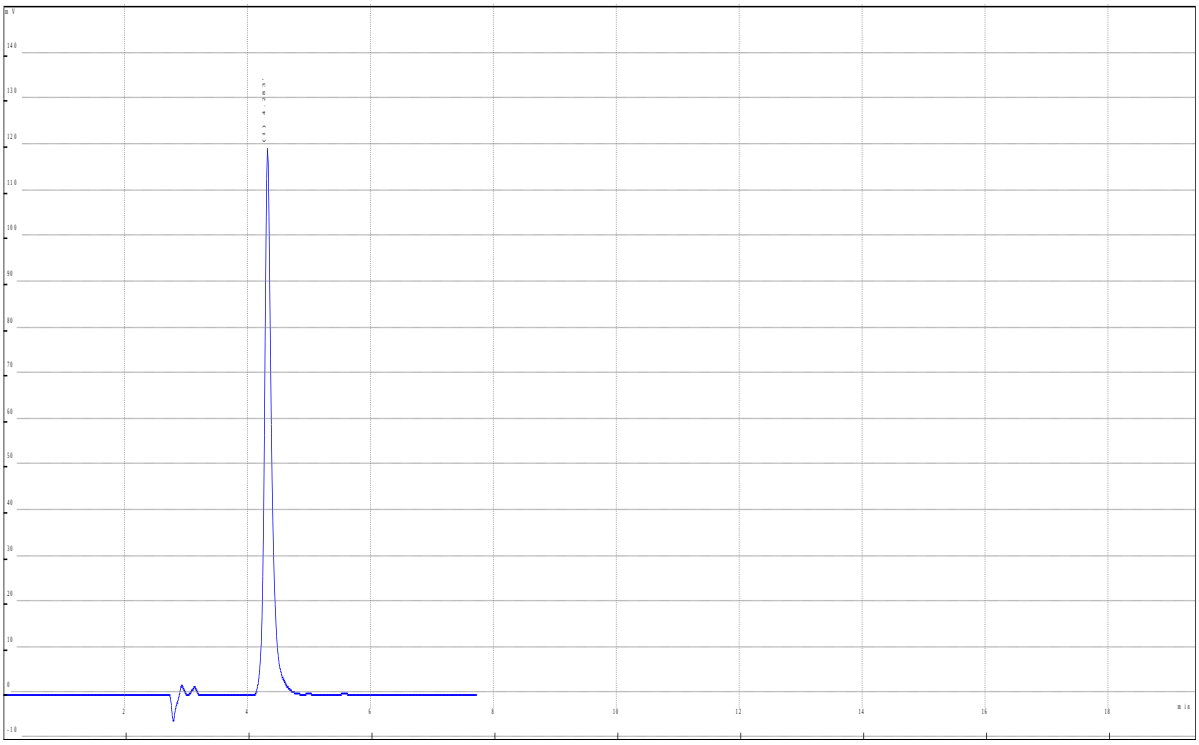
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.69min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.283	126331	0.00	8149	1.29

2) 222 nm

Sample Name: Dapaglipflozin20ppm 02

Wavelength: 222nm

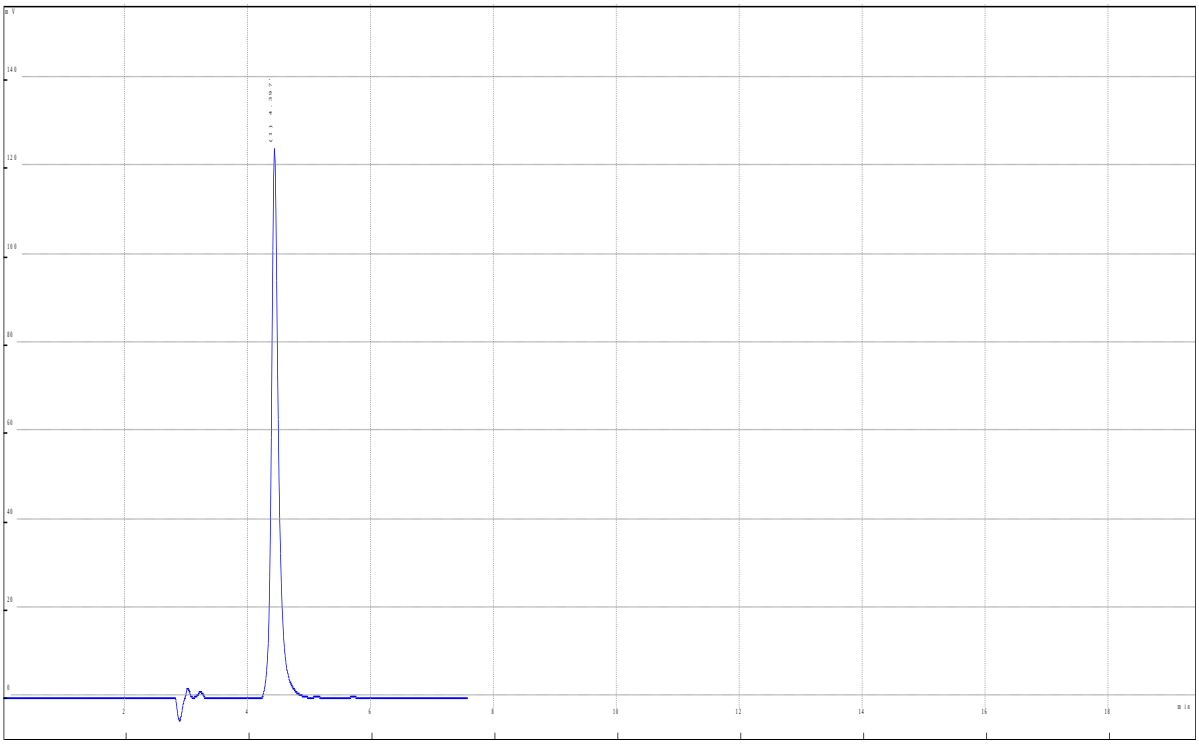
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.55min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.397	1460227	0.00	8562	1.29

