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Research Article

DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD AND IT'S FORCED DEGRADATION STUDIES

SHAKIR BASHA S[1]*, SRAVANTHI P[2]

[1]Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh, India. [2]Department of Pharmacology, Sree Vidyanikethan College of Pharmacy, Tirupathi, Andhra Pradesh, India. Email: shakirbasha72@gmail.com

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ABSTRACT

Objective: To develop and validate a simple, selective, precise, and accurate method for the estimation of dapagliflozin using reversed-phase high-performance liquid chromatography (RP-HPLC) technique in bulk and tablet formulation.

Methods: The proposed method utilizes chromatographic conditions hypersil BDS (250 mm × 4.6 mm, 5 µ), mobile phase was buffer:acetonitrile (60:40) ratio, flow rate was maintained 1 ml/minute, column temperature was set at 30°C, detection wave length was 245 nm, and diluent was mobile phase.

Results: By injecting 5 times of the standard solution system suitability parameters were studied, and results were found well under the acceptance criteria. The linearity study was performed by taking 25-150% levels, and the R^2 value was found to be 0.999, precision was found to be 0.5 for repeatability and 0.31 for intermediate precision. The % recovery was found to be 99.89%. Limit of detection and limit of quantitation were found to be 0.60 µg/ml and 1.81 µg/ml, respectively. The % purity was found to be 99.71%. Degradation study on dapagliflozin was performed and concluded that the purity threshold was more than purity angle and within the acceptable range.

Conclusion: The developed RP-HPLC method for dapagliflozin was found to be simple, precise, accurate, reproducible, and cost effective. Statistical analysis of the developed method conforms that the proposed method is an appropriate and it can be useful for the routine analysis. This method gives the basic idea to the researcher who is working in area such as product development and finish product testing.

Keywords: Dapagliflozin, High-performance liquid chromatography, System suitability, Repeatability, Purity threshold.

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INTRODUCTION

Dapagliflozin is a novel inhibitor of renal sodium-glucose cotransporter 2, which allows an insulin-independent approach to improve Type-II diabetes hyperglycemia [1]. It is a C-aryl glucoside derivative and is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D- glucitol (Fig. 1).

As per the past literature review, dapagliflozin was estimated by different chromatographic methods such as liquid chromatography–mass spectrometry [2], normal phase high-performance liquid chromatography (HPLC) [3,4], and reversed-phase HPLC (RP-HPLC) for simultaneous determination [5,6] and by different spectroscopic methods such as ultraviolet (UV) [7], UV first derivative spectroscopy [6,8], and moreover several methods were there for the determination of its pharmacologic action [9-15].

Hence, the present paper reports that to develop and validate an accurate method for the estimation of dapagliflozin by RP-HPLC method and its forced degradation studies using a different mobile phase ratio that is a mixture of orthophosphoric acid buffer and acetonitrile in (60:40). Through this study, it was found that the mobile phase ratio showed effective results.

METHODS

Chemicals and reagents

Dapagliflozin was obtained as gift sample from Manus Aktteva Biopharma, Gujarat. Acetonitrile, methanol, and water used were of HPLC grade.

Selection of wavelength

Wavelength for detection was selected by obtaining absorption spectra of dapagliflozin in methanol using double beam UV-visible spectrophotometer. The spectrum of dapagliflozin in UV range was showed in Fig. 2 and concluded that dapagliflozin shows strong absorption at 245 nm.

Instrumentation

Chromatography was performed on Waters HPLC 2996 system with Empower- 2 Software, equipped with a photodiode array detector (PDA) detector and chromatographic separation was performed using BDS column (250 × 4.6 mm, 5 µ) [16].

Chromatographic conditions

An HPLC system (make:waters, model 2996) which is operated using a Software Empower - 2, fitted with BDS column and PDA detector (at 245 nm) was used for the analysis. Gradient run with a flow rate 1 ml/minute, temperature 30°C and injection volume 10 µl was preferred for resolving the drug.

Preparation of mobile phase

A mixture (60:40) of orthophosphoric acid buffer and acetonitrile was used as mobile phase.

Buffer preparation

About 1 ml of concentration orthophosphoric acid was diluted to 1000 ml with water.

Diluents

Initially, the drug was dissolved in methanol, and water HPLC grade was used as a diluent for further dilutions.

Stock solution preparation

Weighed accurately about 10 mg of dapagliflozin working standard and transferred into 10 ml volumetric flask. To this 7 ml of diluent was added and sonicated for 5 minutes. Further, volume made with the diluent.

