ejpmr, 2023, 10(12), 630-635

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT OF ANALYTICAL METHOD FOR THE DETERMINATION OF DAPAGLIFLOZIN IN BULK DRUG AND ITS PHARMACEUTICAL FORMULATION

Dr. R. Xavier Arulappa*[1], M. Jerubin Welsingh[2], E. Ebin Blesso, N. A. Nijil, M. Sangeetha, D. Swinton and Dr. G. R. Prasobh

[1]Professor and HOD, Department of Pharmaceutical Chemistry, Sreekrishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala.

[2]Department of pharmaceutical Chemistry, S.A. Raja Pharmacy College, Tirunelveli, Tamil Nadu.

***Corresponding Author: Dr. R. Xavier Arulappa**

Professor and HOD, Department of Pharmaceutical Chemistry, Sreekrishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala.

Article Received on 20/10/2023 **Article Revised on 10/11/2023** **Article Accepted on 01/12/2023**

ABSTRACT

Analytical method development and validation is important in development of Pharmaceutical preparations. The aim of the present work was to develop and validate a simple UV spectroscopic method for the determination of dapagliflozin in pharmaceutical dosage form. The UV spectrum of dapagliflozin in methanol showed λ max at 225nm. Beer's law obeyed in the concentration range of 5- 25µg / ml. This method was carried out according to ICH Q2R1 guidelines by taking the parameters for linearity, accuracy, precision, ruggedness and robustness. The method was rugged and robustness with % relative standard deviation less than 2. The extraction recoveries was found to be higher than 99% in all experimental conditions. Based upon the performance characteristics the proposed method was found accurate, precise and rapid and suitable for the determination of dapagliflozin for routine analysis.

KEYWORDS: Dapagliflozin, UV spectrophotometry, Method development, Validation.

INTRODUCTION

Dapagliflozin[1] is chemically 4-chloro-3(4- ethoxy benzyl)phenyl 1-6(hydroxy methyl) tetrahydro 2 H-pyran-3, 4,5-triol (Fig No 1). It's molecular formula $C_{21}H_{25}O_6Cl$. Dapagliflozin is a sodium glucose co-transporter 2 inhibitor indicated for managing diabetes mellitus type2.t helps to improve glycemic control by inhibiting glucose reabsorption in the proximal tubule of nephron causing glycosuria. It has a role as a hypoglycemic agent and sodium glucose transporter protein such type 2 inhibitor it also results in modest weight loss of about 2- 4 kg. The usual dosage is 10 mg daily, but 5 mg daily is recommended initially in patients with hepatic failure.[2] It is a white to off white amorphous powder[3] soluble in water and slightly soluble in chloroform, DMSO and methanol. As per investigation of literature,[4-10] only HPLC analytical method was developed on determination of dapagliflozin in pharmaceutical dosage form and bulk drug samples. The rationale of this work to develop an simple, accurate, rapid, precise, reproducible and cost effective spectrophotometry method for the direct quantitative determination of the dapagliflozin. In this method we developed a method for determination of dapagliflozin in bulk drug sample and tablet dosage form and validation as per international conference on Harmonizotion (ICH) Guidelines.[11]

CHEMICAL STRUCTURE OF DAPAGLIFLOZIN

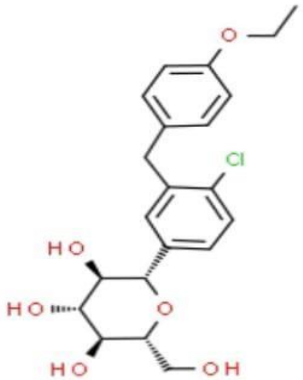

Fig. No.1.

EXPERIMENTAL MATERIALS AND METHODS

INSTRUMENTS

Spectral runs were made on a Systronics UV-spectrophotometer 2704 X Visible double beam was employed with spectral band width of 1nm and wave length accuracy of ± 0.3 nm with automatic wave length corrections with a pair of 10mm quartz cell. Wenser Analytical single pan balance was used. Glasswares usedin each procedure were soaked overnight in a

mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

LOCATION OF λ max

The working standard solution was scanned in UV range (200-400) in 0.1 cm quartz cell againstsolvent blank. The UV spectra of the drug show the spectrum wavelength selected for the estimation of drug was 225 nm as λmax. At 225 nm Dapagliflozin show maximum absorbance. (Fig.No.2).

