Original Article

RP- HPLC Method Development And Validation For Estimation Of Dapagliflozin In Tablet Formulation

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Abstract

A novel, sensitive, suitable and robust strong-cation-exchange RP-HPLC technique was designed and evaluated towards the purpose of identifying dapagliflozin in tablet formulation. The separation was performed with HPLC system containing UV- detector with Openlab EZchrome software and kromasil C18, and methanol and water (75:25 % v/v) as mobile phase flowing at 1.0 ml/min. using UV detector, the detection was performed at 223 nm. Dapagliflozin was eluted with retention time of 5.27 min. The results of analysis have been evaluated with respect to stability, specificity, linearity, accuracy, precision (repeatability and intermediate precision) and robustness. A simple, specific, rapid and reproducible technique has been designed in order to analyse dapagliflozin in tablet formulation.

Keywords: HPLC, Dapagliflozin, Assay, Validation.

INTRODUCTION:

Dapagliflozin (DAPA) (Figure1) is an orally active anti-diabetic drugs, called 'sodium glucose co-transporter 2 (SLGT2)' inhibitor. It is indicated for the treatment of type 2 polydipsia, and functions to improve glycaemic control in adults when combined with diet and exercise. It is a sodium-glucose co-transporter 2 inhibitor which prevents glucose reabsorption in kidney. Dapagliflozin is a first generation, selective SGLT inhibitor that blocks glucose transport with about 100-fold selective for SGLT2 over SGLT1. [1-4]

Chemistry: Chemically dapagliflozin (DAPA) is (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol.

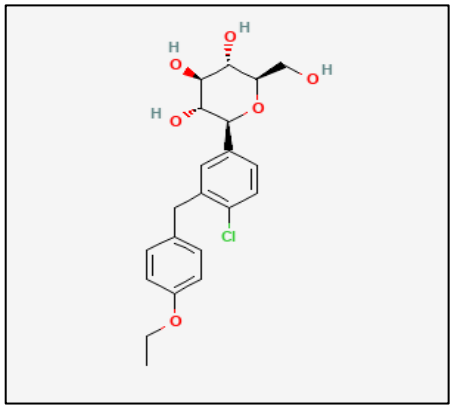


Fig. 1 Chemical structure of dapagliflozin

Mechanism of Action: Dapagliflozin retard the sodium-glucose co-transporter 2 (SGLT2) which is essentially present in the primary part of the nephron. Since SGLT2 aids in 92% of the kidneys' ability to uptake glucose, its blockage enables glucose to be released in urine. Patients with type II diabetes may experience better glucose control and possible weight loss due to this excretion.

Indications: Together with nutrition and lifestyle, dapagliflozin is recommended for type II insulin-resistant diabetes in elderly patients to help with glycemic control.

Solubility: Dapagliflozin is soluble in organic solvent such as methanol, ethanol also dimethyl fumarate. [5-8]

METHODS:

Materials: Qualigens (Thermo fisher scientific) provided HPLC grade methanol and dapagliflozin.

Instrument: An Agilent 1260 Infinity II HPLC system with DEAX02386 pump and autosampler with UV–visible detector served as the chromatographic system (DEACX16446). For data collection and processing, the chromatograms were registered using Openlab EZ Chrome workstation on a windows-based computer system. Concentration of Dapagliflozin were estimated using a Kromasil C_{18} column (250 mm X 4.6 mm internal diameter, 5µm).

Selection of analytical wavelength: With water as a blank, dapagliflozin standard solution (20 PPM) was scanned from 400 nm to 200 nm. Dapagliflozin showed maximum absorption at 223 nm and it was selected as an analytical wavelength for HPLC method development

Preparation of standard stock solution:

Dapagliflozin 500 PPM standard stock solution was prepared by transferring 10 mg dapagliflozin to 15 ml methanol and the quantity was prepared to 20ml with methanol. Further 2 ml of this solution was diluted to 10 ml with water (100 PPM).

Method development

Method was developed by reverse phase liquid chromatography using isocratic elution and UV detection.(Table 1)

Table 1: Developed chromatography parameters

Parameter	Description
Mode	Isocratic
Column Name	Kromasil C18, 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	223 nm
Column Oven temp	40ºC
Mobile Phase	Methanol : Water (75:25 %V/V)
Flow Rate	1.0 ml/min
Run time	8 Minutes

RESULT AND DISCUSSION:

Selection of analytical wavelength: Dapagliflozin showed maximum absorption at 223 nanometer as shown in figure 2. Therefore 223 nm considered as an analytical wavelength for further determination.