3) 226 nm

Sample Name: Dapaglipflozin20ppm 03

Wavelength: 226nm

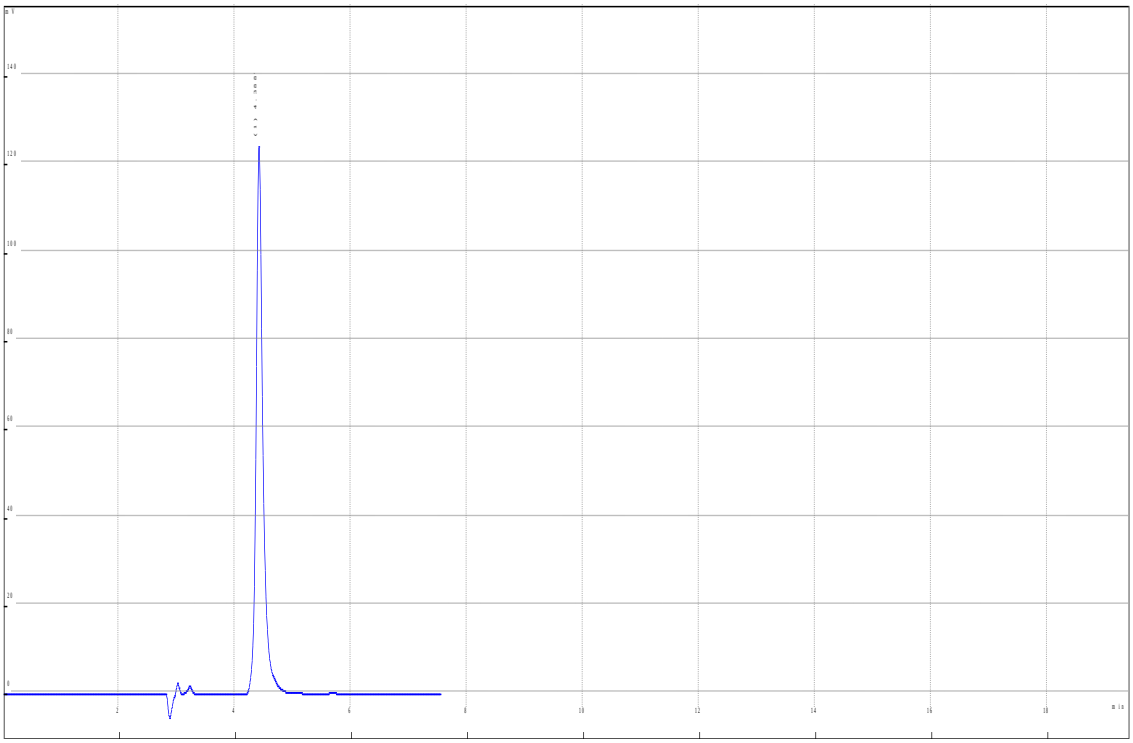
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.53min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.388	1458753	0.00	8872	1.29

Conc.	Conc.	Area	Mean	SD	%SD
	20	1460227			
1	20	1263317	1394099	113263	8.12445402
	20	1458753			

B. Change in flow rate

1) 0.9 ml/min

Sample Name: Dapaglipflozin 20ppm 01

Wavelength: 224nm

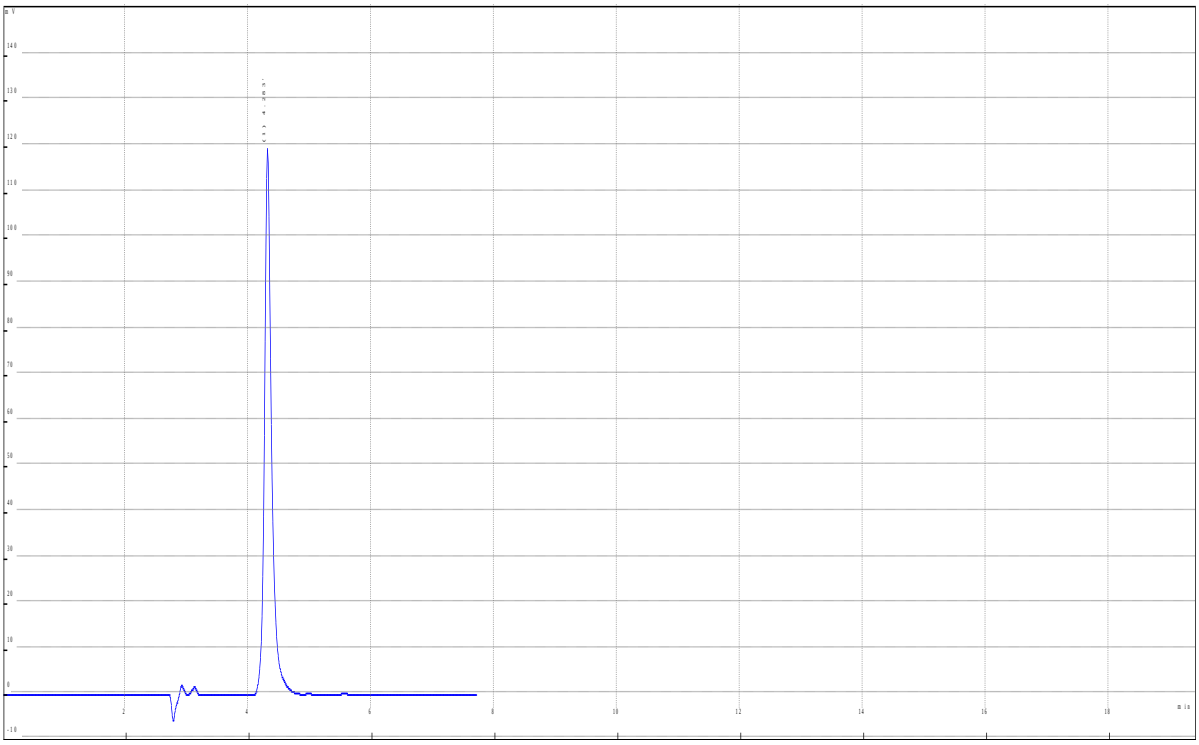
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.69min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.283	1263317	0.00	8149	1.29

2) 0.8 ml/min

Sample Name: Dapaglipflozin20ppm

Wavelength: 224nm

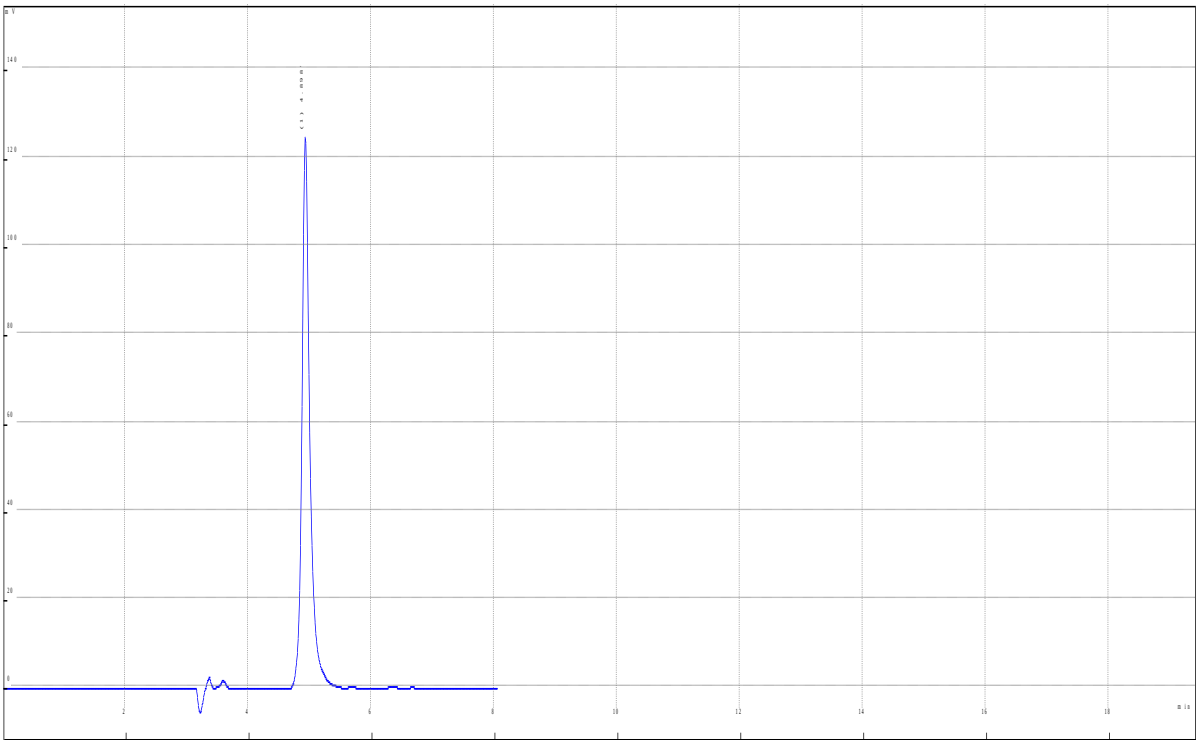
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.8 ml/min

