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[Research article]

RP-HPLC Method Development and Validation of Dapagliflozin in Bulk and Tablet formulation

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ABSTRACT

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, 5µ 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%.

Key Words: Dapagliflozin, RP-HPLC, Validation and method development.

INTRODUCTION

Dapagliflozin is a potent and selective SGLT-2 (SLC5A2) inhibitor with EC50 of 1.1 nM.. Chemically, DGF is (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl] phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol with empirical formula of $C_{21}H_{25}ClO_6$ and Molecular weight is 408.87 g/mol.[2] It has good permeability across Caco-2 cell membranes and is a substrate for P-glycoprotein (P-gp). Dapagliflozin is not a significant P-gp inhibitor.[1] Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor, which prevents glucose reabsorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight

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loss and tiredness.[3] The chemical structure of DGF was shown in Figure.1.

An extreme literature survey revealed that very few analytical methods have been reported such as HPLC for Dapagliflozin individual and combination with other drugs. In order to minimize the batch –to- batch variation, it is very important to develop suitable analytical methods for day –to-date analysis of drugs. It was found that one attempt has been made to develop stability indicating studies and estimation of Dapagliflozin by RP-HPLC at the starting of my work. We here in report a simple, rapid and reliable RP-HPLC for the estimation of DGF in bulk and pharmaceutical dosage forms.

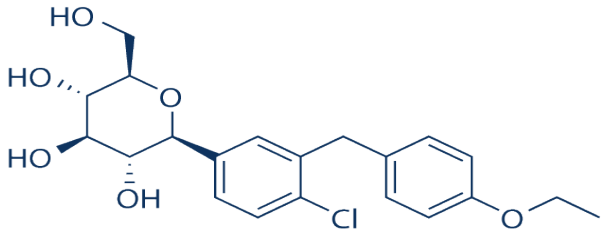

Figure.1: Structure of Dapagliflozin

EXPERIMENTAL

REAGENTS AND MATERIALS

Pure standard of DGF was obtained as gift sample from Dr.Reddy's Lab in Hyderabad. HPLC grade acetonitrile, HPLC grade water, Ortho phosphoric acid HPLC (Merck. Mumbai, India), Potassium dihydrogen phosphate and Triethylamine (RANKEM, Mumbai, India.) and All solvents used in this work are HPLC grade. Forxiga tablets (Astra Zeneca) Containing Dapagliflozin marketed formulation were purchased from the local market, high precision weighing balance (Shimadzu, model: AY-120), micro pipette (in labs 10-100 µl) was employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven whenever required. The working environment was maintained in between 25^{o}C.

HPLC AND CHROMATOGRAPHIC CONDITIONS

The analysis was performed on Waters 2695 RP-HPLC separation module (Waters Corporation, Milford, USA) equipped with PDA detector having back pressure 5000psi, automatic injector and Hypersil BDS C18 (250mm × 4.6 mm, 5µ) was used as stationary phase. Sonicator (Power sonic 405, Labindia Instruments). The chromatographic separation was achieved by using 0.1% Ortho phosphoric acid buffer and acetonitrile 50:50 %v/v as mobile phase at a flow rate of 1ml/min. The mobile phase was filtered through 0.45µm nylon membrane filter and degassed before use. The injection volume was 10 µl and the total run time was set as 5min. The determination of analytes was carried out at 245nm using PDA detector.

PROCEDURE RECOMMENDED

PREPARATION OF STOCK SOLUTION

Accurately Weighed and transferred 10mg Dapagliflozin working Standard into a 10 ml clean, dry volumetric flask, add 5 ml of diluents (Water and acetonitrile 50:50), sonicated for 5 minutes and make up to the final volume with diluents.

PREPARATION OF STANDARD SOLUTION

From the above stock solution, 1 ml was pipette out into a 10ml volumetric flask and then make up to the final volume with diluent.

CONSTRUCTION OF LINEARITY

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.25 – 1.5 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 25 – 150µg/ml. The prepared solutions were filtered through 0.45µm membrane filter and each of the dilutions was injected three times into the column. The calibration curve for DGF was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in

the concentration range 25-150µg/ml with good correlation in between concentration and mean peak area.

ASSAY

10 tablets were weighed and average weight was calculated. Then from the transferred the equivalent to one tablet to 10ml volumetric flask, 7ml of diluent was added and sonicated for 5 min, further the volume was made up with diluent. From the filtered solution, 1ml was pipette out into 10ml volumetric flask and made up to 10ml with diluent. From the solution, 10µl was injected into HPLC system and peak area was recorded with detector at 245nm. The % assay was calculated with obtained peak area of detector response. This indicates that developed method can be used for routine analysis.