Standard solution preparation

Weighed accurately about 10 mg of dapagliflozin working standard and transferred into 10 ml volumetric flask. To this 7 ml of diluent was added, sonicated for 5 minutes and diluted to volume with diluent. Further diluted 1-10 ml with diluent.

Sample preparation

Crushed and powdered 20 tablets. Weighed and transferred equivalent to five tablets powder of the dapagliflozin into 50 ml volumetric flask, 30 ml of diluent was added, sonicated to dissolve for 15 minutes and diluted to volume with diluent. Further diluted the filtrate 1-10 ml with diluent.

Assay of marketed formulation

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system. Then, the percent purity was found by following formula, and the results were shown in Table 1.

$$\text{Assay}=\frac{\text{Sample area}}{\text{Standard area}}\times\frac{\text{Standard dilution factor}}{\text{Sample dilution factor}}\times\frac{\text{Average Wt of tab}}{\text{Label claim}}\times\text{Potency of std}\times 100$$

Validation parameters

The validation of an analytical method confirms the characteristics of the method to fulfill the requirements of the application domain. The method was validated according to the ICH guidelines for specificity, linearity, precision, recovery, and stability [17].

System suitability

A standard solution of dapagliflozin working standard was prepared as per procedure and injected 5 times into the HPLC system. Then, the system suitability parameters were evaluated from standard chromatograms obtained. The % relative standard deviations (RSD) of retention time, tailing factor, theoretical plates, and peak areas from five replicate injections was within range and results were shown in Table 2, and the chromatogram was shown in Fig. 3.

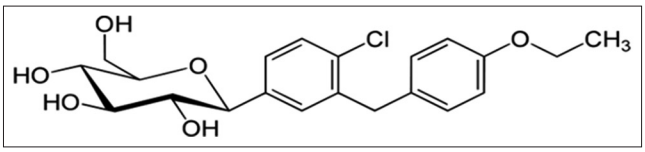

Fig. 1: Structure of dapagliflozin

Specificity

Specificity is the ability of a method to discriminate between the intended analyte(s) and other components in the sample, to check whether there is placebo, blank, impurity, and degradants interference at analyte concentration. Volume of 10 µl of working placebo sample solution was injected, and the chromatogram was recorded. Specificity graph was shown in Fig. 4.

Linearity

To demonstrate linearity of the assay method, five standard solutions with concentrations of about 25-150 ppm of dapagliflozin was injected. Then, a graph was plotted between concentrations and peak area. Linearity plot was shown in Fig. 5.

Accuracy

Three concentrations of 50%, 100%, and 150% were injected in a triplicate manner then % recovery and % RSD were calculated and shown in Table 3.

Table 1: Assay of formulation: (Label claim: 10 mg of dapagliflozin)

Formulation	Label claim (mg)	Assay	Average	SD	% RSD
Dapagliflozin	10	99.13	99.71	0.51	0.51
Dapagliflozin	10	99.46			
Dapagliflozin	10	99.32			
Dapagliflozin	10	100.46			
Dapagliflozin	10	99.75			
Dapagliflozin	10	100.12			

SD: Standard deviation, RSD: Relative standard deviations

Table 2: System suitability parameters of dapagliflozin

Peak name	RT	USP plate count	Peak area	USP tailing
Dapagliflozin	2.789	5030	736119	1.02
Dapagliflozin	2.796	5208	730712	1.02
Dapagliflozin	2.796	5013	734185	1.01
Dapagliflozin	2.797	5072	735891	1.01
Dapagliflozin	2.800	5134	731267	1.02
Dapagliflozin	2.810	5269	729905	1.01
AVG	733012			
SD	2731.3			
% RSD	0.37			