Pressure:9-10MPa

Run time: 8.03min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.896	1465273	0.00	8749	1.29

3) 1.0 ml/min

Sample Name: Dapaglipflozin20ppm

Wavelength: 224nm

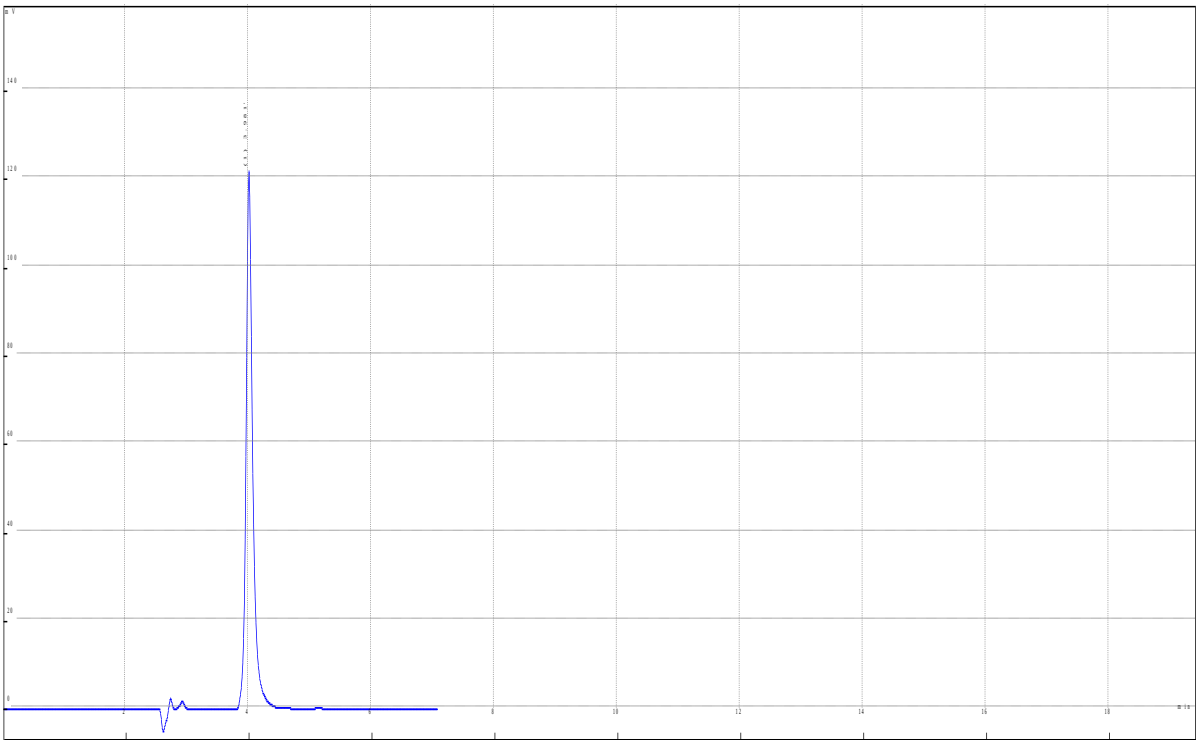
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 1.0 ml/min

Pressure:9-10MPa

Run time: 7.06min



RT(min)	Area	Resolution	T.plate no	Asymmetry
3.981	1461898	0.00	8684	1.28

Conc.	Conc.	Area	Mean	SD	%SD
	20	1263317			
1	20	1465273	1396829	115637	8.27856225
	20	1461898			

RUGGEDNESS

1) 10 ppm

Sample Name: Dapaglipflozin10ppm 04

Wavelength: 224nm

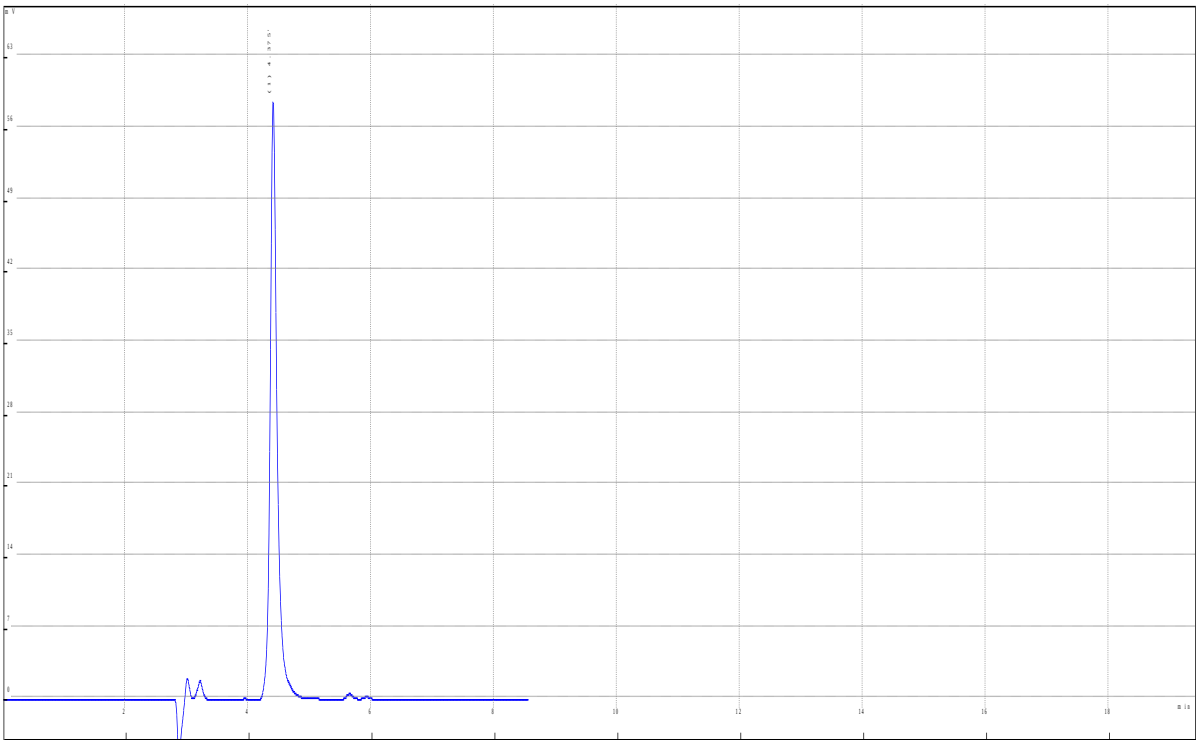
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.53min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.375	816355	0.00	8455	1.28