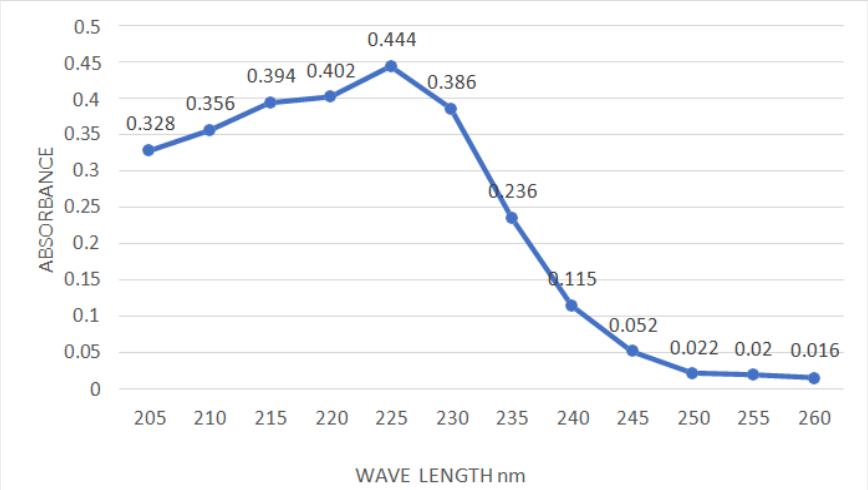

Fig.No.2: UV Spectrum of Dapagliflozin

MATERIAL

Dapagliflozin was kindly gifted from Sahana Pharmaceuticals, Nagercoil, Tamil Nadu, India. The commercially available tablets were obtained from market. Methanol AR grade was used as solvent obtained from Shiv Scientific Industries and distilled water was used obtained fromwater purification unit.

Preparation of Standard Solution

A standard stock solution was prepared by accurately weighed 25 mg of Dapagliflozin in 25 ml of volumetric flask and dissolved in diluent to obtain a concentration of 1mg/ml or 1000µg/ml (standard stock solution-I). Further diluting 5 ml of stock solution to 50 ml with diluent to get desired concentration of 100 µg/ml (standard stock solution – II).

Selection of Wavelength for Analysis of Dapagliflozin

Accurately measured 1 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted 10 ml of given concentration of 10µg/ml and it was used for initial spectral scan in the UV range of 260-205 nm to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution by allegation method.

Preparation of Series Dilutions

The serial dilutions were prepared from the standard stock solution to get a respective concentration of 5,10, 15,20 and 25 µg/ml.

METHOD VALIDATION

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte inthe sample. The range of an analytical procedure to the interval between the upper end lower concentration of an analytein the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n=3) at a concentration range of 5-25 µg/ml. The absorbance obtained at respective concentration was recorded and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software. Fig.No.3

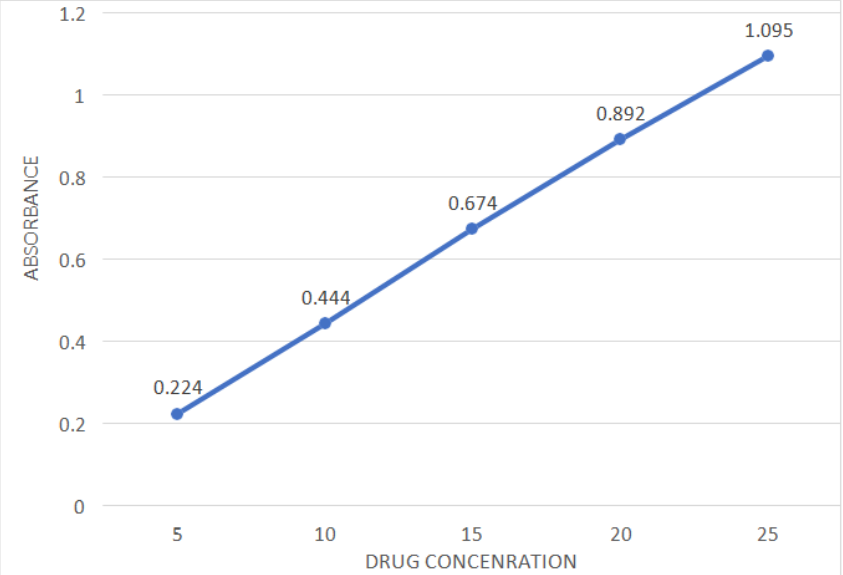

Fig.No.3 Linearity of proposed method.

ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is termed as trueness. The accuracy of proposed method was determinedon the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation). The final concentration of Dapagliflozin was determined at each level of the amount, three determinations were performed. The percentage recovery was calculated as mean ± standard deviation.

PRECISION

The precision of an analytical method is the degree of reproducibility among individual test results when the procedure was applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as standard deviation.

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in asample, which can be detected, but not necessarily quantitated as an exact value.