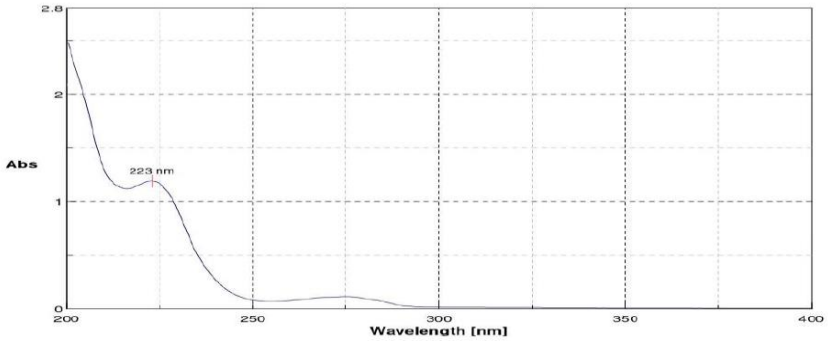


Fig. 2: Dapagliflozin's Ultraviolet Spectra

Process advancement: From the observations of trials first to three, it was concluded that chromatographic conditions in trial three gives better peak, good retention time, good resolution between adjacent peaks and tailing factor therefore chromatographic conditions in trial three was used for method development. (figure 3)

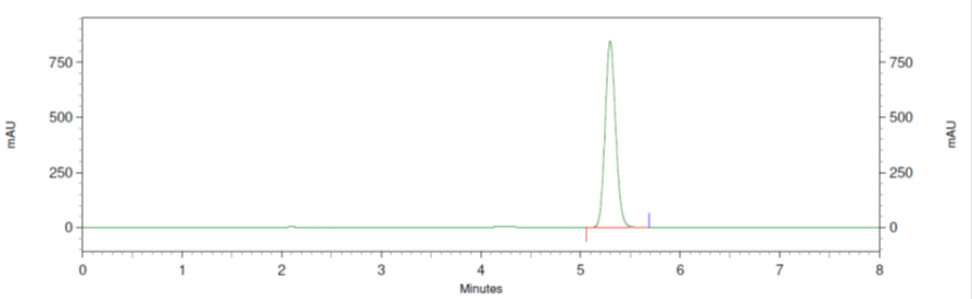


Fig. 3: Optimized chromatogram

System Suitability Test: It was observed that the method complies with system suitability parameters. Hence, it can be concluded that this chromatographic method is good for intended analysis. (Table 2, figure 4)

Table 2: System suitability tests

Parameter	Acceptance Criteria	Result
%RSD	NMT 2.0%.	0.32
Theoretical plates	More than 2000	**12053**
Tailing factor	NMT 2.0	1.12

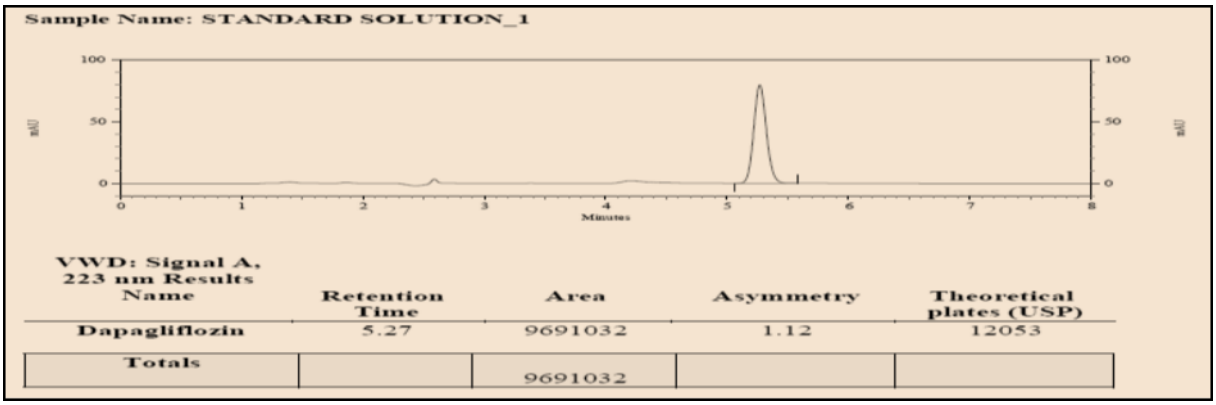


Fig 4: System suitability chromatogram

Stability study: Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analysed at initial, after 12 hours and 24 hours (Table 3, figure 5). It was observed that both the standard and sample solutions remained stable for 24 hours.