2) 20ppm

Sample Name: Dapaglipflozin20ppm 04

Wavelength: 224nm

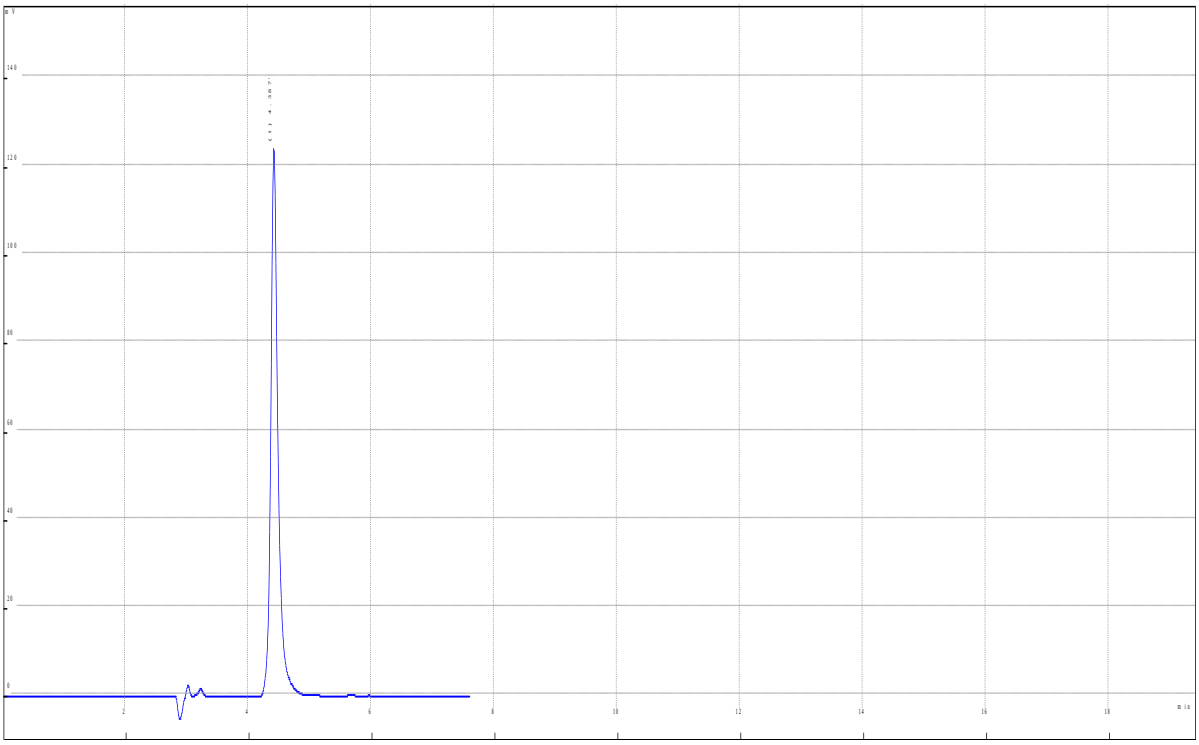
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.58min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.387	1462378	0.00	8540	1.29

3) 30ppm

Sample Name: Dapaglipflozin30ppm 04

Wavelength: 224nm

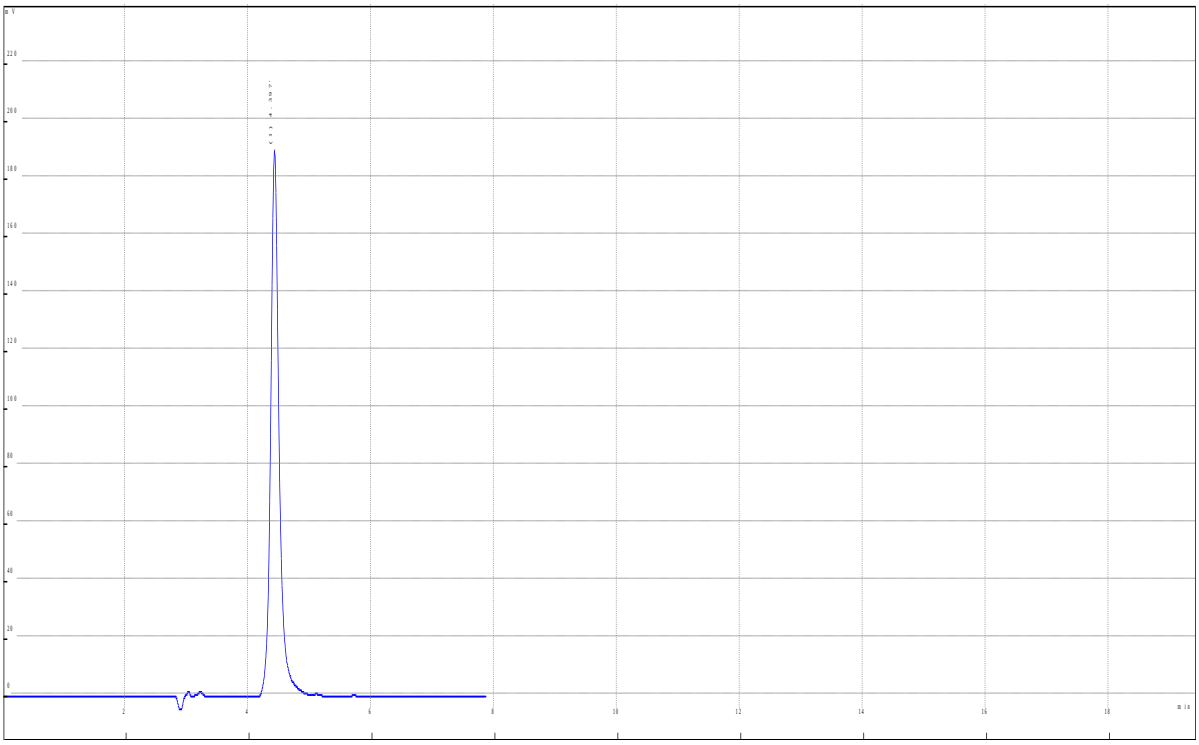
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.85min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.397	2123123	0.00	8068	1.20