The limit of detection (LOD) was determined bypreparing solution of different concentration from 10-20 µg/ml

LOD = 3.3 x SD/S

Where SD = Standard deviationS = Slope

LIMIT OF QUANTIFICATION (LOQ)

The detection limit is the lowest amount of analyte in a sample which can be detected but not quantitates. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve

LOD = 10 SD/S

Where SD = standard deviationS = slope

Sensitivty

The sensitivity of the method was determined by calculating the different parameter likemolar absorptivity and Sandell's sensitivity.

Robustness

The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method the solutions of 10 µg/ml of standard Dapagliflozin solution was prepared and analysed by a change in wavelength. The wavelength was selected λmax ± 1 (i.e.) 224-226nm respectively for the standard Dapagliflozin solution.

Ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days.

To establish ruggedness of the proposed method, thesolution of 10 µg/ml of standard Dapagliflozin solution was prepared and analysed with the change in the different analyst.

RESULTS AND DISCUSSION

The proposed method for determination of Dapagliflozin showed molar 2.3×10^{3} $mole^{-1}$ cm^{-1}. From the calibration curve it was found that it shows linearity in the range 5-25µg/ml with regression coefficient 1.875. Linear regression of absorbance on concentration gave the equation y = 0.7543x+0.0032 with a correlation coefficient (r) of 0.8468. The detection wavelength showing lmax (maximum wavelength) at 225nm.

Accuracy
The percentage recovery and % RSD were calculated the mean percentage recovery and % RSD where found to bewithin limits and its less than 2, which explains the present research paper is accurate in method development of Dapagliflozin. The mean, standarddeviation and percentage relative standard deviation (%RSD) were calculated. The results were shown in table

Precision
Repeatability of the method was studied by precision experiment. The %RSD ofDapagliflozin was found to be 0.3303.

Application of The Proposed Method
The proposed method was successfully developed and validated for the determination of Dapagliflozin in pharmaceutical formulations. The proposed method was compared with the reference method.[5]

Standard Method
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 mins 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 40µg/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 the proposed method was carried out as per ICH and USP guidelines.

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition 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.

The chromatographic separation was achieved by isocratic mode with a mixture of Acetonitrile 0.1%. Triethylamine (PH-6.0) in the ratio of 50:50v/v as mobile Phase using Princeton C18 column at flow rate of 1ml/min and detection wave length 224nm using optimized Chromatographic Conditions, reaction time of drug was found to be 6. 163 min.

The Proposed method obeyed Beer's lambert's in the low 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.

STUDY OF FORMULATIONS
Accurately measured standard stock solution was diluted upto 10ml with diluent to get the concentration range 5 to 25µg/ml. The absorbance of each of the solution was measured at 225nm against blank (diluent). a calibration curve was found to be linear.

QUANTIFICATION OF FORMULATIONS
10 tablets of Dapagliflozin were taken for the analysis. The average weight of tablet was calculated and the tablet was powdered in a glass mortar. Tablet powder equivalent to 25 mg was accurately weighed was dissolved in diluent. It was filtered and the residue was washed with diluent and then the volume was made up to 25 ml with diluent (solution-I) 5ml of solution-I waspipette into 50ml volumetric flask and the volume was made up to 50ml with diluent(solution-II). From this 1mlwas pipetted into 10ml volumetric flask followed by the addition of methanol to produce final drug concentration of 10µg/ml. The absorbance of solution was measured at 225nm against blank. The same procedure was repeated five times. In similar manner standard absorbance was measured with pure drug in same final concentration that of assay method. The readings were recorded in the table no:1

VALIDATION OF PROPOSED UV-SPECTROPHOTOMETRIC METHOD ACCURACY
Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

STANDARD SOLUTION
Standard solution was prepared as per described in standard dilution.

SAMPLE SOLUTION
In order to justify the reliability and suitability of the proposed method of the recovery studieswere carried out. The recovery experiment was performed on the tablet of Dapagliflozin. The powder equivalent to 25mg was weighedaccurately and dissolved in diluent. It was filtered and the residue was washed with diluent an aliquot of 5ml of standard solution (1mg/ml) of pure sample of Dapagliflozin was added to the flask. It was shaken well and the volume was made up to 25 ml with diluent and the procedure for the assay of the tablet was followed. The experiment was repeated 5 times. The results were shown in table no:2. The percentage of recovery was calculated by using the formula,

$$\textbf{\% Recovery} = \frac{A}{B + C} \times 100$$

Where,

A ⟶ Total drug estimated (mg)

B ⟶ wt(mg) of drug contributed by tablet powder

C ⟶ Amount of pure drug added (mg)

Table 1: Result of Analysis of Tablet Dapagliflozin.

