IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2023 Vol.:27, Issue:2

Development and Validation of RP- HPLC Method for Estimation of Dapagliflozin in Formulation

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Konda Mohammed Khalid*, Yogesh. S. Thorat, Varsha. S. Tegeli

D.S.T.S Mandal's College of Pharmacy, Solapur, Maharashtra, India.

Submitted: 20 April 2023
Accepted: 26 April 2023
Published: 30 May 2023

Keywords: RP-HPLC, Validation, Estimation, Dapagliflozin, ICH- guidelines

ABSTRACT

A new sensitive and rapid RP-HPLC method was developed for the determination of dapagliflozin in pharmaceutical dosage forms and it was validated according to ICH guidelines. The Reverse phase high-performance liquid chromatography (RP-HPLC) of Shimadzu SCL-10A_{VP} inbuilt with binary pump (LC-10AT_{VP}), DAD detector (SPD-10A_{VP}), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. 0.1M ammonium acetate-acetonitrile (58:42 v/v)as mobile phase, at the flow rate of 1.0 mL/min. The detection was performed at the wavelength (λ) of 230nm, and the retention time of dapagliflozin was around 8.74 min. The total run time was 10 min. The calibration plot gave linear relationship over the concentration range of 6.12–100µg/ml. The LOD and LOQ were 2.3 and 7.1µg/ml, respectively. UV detection was monitored at 230nm for dapagliflozin as the compound exhibit optimum absorption at this selected wavelength. The accuracy of the proposed method was determined by recovery studies and was found to be in range of 98.63 to 100.86%. The repeatability testing for both standard and sample solutions showed that the method is precise, accurate within the acceptable limits.

INTRODUCTION

Dapagliflozin is used along with diet, exercise, and usually with other glucose lowering medications, to improve glycemic control in adults with type 2 diabetes and to reduce the risk of hospitalization for heart failure among adults with type 2 diabetes and known cardiovascular disease or other cardiovascular risk factors (including high blood pressure, high cholesterol, and smokers). Dapagliflozin was also shown to reduce the rate of decline in kidney function and kidney failure in adults with type 2 diabetes, a finding that has recently been confirmed in both diabetic and nondiabetic populations. Dapagliflozin is also an important treatment option in patients with comorbid type 2 diabetes and heart failure with a reduced ejection fraction (on top of standard medical and/or device therapy), and lowers the risk of hospitalization for heart failure and cardiovascular deaths in this group. In addition, dapagliflozin is indicated for the treatment of adults with heart failure with reduced ejection fraction to reduce the risk of cardiovascular death and hospitalization for heart failure.

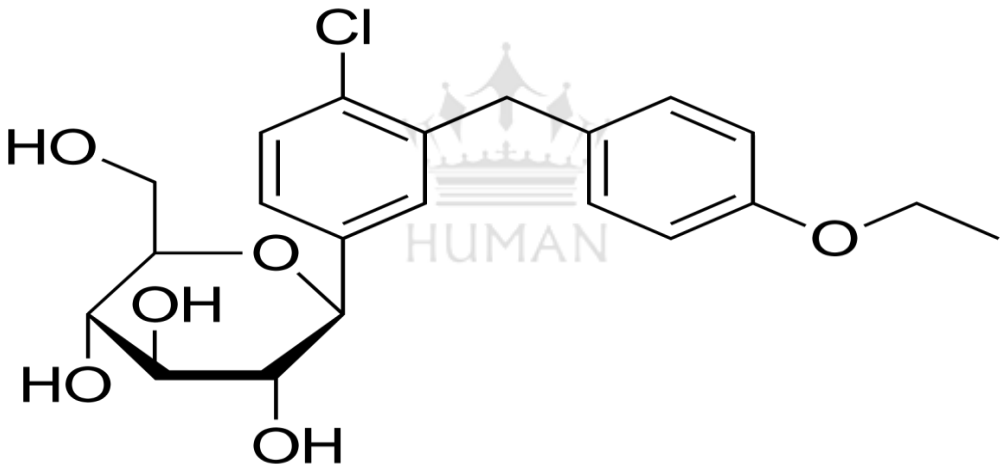

Figure No. 1: Dapagliflozin

MATERIALS AND METHODS

In attempt to improve the peak shape and separation, various eluent compositions, buffers selection and elution mode were tested and evaluated and finally the separation carried out in Acclaimed mix Mode HILIC-1column (150 x 4.6mm ID) with isocratic elution, consisting 0.1Mammonium acetate (AA)-Acetonitrile (58:42 v/v) explicit best results.