Table 3. Stability study

Test solution			Standard solution			Acceptance Criteria
Time Point	**Area**	**% Absolute Difference**	**Time point**	**Area**	**% Absolute difference**	% Absolute difference
Initial	9675912	NA	**Initial**	9689014	NA	NMT 2.0
12Hrs	9641970	0.29	**12Hrs**	9661035	0.30	
24 Hrs	9639107	0.46	**24 Hrs**	9656037	0.48	

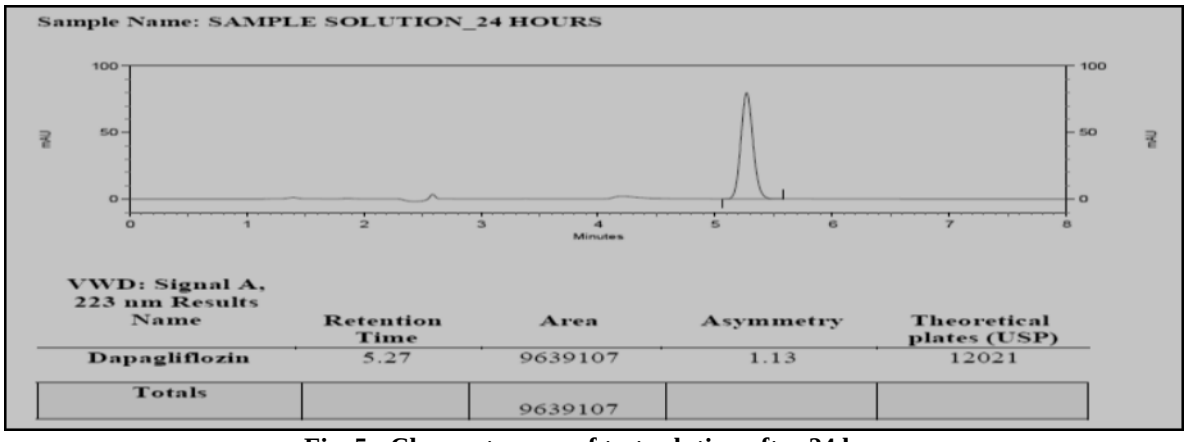


Fig. 5: Chromatogram of test solution after 24 hrs.

ANALYSIS OF MARKETED TEST SAMPLES (ASSAY): % Assay was observed in between 90-110%. The assay result is within the limit for selected marketed test sample and can be used for validation. (Table 4, figure 6).

Table 4: Assay results of dapamac tablet 5 mg

Sample	Area	% Assay	Mean Assay
Sample 1	9430821	98.68	99.01
Sample 2	9490300	99.34	

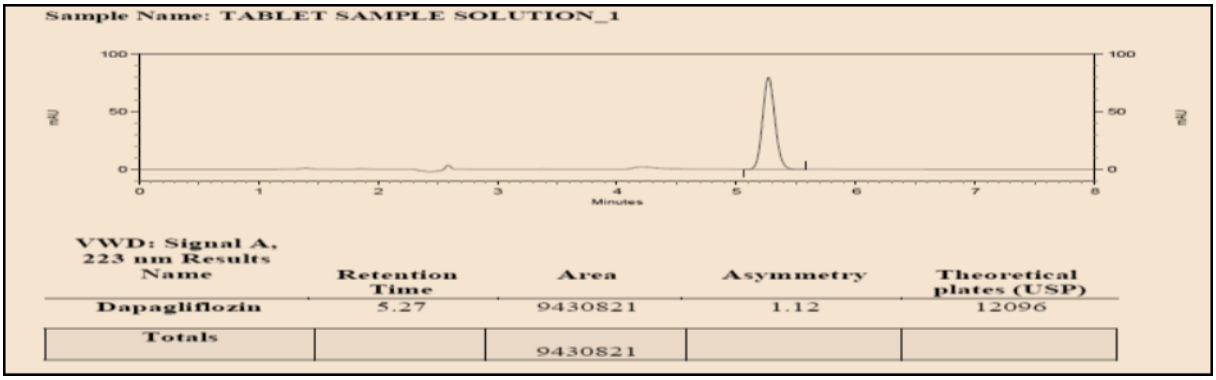


Fig. 6: Chromatogram of dapamac tablet 5 mg

Specificity: Blank and placebo was not having interference at R.T. of Dapagliflozin. Peak purity for standard as well as test solution was well within limits. Hence developed chromatographic method passed the criteria for specificity. (Table 5, figure 7).

Table 5: Results of specificity.