4) 40 ppm

Sample Name: Dapaglipflozin 40ppm 04

Wavelength: 224nm

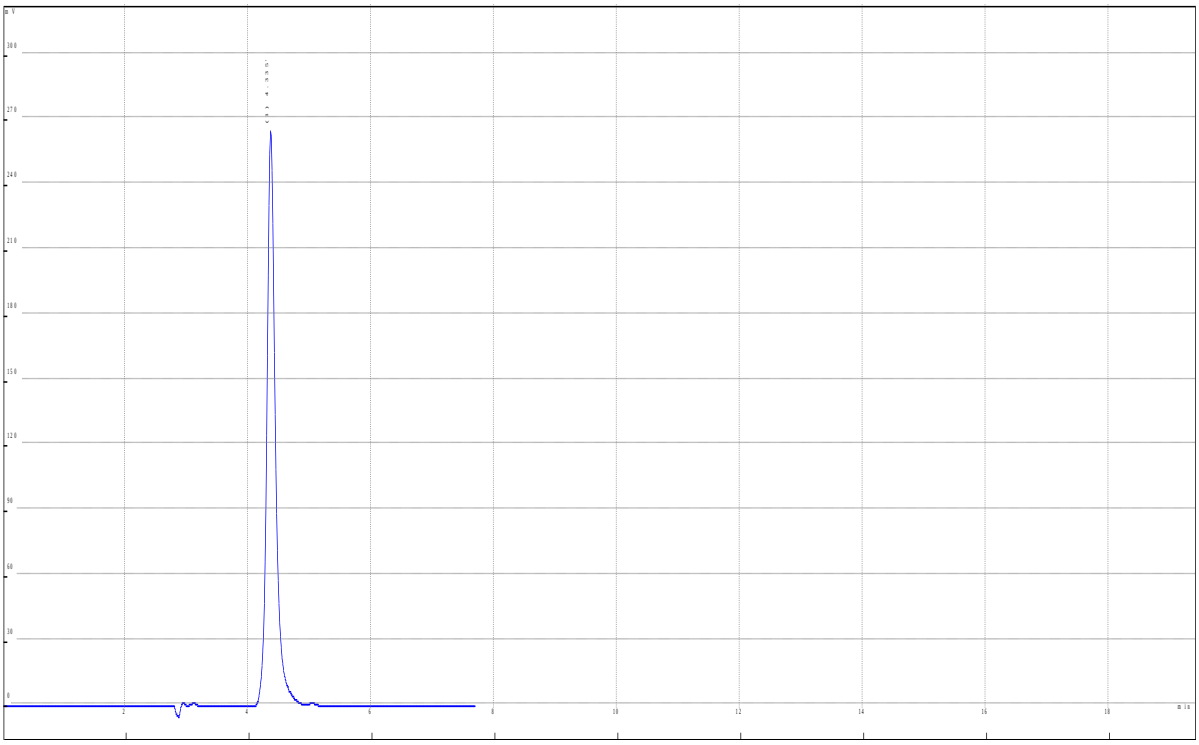
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.67min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.335	2804672	0.00	8926	1.23

5) 50 ppm

Sample Name: Dapaglipflozin 50ppm 04

Wavelength: 224nm

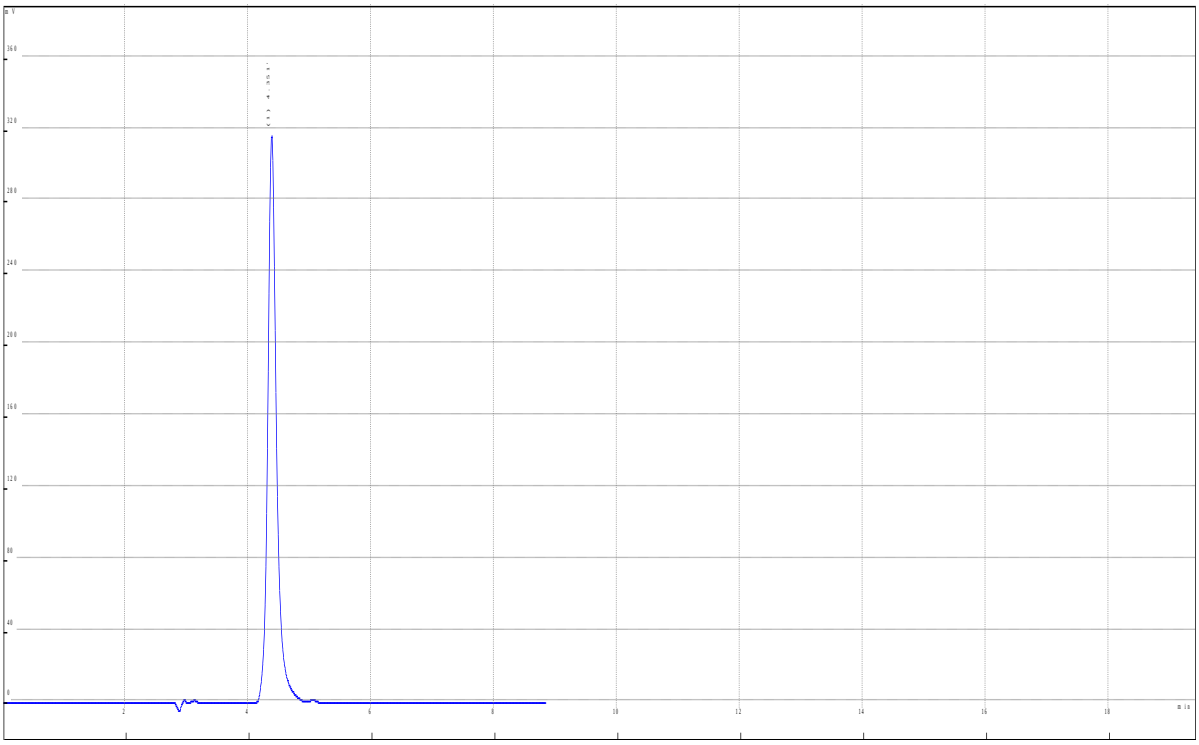
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

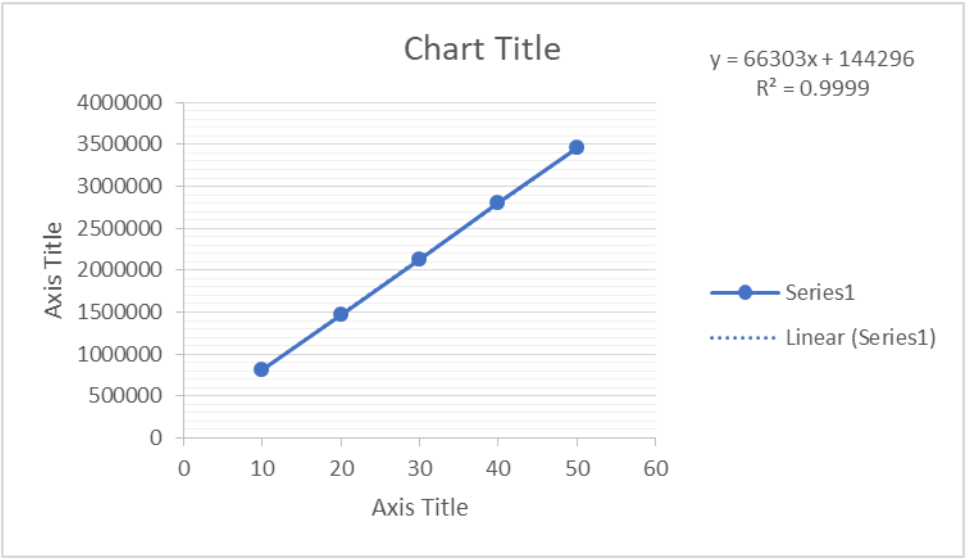
Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.82min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.351	3460335	0.00	7929	1.23



