International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.01, pp 242-248, 2018

RP-HPLC Method for Estimation of Dapagliflozin from its Tablet

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Abstract : Rapid, precise and accurate RP-HPLC method for estimation of Dapagliflozin from its tablet dosage form was developed and validated as per ICH guidelines. The chromatographic separation was achieved by isocratic mode with a mixture of Acetonitrile: 0.1% Triethylamine (pH-5.0) in the ratio of 50:50v/v as mobile phase using Princeton C_{18}column at flow rate of 1mL/min and detection wavelength of 224nm. Using optimized chromatographic conditions, retention time of drug was found to be 5.163min. The proposed method obeyed Beer's-lambert's law in the concentration range of 10-70µg/mL, with correlation coefficient value 0.999.The mean percent amount of drug estimated was 100.57%, found to be good in agreement with label claim of marketed tablet formulation. The validation parameters like accuracy, precision, ruggedness, robustness, linearity and range were studied for proposed method and were found to be within limits. Stress testing under various conditions such as pH (acid/base), oxidation, temperature, light, humidity, etc. was also carried out.

Keywords : Dapagliflozin, HPLC, Validation, Stress testing.

Introduction

Dapagliflozin is a highly selective, orally active and reversible inhibitor of the human Sodium-Glucose Co-Transporter 2 (SGLT2), the major transporter responsible for the renal glucose reabsorption. It improves glyceamic control in patients with Type 2 Diabetes Mellitus by inhibiting the Sodium-Glucose Co-Transporter 2, intern by reducing glucose reabsorption. Dapagliflozin's mechanism of action is complementary to and different from the mechanisms of currently available antidiabetic drugs as it involves the direct and insulin independent elimination of glucose by the kidney. Dapagliflozin selectively block for SGLT2 over SGLT1. It is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol.

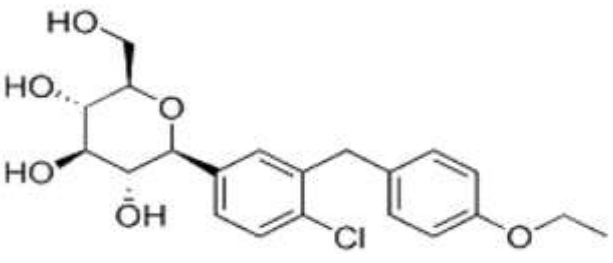

Figure No 1. Structure of DAPA

It has a molecular formula $C_{24}H_{33}ClO_8$ with molecular weight 408.98.Dapagliflozin is a white to half white crystalline powder which is soluble in ethanol, methanol, dimethylsulfoxide and dimethyl formamide.

Literature survey indicated that the drug has been estimated from bulk by RP-HPLC and UV-spectroscopy. The proposed work represents simple, economical, and rapid RP-HPLC method for the quantification of Dapagliflozinin bulk and its tablets. The developed method was validated for accuracy, precision, ruggedness and sensitivity as per ICH guidelines. Stress testing under various conditions such as pH (acid/base), temperature, light, oxidation, humidity, etc. was also carried out.

Material and Methods

Chemicals and Reagents

Pharmaceutical grade Dapagliflozin (DAPA) standard was obtained as generous gift from Indoco Remedies, Mumbai, Maharashtra, India.

Instruments:

HPLC : Shimadzu HPLC series 1100 and Jasco HPLC PU-2089 Plus
Sonicator : PCimumbai, Model No.3.5L 100H
Stability chamber : THERMOLAB, Sr. No.00002008

Development of RP-HPLC Method

Preparation of mobile phase

The mobile phase was prepared by mixing acetonitrile and 0.1% triethylamine (pH-5.0) in ratio 50:50%v/v. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter.

Preparation of standard stock solution

An accurately weighed 10.0mg of DAPA was transferred in a 10.0mL volumetric flask, dissolved in sufficient quantity of diluent to prepare a standard stock solution of 1000µg/mL of DAPA. The working standard solution of 50µg/mL was prepared by appropriate dilution of the stock solution with mobile phase.The average retention time was found to be 5.163 min.