Description	Observation	Acceptance criteria
Blank	No interference at R.T. due to blank	No Interference at R.T.
Placebo	No interference at R.T. of Dapagliflozin due to placebo	No Interference at R.T.
Standard solution	Peak purity was 0.996	Peak purity: NLT 0.95
Test Solution	Peak purity was 0.994	Peak purity: NLT 0.95

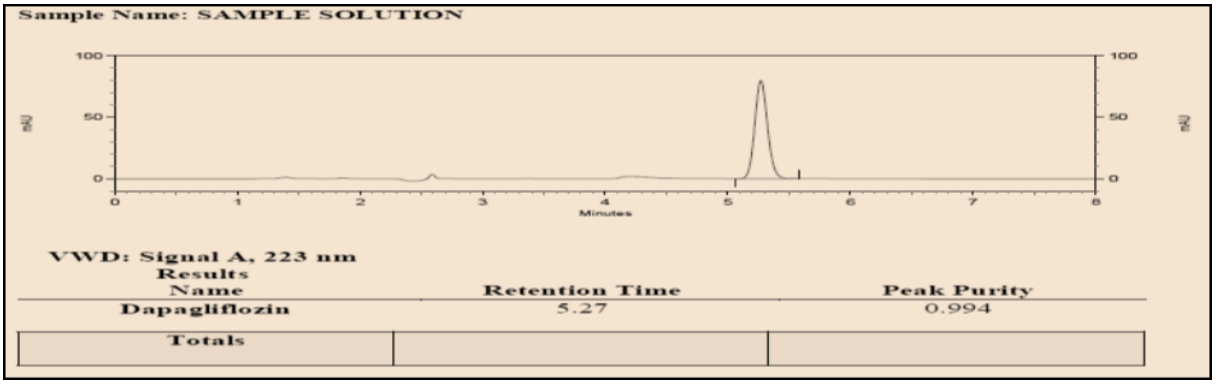


Fig. 7: Chromatogram of peak purity of sample solution.

Linearity

From the calibration curve it was concluded that the Dapagliflozin shows linear response within the region from 1.0-15.0 µg /ml. The Regression value was found well within the limit. (Table 6 & 7, figure 8).

Table 6: Results of HPLC linearity data for Dapagliflozin:

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	1.00	978960	973982	0.466
		970081		
		972905		
50%	5.00	4852621	4833059	0.554
		4844039		
		4802518		
100%	10.00	9682378	9638481	0.434
		9634029		
		9599035		
125%	12.50	11911312	11956360	0.385
		12003248		
		11954521		
150%	15.00	14518395	14487058	0.522
		14541930		
		14400850		

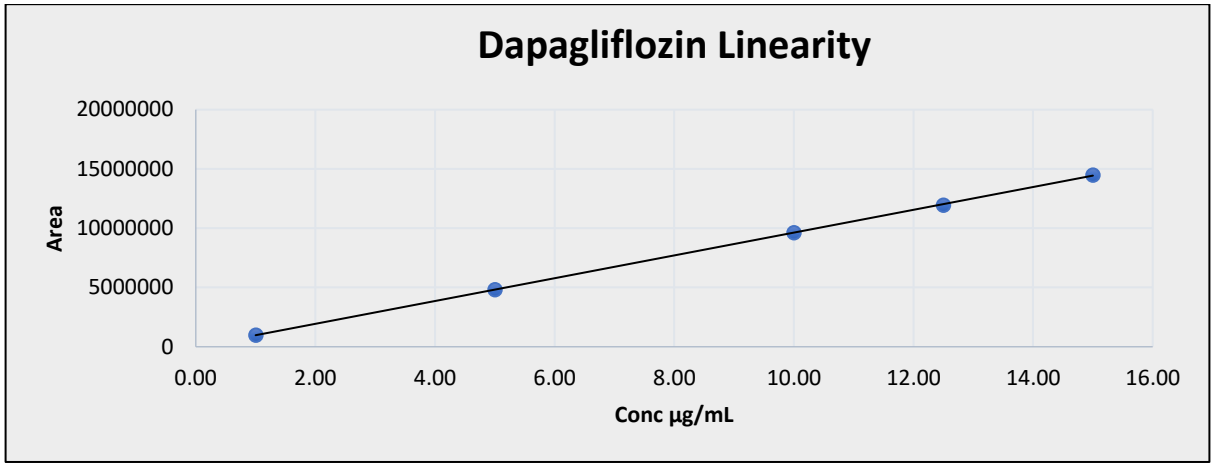


Fig. 8: Calibration curve of dapagliflozin.

Table 7. Summary HPLC linearity of dapagliflozin

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	1.0-15 µg/mL	NA
2	Correlation coefficient (R^2)	0.99996	NLT 0.98
3	% RSD for area at each level	NA	NMT 2.0

Accuracy (Recovery):

Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not get hampered by change in analyte concentration. (Table 8, figure 9,10,11).