Column : Acclaimed Mix-Mode; 5µ, 150 X 4.6 mm. ID.

Mobile Phase : 0.1M ammonium acetate-acetonitrile (58:42 v/v)

Flow rate : 1mL.min^{-1}

Elution mode : Isocratic elution mode

Wavelength selected : 230 nm

Temperature : Room temperature

Run time : 11 minutes

Retention time : dapagliflozin (8.74 min)

Method Validation

The method was validated as per ICH Q2 (R1) guideline, and the validation parameters included Specificity, Linearity, Range, Accuracy, Precision, System suitability, LOQ, LOD and Robustness.

System suitability

The purpose of the system suitability test is to ensure that the complete testing system, including instruments, reagents, columns, analysts etc., is adequate for the intended analysis. The following parameters are usually determined: theoretical plate count, tailing factors, resolution, and reproducibility.

Peak	Ret. Time	Area	Area%	T. Plate	Resolution	Tailing F.
Dapagliflozin	8.74	23799028	52.3462	2407.625	6.748	1.211

Specificity

Specificity is the ability of the analytical method to discriminate between the analyses and the other components in the mixture.

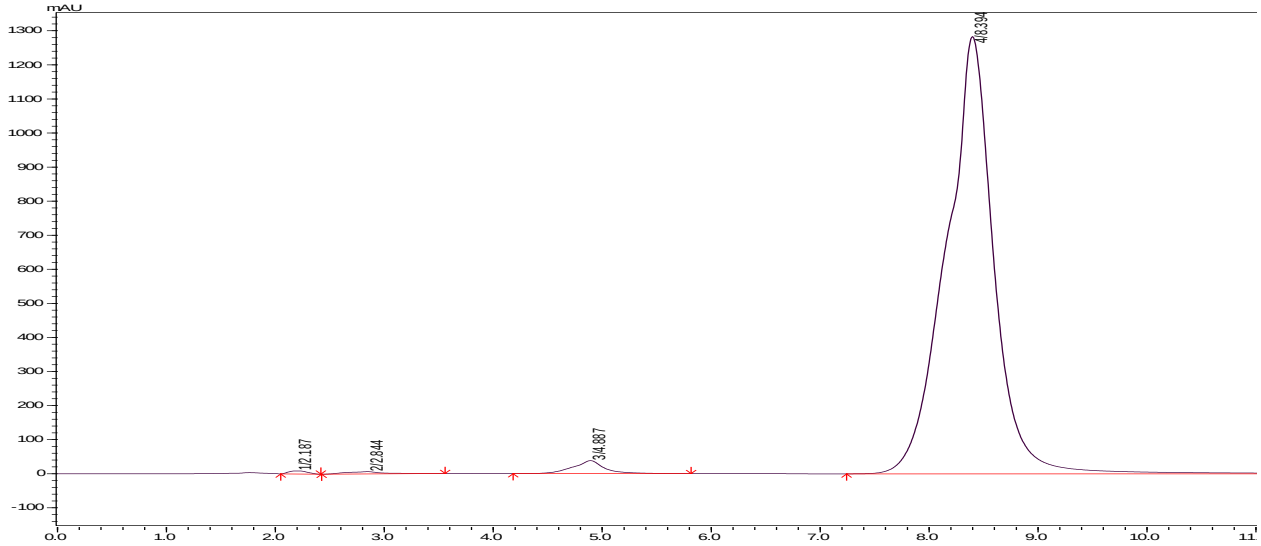

Figure No. 2: Chromatogram of dapagliflozin standard solution

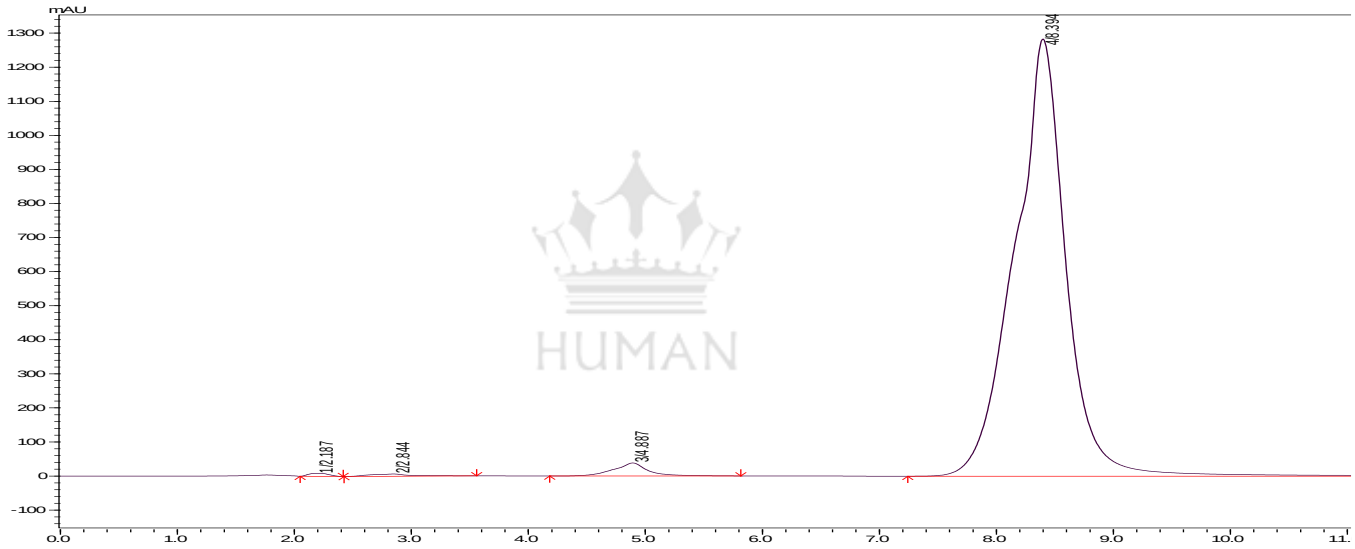

Figure No. 3: Chromatogram of dapagliflozin sample solution

Repeatability

Implementing the procedure mentioned under section (5.6), the homologous mixture of DAP (100ppm) were tested for six injections within the same day. The % RSD was calculated and found it is less than 2% for both selected drugs; shown in (Table 1).