SI.No	Brand Name	Avg. Wt.of tablet(mg)	Wt.ofstd drug (mg)	Std abs	Wt .of tablet powder(mg)	Test abs	Contentdrug in tablet (mg)	Avg. Content (mg)
1	SANXIGA	150	25	0.446	390.82	0.442	9.89	9.9
					391.27	0.443	9.91	
					391.50	0.441	9.86	
					389.92	0.442	9.90	
					392.25	0.444	9.95	

±SD =0.032711 %RSD=0.330346

Table 2: Result of Recovery study.

SI No	Brand name	Wt.ofstd drug (mg)	tdabs	Avg wt. of tablet powder (mg)	Wt .of tablet powder(mg)	Amt.of pure drug added	Absorbance of recovery Sample	% of recovery
1	SANIXGA	25	0.446	391.15	391.82	5	0.462	102
2					390	5	0.470	
3					391.50	5	0.467	
4					391	5	0.470	
5					391	5	0.474	

Table 3: Assay of reference Method.

SI.No	Wt. of tablet powder	% Labelclaim	MEAN	S.D ±	% RSD
1.	245.4	100.22	100.48	0.2825	0.2808
2.	245.2	100.38			
3.	245.5	100.57			
4.	245.4	100.22			
5.	245.5	100.71			

Table 4: Optical Characteristic, Data, Precision and Accuracy of the Proposed Methodfor Dapagliflozin.

Parameter	Meter
λ Max	225
Beer's law limits (µg/ml)	5-25
Molar absorptivity (Lit $mole^{-1}$ cm^{-1})	1.68×10^{4} $mole^{-1}$ cm^{-1}
Sandall's sensitivity (µgkm^{2}/0.001 abs unit)	0.0369
Regression equation (y=a+bc)	0.1875
Slope (b)	0.1543
Correlation coefficient	0.8468
% Relative standard deviation*	0.3303%

*Average of five determinations.

CONCLUSION

The proposed UV-Visible Spectroscopy is a simple, low-cost method can be easily be applied to Dapagliflozin control sample analysis in bulk and pharmaceutical formulations. It has a morecomprehensive dynamic range for the study with excellent accuracy and precisionvalue. The proposed method does not require any laborious clean up procedure before analysis and simple methodology for its determination. Therefore, it can easily accommodate in the laboratories of research, and pharmaceutical industries for the quantification of Dapagliflozin in pure and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

I hereby express my sincere gratitude to **Dr. JACOB RAJA M.D.S, FIDA**, **Chairman, Rajas Medical Institutions**, Kavalkinaru, Tirunelveli District for providing laboratory facilities and for instructive suggestions.

The authors would like to express gratitude to **Dr. M.G. ETHAYARAJAN M.B.B.S, M. Diab., M.D.,Ph.D.,(cardio) Managing director** and **Mr. T. RAMESH, B.Pharm, General Manager, Sahana Pharmaceuticals**, Nagercoil, Kanyakumari, Tamilnadu for providing gift sample of Dapagliflozin.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCE

1. Bertram. G. katzung. MD. PhD. Basic and clinical pharmacology 14th edition released 2017. MC Grau - Hill Education / Medical Publisher, 261-262.
2. Current Index of Medical Science, 44th year; Oct-Dec, 2022; 363.
3. BRITISH NATIONAL FORMULARY (BNF) 82nd Edition Royal Pharmaceutical Society, September-March, 2021-2022; 747.

4. Manasa Sanagapti, Dhanalakshmi. K, Nagarajuna Reddy G, Sreenivasa S; Journal of Advanced Pharmacy Education and Research, 2014; 4(3): 350-353.
5. Jitendra Debata, Sundeep Kumar, Sajal Kumar Jha, and Amjad Khan; International Journal of Drug Development and Research, 2017; 9(2): 48-51.
6. Shakir Basha S, Sravanthi P; Asian Journal of Pharamaceutical and Clinical Research, 2017; 10(11): 101-105.
7. Mitali V. Verma, Chirag J. Patel, M.M Patel; International Journal of Applied Pharmaceutics, 2017; 9(5): 33-41.
8. Thiyagarajan Deepan, Magharla Dasaratha Dhanaraju; Current Issues in Pharmacy and Medical Sciences, 2018; 31(1): 39-43.
9. G.V. Mante, A.T. Hemke and M.J. Umekar; International Journal of Chem Tech Research, 2018; 11(1): 242-248.
10. B. Reddy Padmaja, B. Sivagami, R. Chandrasekar, M. Niranjanbabu; International Journal of Pharmaceutical sciences and Drug Research, 2018; 10(5): 372-378.
11. International Conference on Harmonization (ICH), Q2A. Validation and Analyticalprocedures: methodlogy, Geneva, 1994.