Table 8: Statistical data of accuracy of dapagliflozin

Level (%)	Area	% Recovery	Mean Recovery	% RSD	Acceptance Criteria
50	4879958	100.20	99.24	0.8796	% Recovery: 98.00 %to 102.0 %
	4991046	98.49			
	4920318	99.04			
100	9629543	98.82	99.51	0.6483	
	9662037	100.10			
	9610358	99.60			
150	14439520	98.76	99.04	0.6197	
	14388423	99.74			
	14225848	98.61			

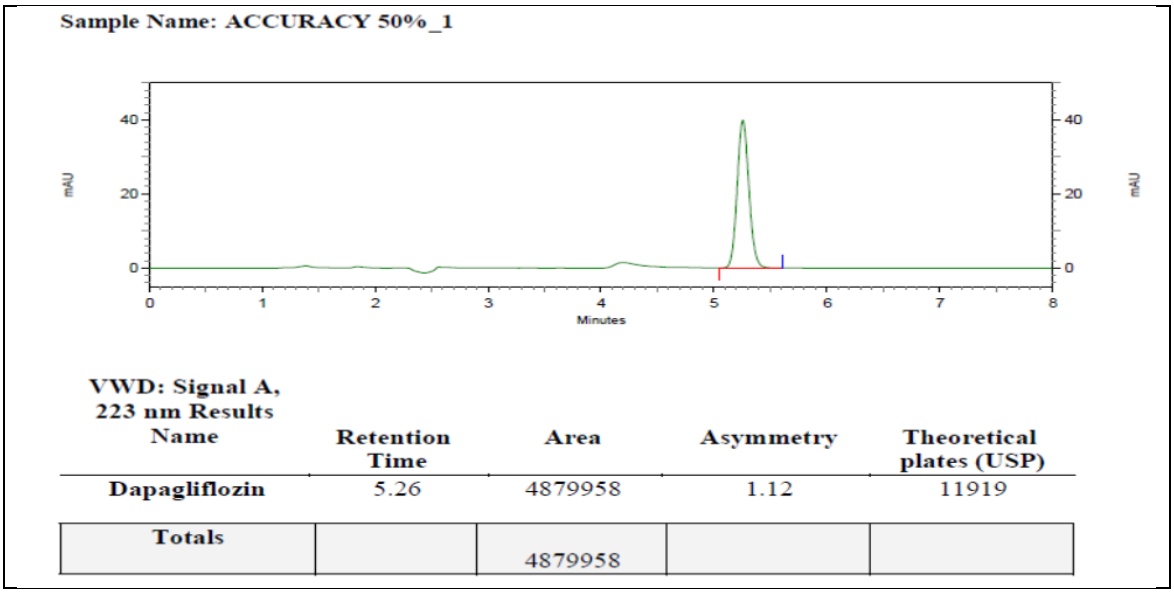
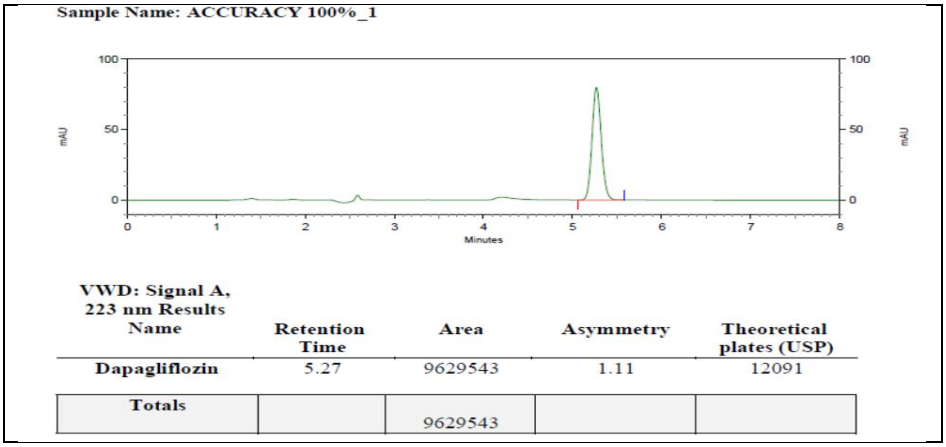