Table No. 1: Repeatability data of Dapagliflozin

S. No.	Dapagliflozin
1	16088619
2	16264089
3	16077241
4	16093507
5	16216629
6	16125961
Mean	**16144341**
STD. DEV.	**77581.45**
RSD (%)	**0.48**

Precision studies

The precision of RP-HPLC method reflects its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions.

Table No. 2: Precision data of dapagliflozin

S. No.	Concentration (ppm)	Area
1	100	13577128
2	100	13566060
3	100	13542511
1	100	13558485
2	100	13542511
3	100	13899881
1	100	13706239
2	100	13827994
3	100	13895026
	Mean % RSD	**1.48**

Linearity and range

The linearity of any RP-HPLC Method represent Under linearity or calibration studies, a linear relationship between area under peak values and selected drug concentration (µg.mL.min^{-1}) was plotted for five-six chosen concentrations of each drug. The regression equations, correlation coefficient values (R^2), standard error of intercept (S_e), standard deviation of intercept (S_a), limit of detection (LOD) and limit of quantification (LOQ) have been calculated. The linearity of the calibration curves was validated by the high value of correlation coefficient, acceptable values of regression coefficient, standard deviation of the slope and standard deviation of the intercepts.

Table No. 3: Linearity data of Dapagliflozin

S. No.	Concentration (µg.mL^{-1})	Area	Average (Mean)
1	100	20650831	20650831
2	50	10420883	10420883
3	25	5211836	5211836
4	12.25	2619084	2619084
5	6.12	1320090	1320090