Sr.no	Conc	Area
1	10	816355
2	20	1462378
3	30	2123123
4	40	2804672
5	50	3460336

A. limit of detection (LOD)

It may be calculated based on standard deviation of the response and slope of the curve

LOD=3.3(SD)/S

Where,

SD=standard deviation

S=slope

Calculation of dapaglipflozin

LOD: 3.3 x 1675.345636/68457

LOD: 0.80761 µg/ml

LOD of dapaglipflozin is 0.80761µg/ml

B. Limit of Quantification (LOQ)

It may be calculated based on standard deviation (SD) of the response and slope of the curve (S)

LOQ=10 (SD)/S

S/N=10/1

Where,

SD=standard deviation

S=slope

Calculation of dapaglipflozin

LOQ=10 x 1675.345636/68457

LOQ=0.24473 µg/ml

LOQ of dapaglipflozin is 0.24473µg/ml

Name	LOD (µg/ml)	LOQ (µg/ml)
Dapaglipflozin	0.80761	0.24473

C. forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. all solutions for use in stress studies were prepared at an initial concentration of 1mg/ml of dapaglipflozin hydrochloride and refluxed for 30 min at 60^0c. all

samples were then diluted in mobile phase to give a final concentration of 50 µg/ml and filtered before injection.

1) hydrolytic degradation under acidic condition

Acidic degradation was performed by treating the drug solution(1mg/ml) with 0.1 N hydrochloric acid for 30 min in a thermostat maintained at 60^0c,cooled and then stressed sample was neutralized and diluted with mobile phase as per requirement before injecting into the HPLC system.

2) hydrolytic degration under alkaline condition

Alkaline degradation was performed by treating the drug solution(1mg/ml) with 0.1 N NAOH for 30 min in a thermostat. maintained at 60^0c,cooled and then stressed sample was neutralized and diluted with mobile phase for injecting into the HPLC system.

3) oxidative degradation

Oxidation degradation was performed by treating the drug solution (1mg/ml) with 3% H2O2 for 30 min in a thermostat maintained at 60^0c, cooled and then stressed sample was diluted with mobile phase and injected into the HPLC system.

4) photochemical degradation

The drug solution(1mg/ml) for photostability testing was exposed to UV light chamber for 24 hrs and then analysed.

D.chromatograms of forced degradation studies

- **Sample dapaglipflozin 50 ppm treated with 0.1 N HCl at 60^0c**

Sample Name: Dapaglipflozin 50ppm treated with 0.1N HCl at 60C for 30min

Wavelength: 224nm

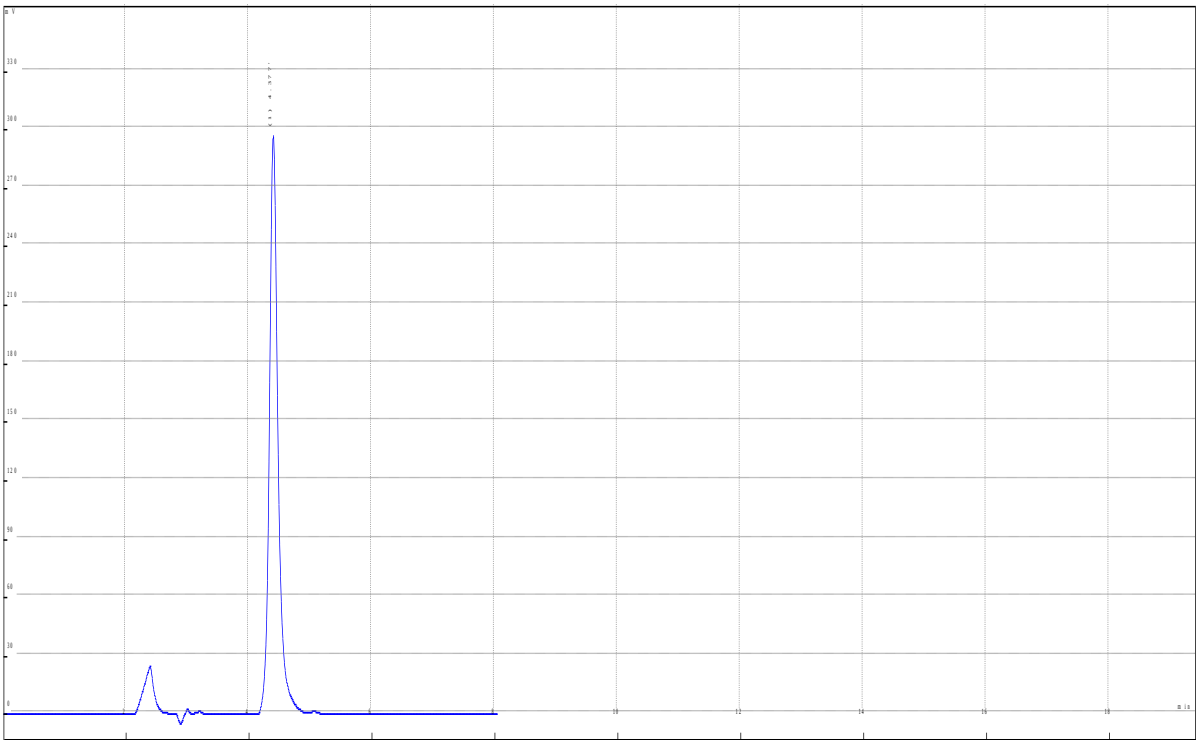
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.03min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.377	3158509	0.00	8077	1.23