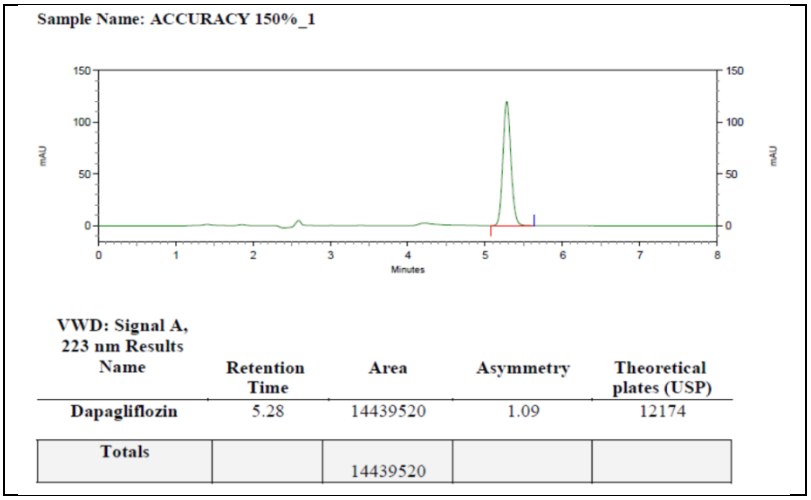
Fig. 9: Chromatogram of recovery 50%.



VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	5.27	9629543	1.11	12091
Totals		9629543		

Fig. 10: Chromatogram of recovery 100%.



VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	5.28	14439520	1.09	12174
Totals		14439520		

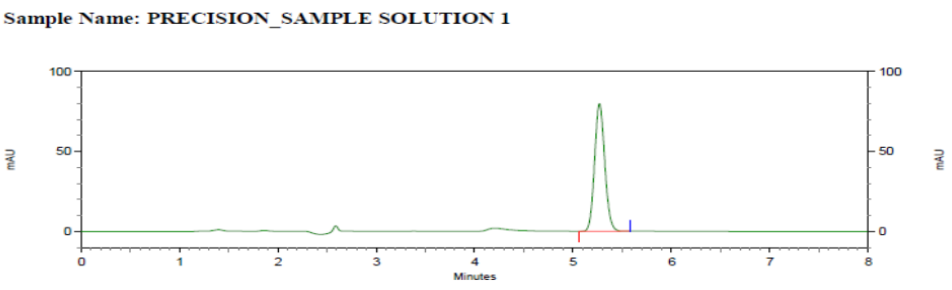
Fig. 11: Chromatogram of recovery 150%.

Precision

% Assay and % RSD was found well within acceptance limit and hence method is precise (Reproducible). (Table 9, figure 12,13).

Table 9. Result of intraday and inter- day precision for dapagliflozin test sample assay.

Parameters	Intraday Precision	Interday Precision	Acceptance criteria
Mean	98.89	98.60	% RSD for six samples is not more than 2.0
SD	0.804255	0.746987	
%RSD	0.813	0.758	



VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	5.27	9516821	1.12	12027
Totals		9516821		

Fig. 12: Chromatogram of repeatability precision (Sample 1).

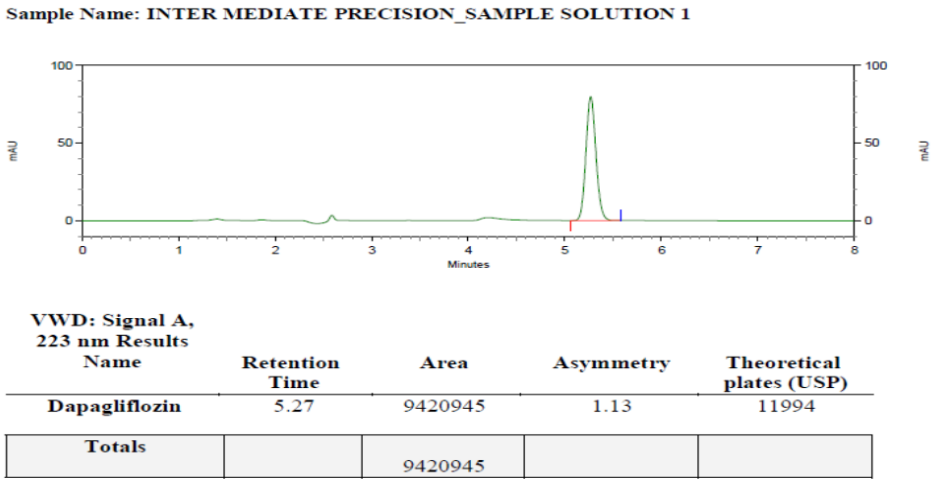


Fig. 13: Chromatogram of inter-day precision (Sample 1).

Robustness:

Robustness conditions like wavelength, Flow rate, column temperature were studied and samples were injected in duplicate manner. Process acceptability tests were not much influenced. %RSD was within the limit (Table 10, Fig. 14 - 19).