RSD: Relative standard deviations, SD: Standard deviations

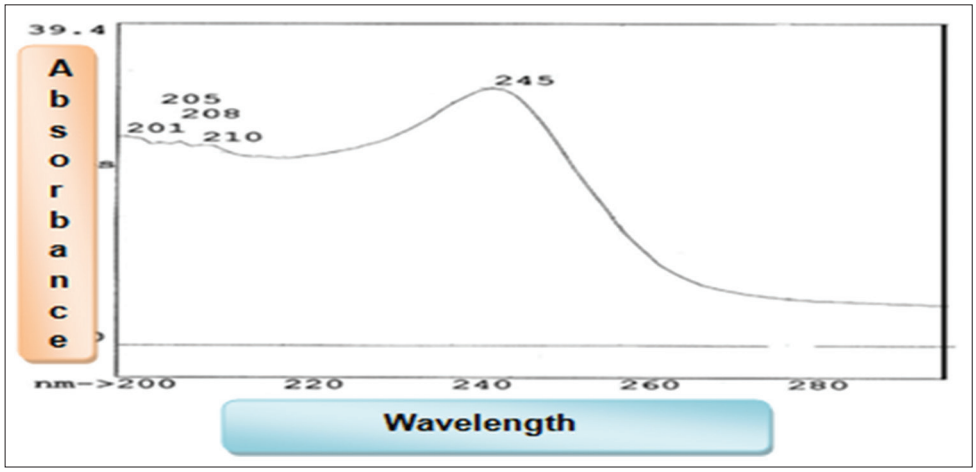

Fig. 2: Ultraviolet spectrum of dapagliflozin

Precision

Precision was estimated by studying repeatability, intra- and interday tests by injecting 100 ppm concentration of dapagliflozin. The results were calculated as standard deviation, relative standard deviation and shown in Table 4.

Limit of detection (LOD)

LOD is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated. It can be calculated from the below formula, and the results are shown in Table 5.

LOD = 3.3 σ/S

Where,
σ = Standard deviation of the response,
S = Slope of calibration curve.

Limit of quantitation (LOQ)

LOQ is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It can be calculated from the below formula, and the results are shown in Table 6.

LOQ = 10 σ/S

Where,
σ = Standard deviation of the response,
S = Slope of calibration curve.

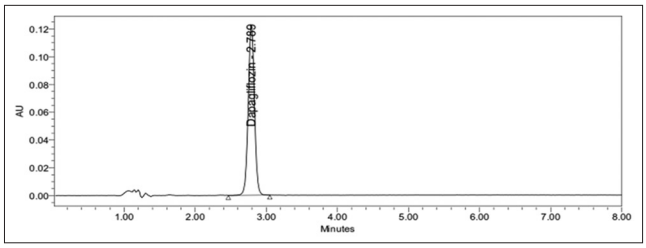

Fig. 3: System suitability chromatogram of dapagliflozin

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 temperature, mobile phase composition and flow rate. The results were calculated as % RSD and were given in Table 7.

Degradation studies

Acid degradation studies

To 1 ml of stock solution of dapagliflozin, 1 ml of 2 N hydrochloric acid was added and refluxed for 30 minutes at 60°C. Then, the resultant solution was diluted to obtain 100 µg/ml solution. Then, 10 µl solution was injected into the system. The chromatogram was recorded to assess the stability of sample which is shown in Fig. 6; % of degradation was calculated and shown in Table 8.

Alkali degradation studies

To 1 ml of stock solution dapagliflozin, 1 ml of 2N sodium hydroxide was added and refluxed for 30 minutes at 60°C. Then, the resultant solution was diluted to obtain 100 µg/ml solution, and 10 µl was injected into the system, and the chromatograms were recorded to assess the stability of sample which is shown in Fig. 6, % of degradation was calculated and shown in Table 8.

Oxidation degradation studies

To 1 ml of stock solution of dapagliflozin, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 minutes at 60°C. Then, the resultant solution was diluted to obtain 100 µg/ml solution, and 10 µl was injected into the system, and the chromatograms were recorded to assess the stability of sample which is shown in Fig. 6, % of degradation was calculated and shown in Table 8.

Thermal degradation studies

The standard drug solution was placed in oven at 60°C for 6 hrs to study dry heat degradation. Then, the resultant solution was diluted to 100 µg/ml solution, and 10 µl was injected into the system, and the chromatograms were recorded to assess the stability of sample which is shown in Fig. 6, % of degradation was calculated and shown in Table. 8.