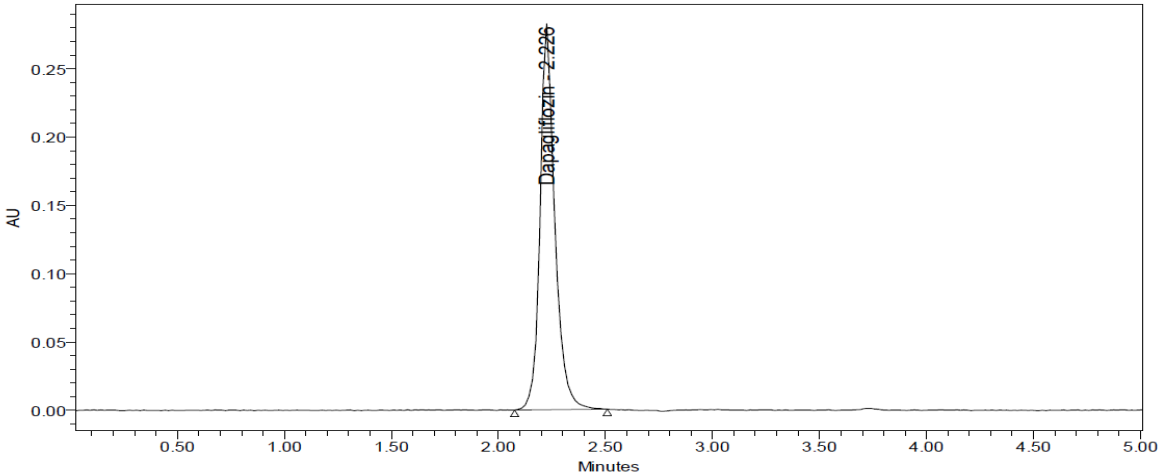

Figure.2: A typical chromatogram of DGZ

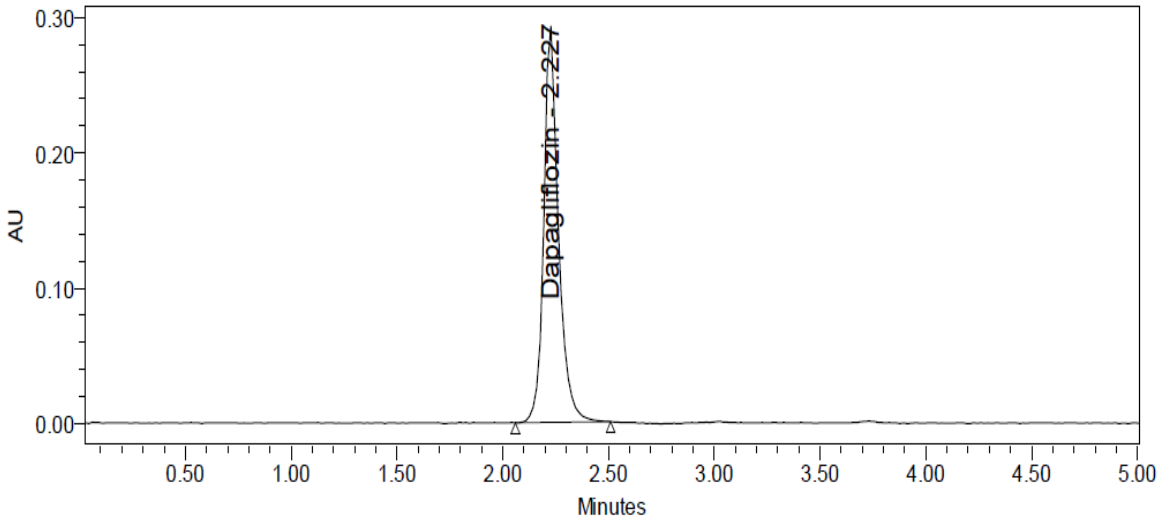

Figure.3: Chromatogram showing the assay of DGZ marketed dosage form.

METHOD VALIDATION

Method validation is the process of determining the performance of the developed method to meet the requirements for its analytical application. The proposed assay method was successfully validated according to ICH guidelines. The parameters studied for validation were specificity, accuracy, linearity, precision, robustness, limit of detection, limit of quantification and system suitability.[4-8]