Table 10. Test results of robustness research

Change in parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 nm (226 nm)	5.27	9458244	1.09	12051
Wavelength by -3 nm (220 nm)	5.27	9367023	1.09	12058
Flow rate by +10% (1.1ml/min)	4.79	8823790	1.11	11228
Flow rate by -10% (0.9ml/min)	5.85	10769264	1.11	12984
Column temp by +2ºC (42 ºC)	5.27	9535059	1.13	11986
Column temp by -2ºC (38 ºC)	5.27	9580935	1.12	12008

A. Change in wavelength by +3 nm:

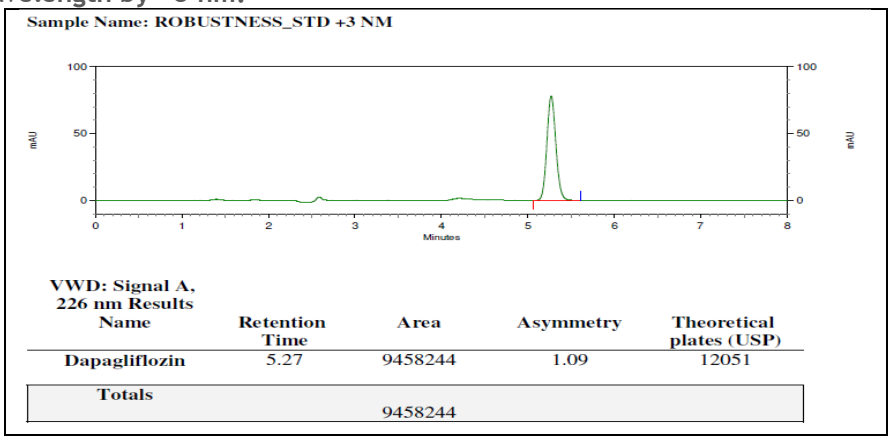


Fig. 14: Chromatogram of standard +3 nm.

B. Change in wavelength by -3 nm:

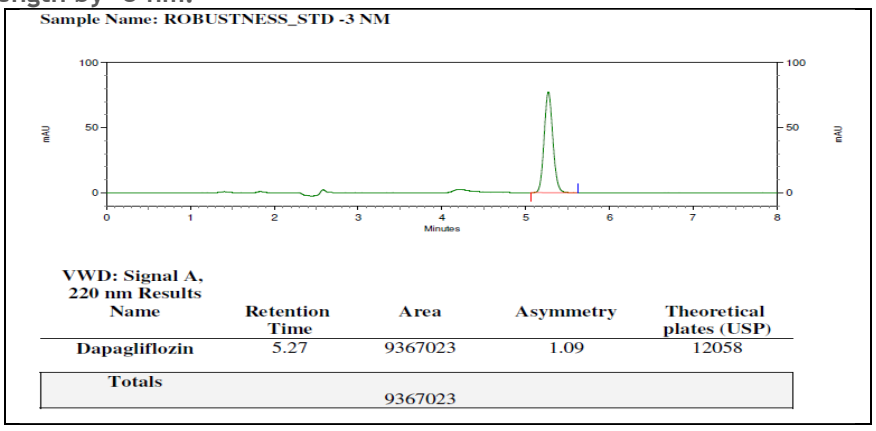
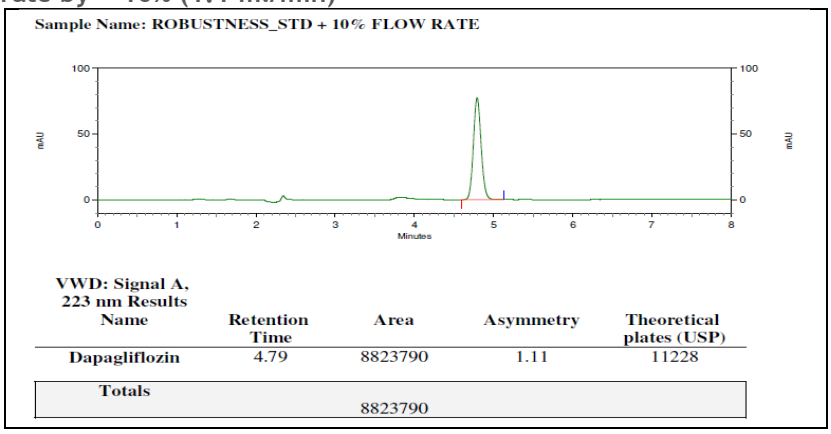


Fig. 15: Chromatogram of standard -3 nm.