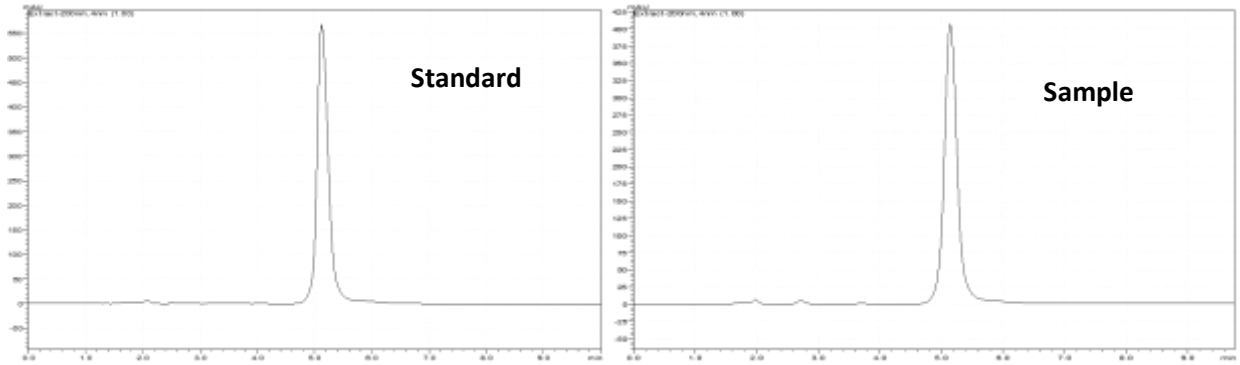

Figure No 2. Typical chromatograms of DAPA

Study of system suitability parameters

After equilibration of column with mobile phase, six replicate injections of working standard solution (50µg/mL) was injected through the manual injector and the chromatogram was recorded and the peak area was measured.

Table No 1. System suitability parameters

Results	Area (mV)	Retention time (Min)	Theoretical Plate	Asymmetry
Mean	1528.45	5.163	23265	1.235
±SD	15.7268	0.0253	298.96	0.0217
%RSD	1.02	0.4554	1.285	1.763

Each value is the mean of six observations.

Study of Beer-lamberts' law

Appropriate dilutions of standard stock solution were made to get final concentration in the range of 10-70µg/mL. Peak area was measured of each prepared solution at above selected wavelengths. The calibration curve was plotted between concentrations*vs.* peak area, having correlation coefficient 0.999.

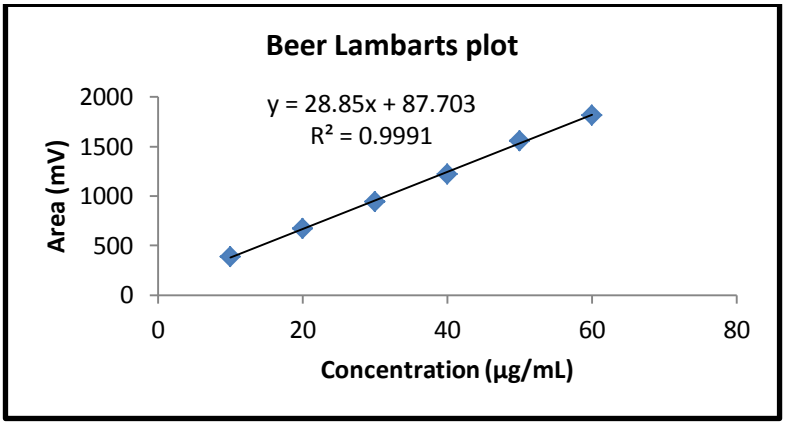

Figure No 3. Beer's Lambart's plot of DAPA

Preparation of sample

Weigh and finely powdered 20 tablets and transfer the quantity of powder containing equivalent to 10.0mg of DAPA to 10.0mL volumetric flask, sonicated for 15 min with sufficient quantity of mobile phase and volume was made up to mark with mobile phase. The content of the flask was filtered through 0.45µm nylon filter.From the filtrate, measured volume was taken and diluted with mobile phase to get the final concentration of 40*µ*g/mL. After equilibration of stationary phase, such six sample solutions were injected separately and chromatograms were recorded and the content of DAPA in each sample was determined.

Validation of Proposed HPLC Method

Validation of the proposed method was carried out as per ICH and USP guidelines.

Accuracy:

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. Weighed the pre-analyzed tablet powder equivalent to 2.5mg; a known amounts of standard drug was added at different levels 50-150%. The resultant solutions were then re-analyzed by the developed method. At each concentration, each sample was analyzed thrice at each level to check repeatability and from the data it was analyzed that the methods were found to accurate.