- **Dapaglipflozin 50ppm treated with 0.1N NaOH at 60^0c for 30min**

Sample Name: Dapaglipflozin 50ppm treated with 0.1N NaOH at 60C for 30min

Wavelength: 224nm

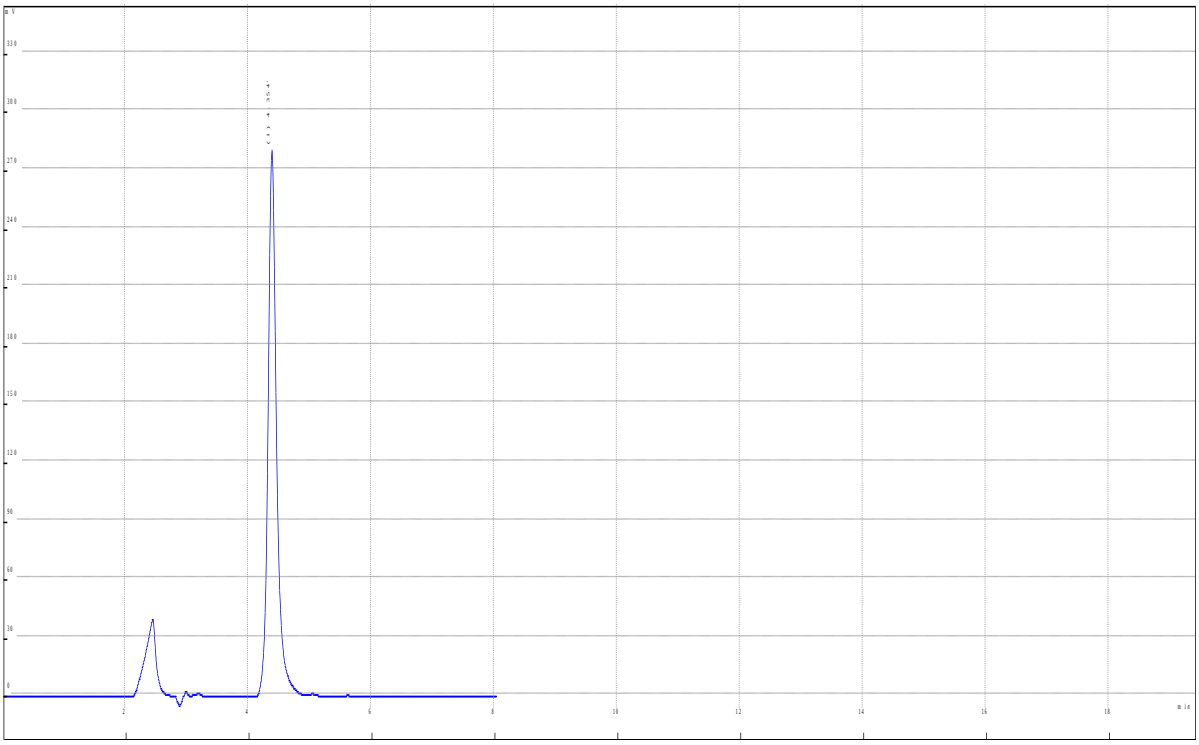
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.02min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.354	3092061	0.00	8172	1.22

- **Dapaglipflozin 50ppm treated with 3% H2O2 at RT for 24Hr**

Sample Name: Dapaglipflozin 50ppm treated with 3% H2O2 at RT for 24Hr

Wavelength: 224nm

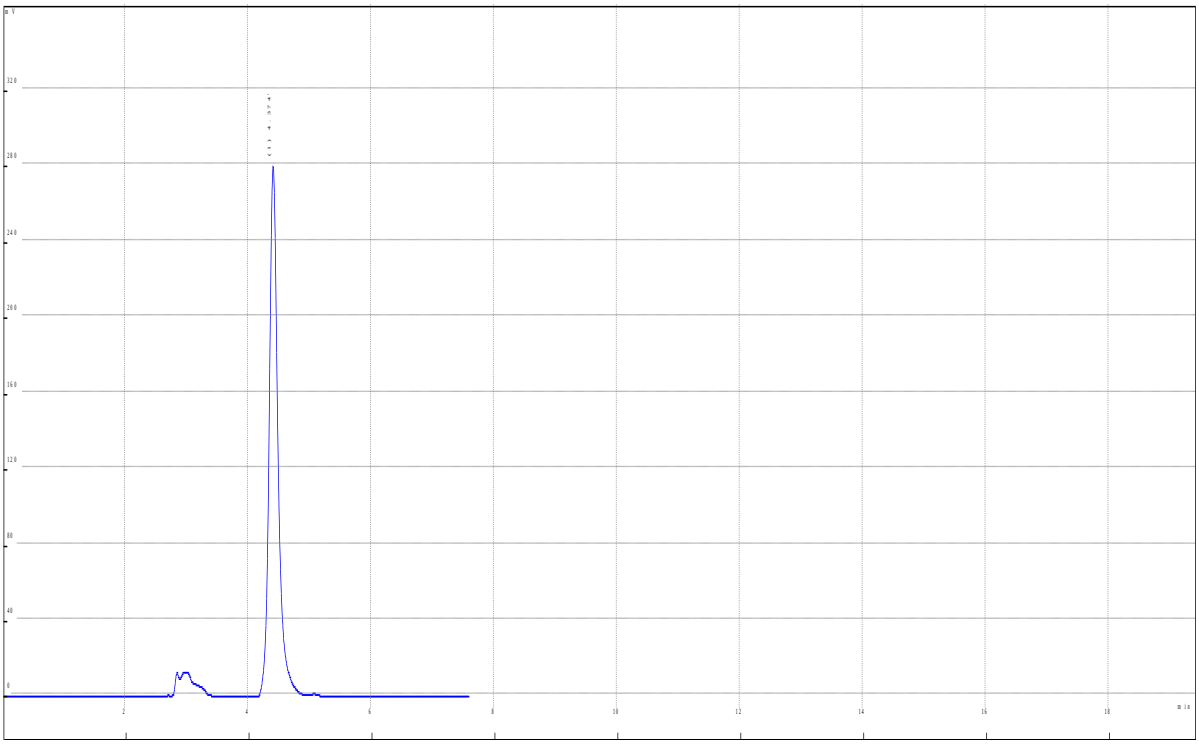
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 7.57min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.374	3339164	0.00	8398	1.26