RESULTS

The present RP-HPLC method for the quantification of DGF in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method with significant shorter retention time of 2.226min. The linearity for the detection of DGF was 25-150µg/ml with (R^2 = 0.9998; y = 15079x + 4232.3) the coefficients of variation based on mean peak area for three replicate injections were found to be 0.9998. Results were shown in table-1 and statistical data

of calibration curves were shown in table-2. The intraday and inter day variations of the method were determined using five replicate injections analyzed on the same day and next day over a period of 24 hours. The result revealed the precision with %RSD of 0.6 and 0.29, respectively for intraday and inter day. Results were shown in table-3. To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting the solution about three times, at three different concentrations equivalent to 50%, 100% and 150% of the active ingredients, by adding a known amount of DGF standard to a sample of known concentration and calculating the recovery of DGF with RSD (%) and recovery for each concentration. The mean % recoveries were in between 100.12% and were given in table- 4. The assay for the marketed tablets of Foexiga tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.01% of the labeled claim and no interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in table – 5. To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factors, theoretical plates, limit of detection and limit of quantification and values were shown in table-6. Robustness of the proposed method was estimated by changing mobile phase composition from buffer: acetonitrile (50:50) v/v to buffer: acetonitrile 55:45 (v/v) and 45:55(v/v), changing the flow rate from 1ml to 0.9 ml/min and 1.1 ml/min, changing the temperature ($\pm$ 5^{o}c) and system suitability parameters were found to be within acceptable limits. The results were shown in table-7 and indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. Limit of detection (LOD) and quantification (LOQ), the limits of detection and quantification were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = 3.3σ/s LOQ=10 σ/s. The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method. The typical chromatograms of DGF standard and tablet dosage form were shown in Figure 2,3.

Table.1: Linearity results of Dapagliflozin

Concentration (µg/ml)	Area	Average area	% RSD
25	385637		
	380158	381984	
	380158		0.82
50	757872		
	764259	761708	
	762992		0.44
75	1120567		
	1120527	1124143	
	1131335		0.55
100	1522931		
	1543878	1529862	
	1522777		0.79
125	1870059		
	1871352	1869125	
	1865965		0.15
150	2272551		
	2272709	2275299	
	2280636		0.20

Table 2; Statistical Data of Calibration Curves of DGF

Parameters	DGF
Linearity	25 – 150 µg/ml
Regression Equation	15079x – 4232.3
Average of Slope	15079
Average of intercept	4232.3
Correlation coefficient (r^2)	0.9998

Table 3; Precision of method

Parameters (n=6)	Intraday	Interday
Mean Peak Area	1540588	1510975
Standard deviation	8773.6	6231.3
%RSD	0.6	0.29

Table.4: % Recovery results of Dapagliflozin

Conc.	Dapagliflozin		
	Amount added (µg/ml)	**Amount recovered (µg/ml)**	**%Recovery**
50%	50	50.07	100.14
	50	50.30	100.59
	50	49.94	99.87
100%	100	99.77	99.77
	100	100.23	100.23
	100	101.72	101.72
150%	150	148.43	98.95
	150	148.57	99.04
	150	151.12	100.75

Table.5: % Assay results of Dapagliflozin in formulation

Tablets	Drug	Dosage (mg)	Sample concentration (µg/ml)	Amount found (µg/ml)	% Assay
1	Dapagliflozin	10	100	100.01	100.01

Table 6: System suitability Parameters

Parameters	DGF
Retention time (min)	2.226
Theoretical plates	5580
Linearity Range (µg/ml)	25 - 150
Limit of Detection (LOD) (µg/ml)	0.040
Limit of Quantification (LOQ) (µg/ml)	0.121
Relative standard deviation (RSD)	0.9998

Table 8: Robustness of method

Parameter	Variation	Average Area	%RSD
Standard	-	1540588	0.60
Flow rate	0.9 ml	1571462	0.81
	1.1 ml	1457151	0.59
Mobile phase	55:45	1612324	0.38
	45:55	1572705	0.66
Temperature	-5^{o}C	1556328	1.27
	+5^{o}C	1372587	1.90

DISCUSSION

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 drug products. The goal of this study was to develop and validate a RP-HPLC method for the estimation of DGF in bulk and pharmaceutical commercial preparations. The main objective of method development was to determine the drug content present in the formulation and its % purity. The chromatographic conditions like mobile phase composition, flow rate was optimized and the method was developed, validated successfully. The selected mobile phase system gave a single sharp peak without interfering peaks. Initial development of the method various mobile phases were tried to get sharp peak, finally buffer: acetonitrile in the ratio of 50:50 (v/v) was selected which gave a single sharp peak with retention of 2.226min. Commercial marketed formulation of DGF was analyzed for its contents and % 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. The proposed method was following linearity in the concentration range of 25-150 µg/ml and obeys the beers lamberts law and above 25-150 µg/ml the linear plot showing deviation from beers law. Every concentration was injected into the chromatographic system about three times and peak areas were noted. Greater reproducibility was obtained for calibration plots and it was determined by calculating the slope, intercept and %RSD for each standard plot. The method was found to be robust as there was no significant change in the peak area and retention time. The system suitability tests were performed to assess the quality performance of the method. The method was found to be more specific, robust and rugged and most suitable for routine analysis.

CONCLUSION

The proposed method was simple, accurate and sensitive HPLC method for the estimation of DGF in bulk and pharmaceutical dosage forms.

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