Precision:

Precision of any analytical method was expressed as SD and %RSD of series of measurements. Precision of estimation of DAPA by proposed method was ascertained by replicate analysis of homogeneous samples of tablets.

Ruggedness:

Ruggedness of proposed methods was performed to examine effect of non procedure related factors such as instruments and analysts. For this study Dapagliflozin (40μg/mL) was analyzed by proposed methods using two different analyst restraining similar operational and environmental conditions.

Linearity and Range:

Accurately weighed tablet powder equivalent to 80, 90, 100,110 and 120% of label claim was taken and dilutions were made as described under marketed formulation. Then each solution was injected and chromatograms were recorded. A graph of concentration Vs. Area under curve was plotted for the drug.

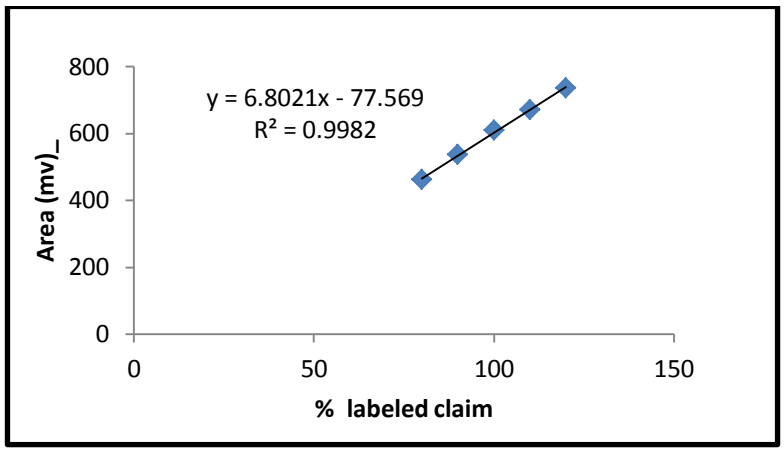

Figure No 4. Linearity and range plot of DAPA

Robustness:

It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The analysis was performed by slightly changing the pH, mobile phase composition, and detection wavelength and flow rate.

Force degradation study

Preparation of sample solutions

Solution state analysis: An accurately weighed 10.0mg of standard DAPA and quantity of tablet powder equivalent to about 10.0mg of DAPA was transferred to series of 10.0mL volumetric flasks. To each flask 10.0mL of reagent (0.1N HCl/0.1N NaOH/ 3% H_2O_2/ Distill water) was added and kept at 60^0C for a period of 180 min. The sample solutions were withdrawn at 30min interval upto 180 min for all stress conditions. The 1.0mL stressed samples were diluted to 10.0mL with mobile phase (Conc. 100µg/mL). The content of each flasks were sonicated for 15min and samples were filtered separately. A 6.0mL portion of the above sample solutions were further diluted to 10.0mL with mobile phase (Conc. 60µg/mL). (The acidic and alkaline stressed samples were neutralized prior to dilution with mobile phase). A 20µL volume of each finally diluted stressed solution was injected separately and the results of % degradation in alkaline, acid, oxidative and neutral hydrolysisare shown in Table No.4.