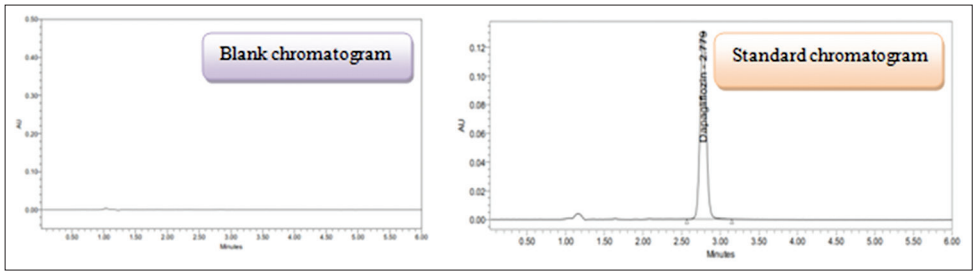

Fig. 4: Specificity chromatogram of standard dapagliflozin

Table 3: Accuracy data

Spiked concentration (%)	Amount taken PPM	Amount recovered PPM	% Recovery	Average	SD	% RSD
50	50	50.01	100.02			
50	50	50.17	100.34	99.82	0.63	0.63
50	50	49.55	99.11			
100	100	100.30	100.30			
100	100	99.84	99.84	100.00	0.26	0.26
100	100	99.87	99.87			
150	150	150.97	100.65			
150	150	149.18	99.40	99.83	0.71	0.71
150	150	149.17	99.45			
AVG	99.89					
SD	0.50					
% RSD	0.50					

RSD: Relative standard deviations, SD: Standard deviations

Photo stability (UV) degradation studies

The photochemical stability of the drug was also studied by exposing the 100 µg/ml solution to UV light by keeping the beaker in UV Chamber for 7 days or 200 Watt hrs/m^2 in photo stability chamber. For HPLC study, from this, 10 µl was injected into the system, and the chromatograms were recorded to assess the stability of sample which is shown in Fig. 6, % of degradation was calculated and shown in Table 8.

Neutral (water) degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 105°C. For HPLC study, the resultant solution was diluted to 100 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample which is shown in Fig. 6, % of degradation was calculated and shown in Table 8.

RESULTS AND DISCUSSION

The selected drug dapagliflozin was estimated by RP-HPLC method and the forced degradation studies were performed as per ICH Guidelines. The method was optimized in the mobile phase ratio of Buffer (Ortho phosphoric acid buffer): Acetonitrile (60:40). The detection was carried out at the wavelength of 245 nm (shown in Fig. 2) with a retention time 2.789 min and with peak asymmetry of 1.02 (shown in Fig. 3 and Table 2).

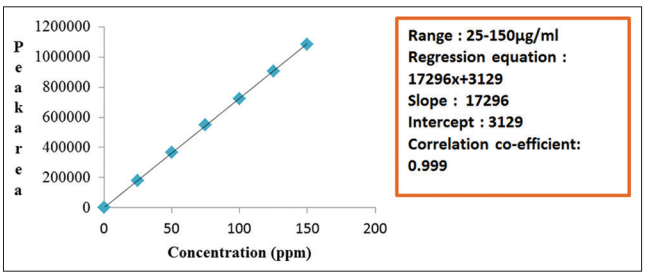

Fig. 5: Linearity plot of dapagliflozin

The method was validated as per ICH guidelines. The linearity range for Dapagliflozin was 25–150 µg/ml with R^2 (correlation co-efficient) value of 0.999 (shown in Fig. 5). The percentage RSD for method and system precision was <2% (shown in Table 2). The method has been validated in assay of dosage forms. The accuracy of the method was validated by recovery studies and was found to significant and under specification limits, with % Recovery 99.11– 100.65 % (within acceptable range (98–102%) (shown in Table 3). The assay results were found to be 99.71% (shown in Table 1) (i.e., within 98–102%). LOD and LOQ were found to be 0.60 µg/ml and 1.81 µg/ml respectively (Table 5 and Table 6). The method also passes the specifications for robustness parameters (shown in Table 7).

The sample solutions were subjected to acid, base, oxidative, thermal, UV, Neutral degradations. The % degradation in acid (7.35 %), basic

Table 4: Precision reports for dapagliflozin

Precision	SD	% RSD	Acceptance limit
System precision	2431.1	0.34	NMT 2%
Repeatability	3608.3	0.5	NMT 2%
Intermediate precision	2156.2	0.31	NMT 2%