C. Change in flow rate by + 10% (1.1 ml/min)

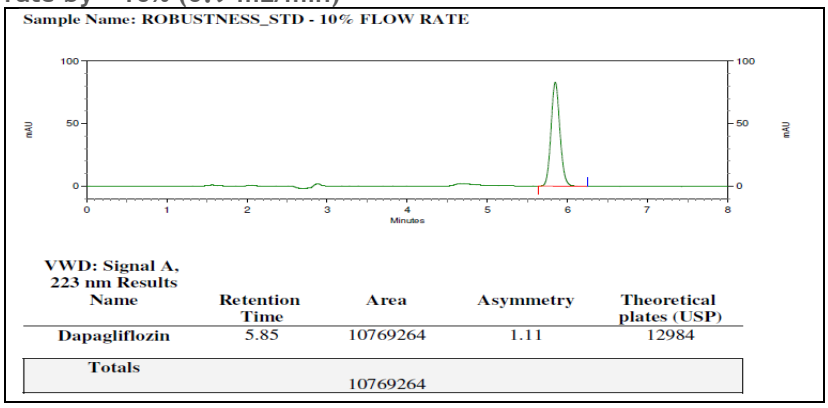


VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	4.79	8823790	1.11	11228
Totals		8823790		

Fig. 16: Chromatogram of standard +10 F.R.%.

D. Change in Flow rate by - 10% (0.9 mL/min)

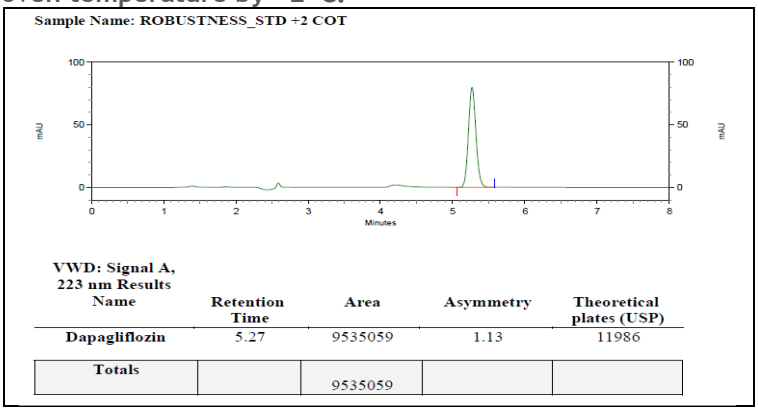


VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	5.85	10769264	1.11	12984
Totals		10769264		

Fig. 17: Chromatogram of standard -10 F.R.%.

E. Change in column oven temperature by +2°C:

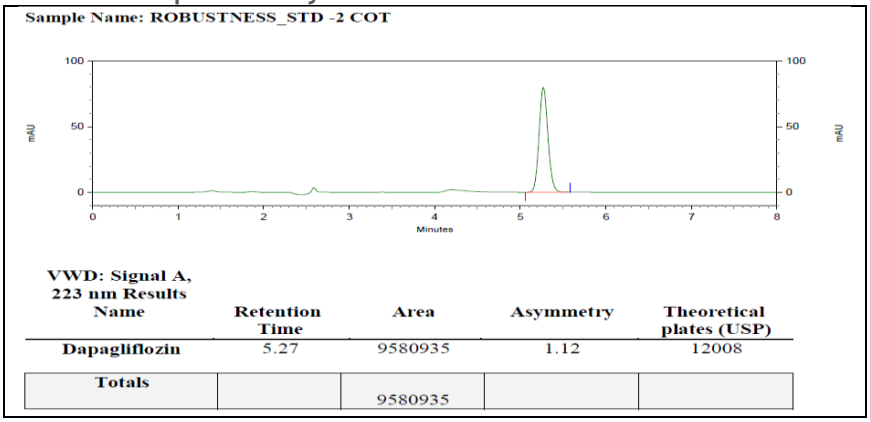


VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	5.27	9535059	1.13	11986
Totals		9535059		

Fig. 18: Chromatogram of standard +2°C C.O.T.

F. Change in column oven temperature by -2°C:



VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dapagliflozin	5.27	9580935	1.12	12008
Totals		9580935		

Fig. 19: Chromatogram of standard -2ºC C.O.T.

CONCLUSION:

In the present study, a new, sensitive and suitable strong-cation-exchange RP-HPLC technique was designed and evaluated for the analysis of Dapagliflozin in tablet formulation. The results of analysis in the developed method were verified with respect to linearity, accuracy, precision, robustness. The developed method has several advantages, including reproducibility of results, rapid analysis, improved selectivity as well as sensitivity. The regression coefficient (r^2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2% showing high degree of precision of the proposed method. Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for estimation of dapagaflozin in tablet formulation.

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