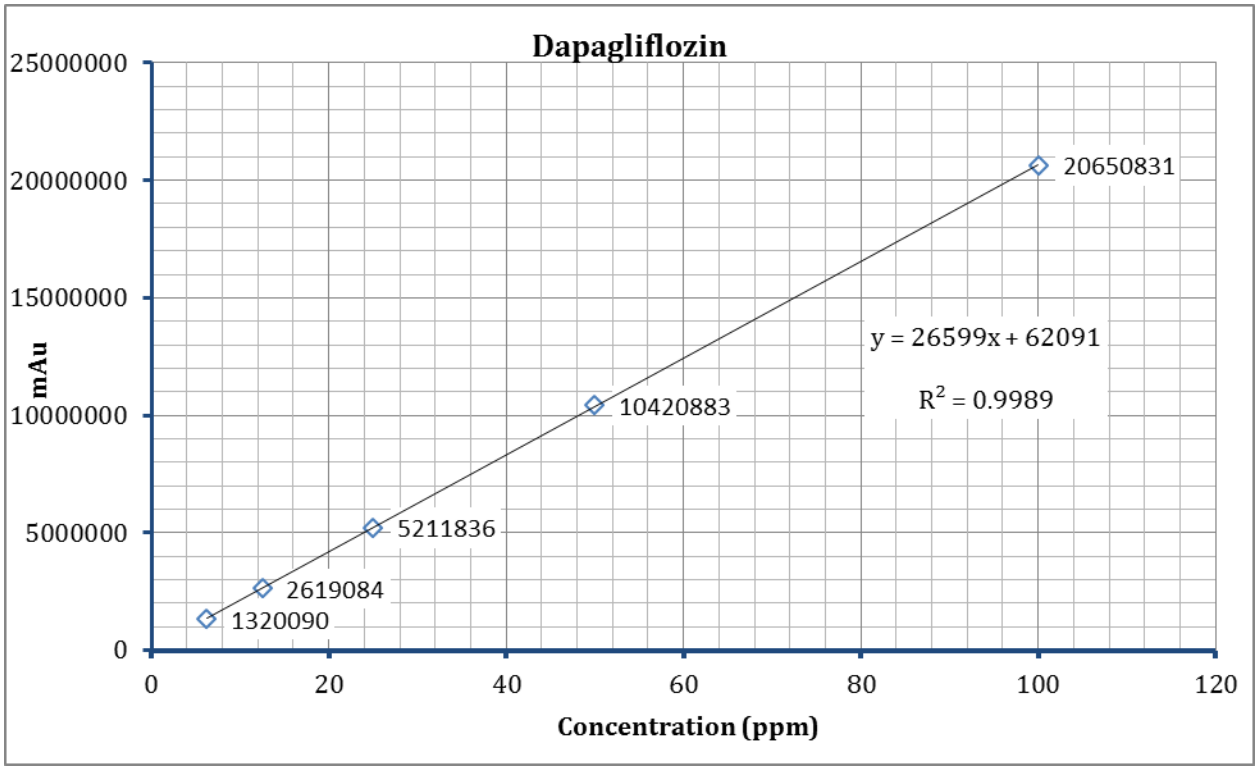

Figure No. 4: Calibration curve of dapagliflozin

Robustness for the chromatographic method

Robustness of RP-HPLC Method represents its ability to remain unaffected by small but deliberate variations in separation parameters to ascertain its reliability during routine analysis. From all above studies, after making deliberated changes in flow rate (± 0.2mL.min^{-1}), organic modifier concentration; acetonitrile (±2%) and wavelength (±2nm) have not made any significant changes in resolution, capacity factor and tailing factor.

Table No. 4: Robustness data of dapagliflozin

S. No.	F. (-0.2 ml.mL^{-1})	F (+0.2 ml.mL^{-1})	A (-2 ml)	A (+2 ml)	WL (-2 nm)	WL (+2 nm)
Resolution	6.27	6.27	6.27	6.27	6.32	6.27
Tailing factor	1.23	1.23	1.17	1.15	1.18	1.52
Capacity factor	1.81	1.79	2.59	1.27	1.18	1.52
Theoretical Plates	2552	3015	3165	2926	2989	3150

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

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the regression equation. As observed, the LOD and LOQ of dapagliflozin were 2.3 and 7.1µg/ml.

Accuracy

Percentage recoveries of three different concentrations; 80%, 100% and 120% (injected thrice) to determine the Metformin was calculated to determine the drug recovery (%) and variation in RSD% and results obtained were reported.

Table No. 5: Accuracy data of Dapagliflozin

Conc. (%)	S. No.	S. amt. (µg.mL^{-1})	D. added (µg.mL^{-1})	Amt. rec. (µg.mL^{-1})	% recovery	Mean±SD	% RSD
80%	1	100	80	180.1	100.06	100±0.01	0.01
	2	100	80	180.12	100.07		
	3	100	80	180.11	100.06		
100%	1	100	100	195.25	99.63	99±1.39	1.40
	2	100	100	200.05	100.03		
	3	100	100	200.07	100.04		
120%	1	100	120	220.17	100.08	100±0.03	0.03
	2	100	120	220.24	100.11		
	3	100	120	220.13	100.06		

CONCLUSION

The developed HPLC method is fast & simple and found specific, linear, accurate, precise, and robust. Hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for dapagliflozin. Hence the present RP-HPLC method used for routine analysis of raw material of dapagliflozin and its formulation.

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