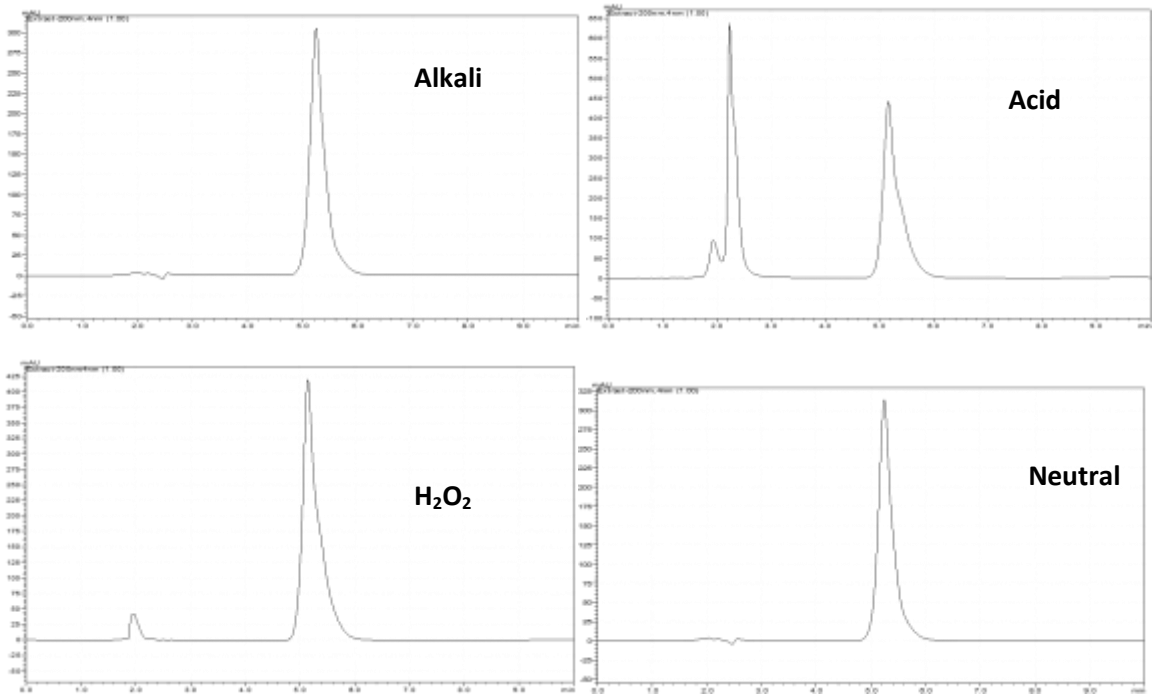

Figure No 5. Solution state degradation peak of DAPA

Solid state analysis: Standard DAPA and tablet powder was spread on petri dish kept in the oven at 60ºC, humidity chamber at 40 ºC, 75% RH and UV chamber at expect the use of UV radiation 254nm separately. After 48 hours, an accurately measured quantity of 10.0mg of Std. DAPA and weight of tablet powder equivalent to 10.0mg of DAPA was withdrawn and transferred to 10.0mL volumetric flask and volume was made up to the mark with mobile phase separately. The content of the flasks was filtered through 0.45µm nylon filter paper. A 1.0mL portion of the filtrate was further diluted to 10.0mL with mobile phase. A 6.0mL portion further diluted to 10.0mL with mobile phase (60µg/mL). A 20µL volume of each final diluted stressed solution was injected separately and the results of % degradation for solid state analysis are shown in Table No.4.