RSD: Relative standard deviations, SD: Standard deviations

Table 5: LOD of dapagliflozin

Drug name	SD	Slope	LOD (µg/ml)
Dapagliflozin	3129	17296	0.60

SD: Standard deviation, LOD: Limit of detection

Table 6: LOQ of dapagliflozin

Drug name	SD	Slope	LOQ (µg/ml)
Dapagliflozin	3129	17296	1.81

SD: Standard deviation, LOQ: Limit of quantification

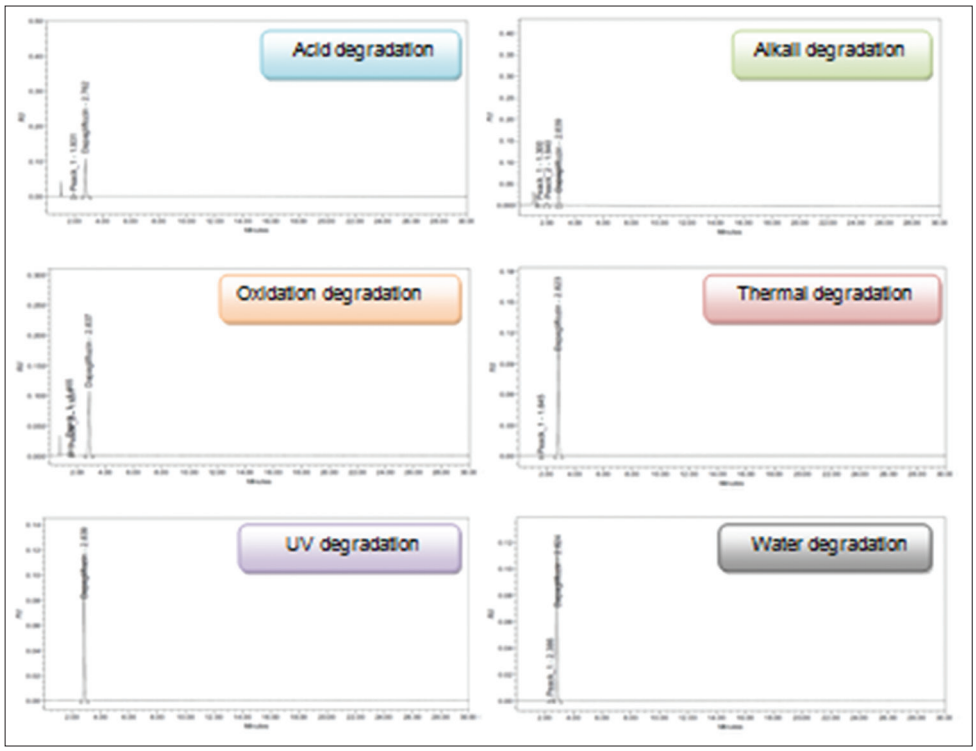

Fig. 6: Degradation studies of dapagliflozin

Table 7: Robustness data of dapagliflozin

Parameter	Modification (ml/minutes)	RT	USP plate count	USP tailing	Peak area	SD	% RSD
Flow rate	0.9	3.141	6646	1.05	721537.9	7083.5	0.98
	1.0	2.780	6846	1.19	772345.6		
	1.1	2.580	5853	1.02	635920.4		
Change in mobile phase ratio	50:50	2.679	6102	1.03	685932.8	3428.4	0.07
	60:40	2.832	6543	1.16	724563.7		
	55:45	3.011	6270	1.13	716432.5		
Temperature	25°C	2.780	6298	1.02	716014.6	3235.6	0.45
	30°C	2.782	6743	1.04	724634.7		
	35°C	2.763	6410	1.02	659511.6		

RSD: Relative standard deviations, SD: Standard deviations

Table 8: Results of degradation data of dapagliflozin

Degradation condition	Exposure	Time	% Drug degraded	Purity angle	Purity threshold
Acid	2N HCl/60°C	30 minutes	7.35	0.115	0.311
Alkali	2N NaOH/60°C	30 minutes	6.90	0.236	0.361
Oxidation	20% H_2O_2/60°C	30 minutes	5.53	0.361	0.517
Thermal	At 60°C	6 hrs	4.43	0.271	0.320
UV	UV chamber	7 days	1.91	0.113	0.303
Water	At room temp	6 hrs	0.78	0.111	0.308

UV: Ultraviolet

(6.90%), oxidative (5.53%), hydrolytic (0.78%), thermal (4.43%), photolytic (1.91%) was found (shown in Table 8).

CONCLUSION

The developed RP-HPLC method for dapagliflozin was found to be simple, precise, accurate, reproducible, and cost effective. Statistical analysis of the developed method conforms that the proposed method is an appropriate and it can be useful for the routine analysis. This method gives the basic idea to the researcher who is working in area such as product development and finish product testing. The developed method can also be used regular for the in-process quality control of the sample. This method gives the sound knowledge about stability studies. The results of the forced degradation studies show the major route of degradation is in acid hydrolysis followed by alkali, oxidation, thermal, photolytic, and neutral, respectively. The developed method concludes that the dapagliflozin was found to be stable in neutral, photolytic, thermal and oxidative stress conditions, and unstable in acidic and alkali conditions.

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