- **Dapaglipflozin 50ppm treated Photolytically at RT for 24Hr**

Sample Name: Dapaglipflozin 50ppm treated Photolytically at RT for 24Hr

Wavelength: 224nm

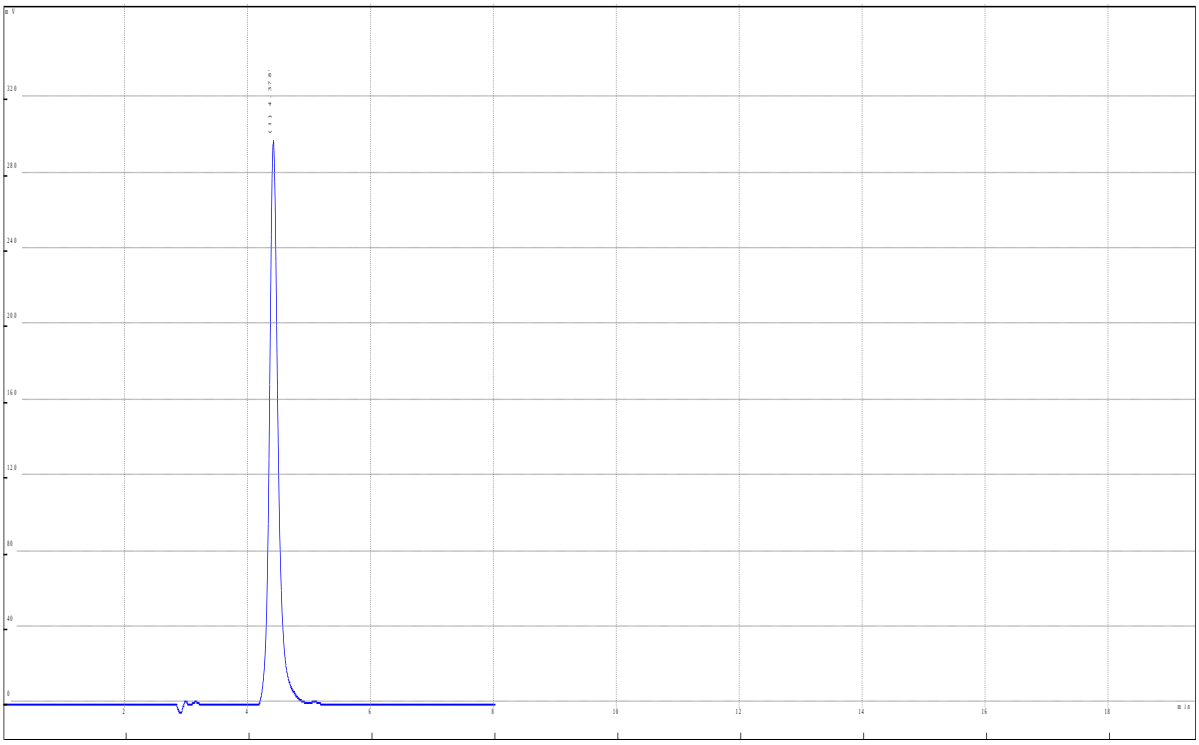
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.00min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.378	3452615	0.00	8707	1.23

- **Dapaglipflozin 50ppm treated Thermolytically for 24Hr**

Sample Name: Dapaglipflozin 50ppm treated Thermolytically for 24Hr

Wavelength: 224nm

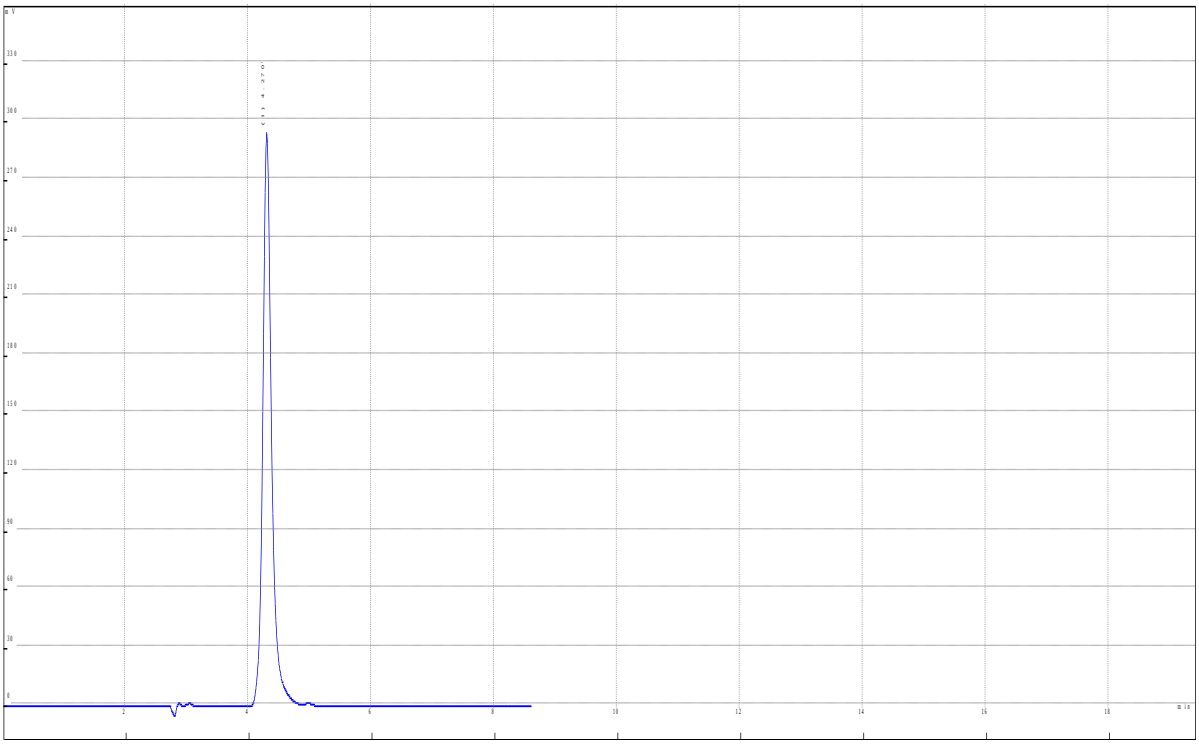
Mobile Phase: Methanol:Water (85:15)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10MPa

Run time: 8.58min



RT(min)	Area	Resolution	T.plate no	Asymmetry
4.270	3449040	0.00	8676	1.23

Sr. NO.	Degradation	Area of Standard	Area of degraded Sample	Degraded upto %	Actual % degradation
1	Acid Degradation	3467127	3158509	91.09873968	8.901260323
2	Basic Degradation	3467127	3092061	89.18222494	10.81777506
3	H2O2 Degradation	3467127	3339164	96.3092497	3.690750296
4	Thermal	3467127	3449040	99.47832889	0.521671113
5	Photolytic	3467127	3452615	99.5814402	0.418559805

Data interpretation

The degradation in acid, base, light was within limit. degradation products produce as a result of stress studies did not interfere with the detection of dapaglipflozin and the method can thus be considered stability indicating.

Acceptance criteria

1) maximum 5% to 20% degradation shall be achieved.

2) no interference from any degradation product with the drug peaks.

SUMMARY AND CONCLUSION

Based on experiments, we can conclude that the RP-HPLC developed for the determination of dapaglipflozin can be used for routine analysis.

Determination of dapaglipflozin were estimated by RP-HPLC using Methanol:buffer (85:15) pH 3 adjusted with potassium dihydrogen phosphate and column cosmosil C18,(250x4.6mm 5µm) as a stationary phase and the chromatogram of dapaglipflozin has shown in trial.5 and peak was observed at 224 nm which wass selected as a wavelength for quantitative estimation. after development of the method it was validated for linearity, precision, robustness, accuracy studies.

The precision was found to be within the limits. the limit was not more than % RSD<2%. precision %RSD was found to be 0.18% for dapaglipflozin. this indicates that the method is precise and accurate. the chromatograms for precision are shown under precision title and the data regarding the precision are shows in table.

The calibration curve was linear over the range 10 to 50 µg/ml. the linearity of method was statistically confirmed. the correlation coefficients (r^2) for calibration curves were not less than 1. the relative standard deviation (R.S.D) values of the slope were not more than 2%. from the results shown in the accuracy table it was found that recovery value of pure drug from the reanalysed solution of formulation were between 98.0% to 102% (99.84%) which indicates that the method is accuate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods. the system suitability parameter also reveals that the values within the specified limit for the proposed method.

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