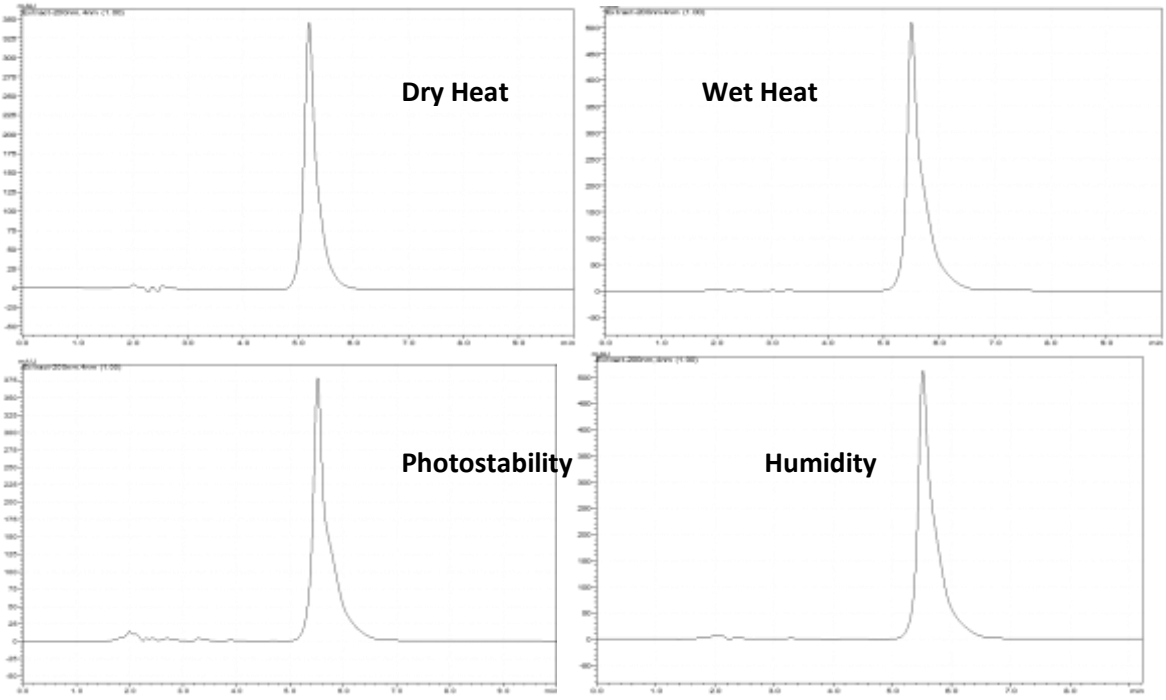

Figure No 6. Solid state degradation peak of DAPA

Result And Discussion

Dapagliflozin was found to be highly soluble in methanol and stable acetonitrile:0.1% triethylamine (pH-5.0) mixture. Using these solvents working standard solutions were prepared of desired concentration for RP-HPLC estimation of Dapagliflozin. The mean percentage amounts of Dapagliflozin estimated from tablet formulation using RP-HPLC method was found to be 100.57%.

Table No 2. Result of % estimation of DAPA

Sr. No	Wt. of tablet powder (mg)	% Label claim
1.	245.4	100.22
2.	245.2	100.38
3.	245.5	100.57
4.	245.4	100.55
5.	245.5	100.71
6.	245.3	101.04
Mean		**100.57**
±SD		**0.2825**
%RSD		**0.2808**

The % amount estimated from tablet formulation indicates that there was no interference from excipients present in it. The linearity was found to be 10-70µg/mlshowing the correlation coefficient 0.999. The method was validated for accuracy, linearity, precision, LOD, LOQ, and robustness.

Table No 3. Results of validation parameter of DAPA

Parameters	Results
Accuracy (%Mean, %RSD)	100.19, 0.59
Precision (%RSD)	0.28
Intraday (%RSD)	0.94
Interday (%RSD)	0.56
Linearity and range (r^2)	0.9978
Different analysts (%RSD)	1.12
Robustness (mean %RSD)	1.57
LOD (µg/mL)	2.1
LOQ (µg/mL)	6.39

Stress testing under various conditions such as pH (acid/base), temperature, light, oxidation, humidity, etc. was also carried out.

Table No 4. Results of solution and solid state analysis by RP-HPLC

Sr. No	Conditions	% Degradation with respect to unexposed sample
Solution state analysis		
1	Acidic hydrolysis	18.06
2	Alkaline hydrolysis	8.67
3	Oxidative study	9.67
4	Neutral hydrolysis	11.13
Solid state analysis		
5	Dry heat study	0.75
6	Wet heat study	9.78
7	Humidity study	4.81
8	Photochemical study	2.69

Table No 5. Results for kinetics of solution state analysis

Degradation medium	**Condition (At 60^0 C, 180 min)**	**Value of 'r^2'**	**Order of reaction**
Acidic	0.1N HCl	0.9288	Zero -order
Alkaline	0.1N NaOH	0.8018	Zero-order
Oxidative	3% H_2O_2	0.8712	Second-order
Neutral	Distilled water	0.9481	Zero-order

Considering the kinetic results, value of $\mathbf{r^2}$ demonstrating a lower stability of the drug in oxidative media.

Conclusion

The RP-HPLC method was developed for the determination of Dapagliflozin in tablet dosage form. The method was validated and found to be simple, sensitive, accurate, and precise. Hence, it can be used successfully for routine analysis of Dapagliflozin from its tablets. The developed method was found to be superior with respect to resolution of drug from its degradation products under applied stress conditions. Hence, methods may be adopted for routine assay of selected drug free of interferences from its degradation products in bulk and tablet formulation. The proposed validated HPLC methods in true sense can be said to Stability indicating assay method for selected drug in their bulk and tablet formulation.

Acknowledgement

The authors were thankful to Principal of Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur (MS), India for providing necessary help for the work.

Abbreviation Used

AUC: Area under curve, **API**: Active pharmaceutical ingredient, **RSD**: Relative standard deviation, **SD**: Standard deviation.

References

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