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Research Article

Quantitative Estimation of Dapagliflozin in Blood Plasma by Using UV Spectroscopy

Bhagwat J Bodade, Dhiraj A Kanade and Sandip S Chaudhari*

Department of Pharmaceutical Chemistry, KYDSCT's College of Pharmacy, Sakegaon Bhusawal, Maharashtra, India

ABSTRACT

An accurate, precise and reproducible quantitative estimation of dapagliflozin in blood plasma method were developed. Spectrophotometric estimation was done by absorption method followed by using sample of blood plasma of patient who are given dapagliflozin tablet. In this method λ_{max} for DAPA was selected at 224 nm at concentration range of 20-100 µg/ml for DAPA in UV spectrophotometric method (the value of r^2=0.994). The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Therefore this method is useful for quantitative estimation of drug and amount of drug absorb in blood plasma.

Keywords: Dapagliflozin; Bioanalytical; Blood plasma; ICH

INTRODUCTION

Dapagliflozin is a drug of the Gliflozin class, used for the treat type 2 diabetes. Dapagliflozin tablets are approved as a once-daily oral medication in adult patients with Type 2 diabetes mellitus.

Dapagliflozin inhibits subtype 2 of the Sodium-Glucose Transport Proteins (SGLT2) which are responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine. It was developed by Bristol-Myers Squibb in partnership with AstraZeneca Company [1].

Dapagliflozin is an inhibitor of the Sodium Glucose Co-Transporter-2 (SGLT-2), which is found almost exclusively in the proximal tubules of nephronic components in the kidneys (Figure 1).

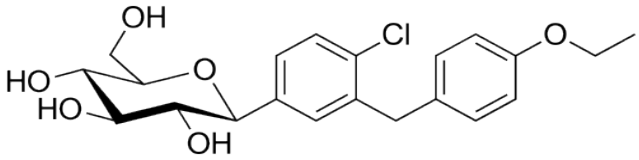

Figure 1: Chemical structure of Dapagliflozin.

MATERIALS AND METHODS

Chemicals and reagents

Dapagliflozin Tablet (Forxiga) 10 mg was obtained from local pharmacy and gives to patient and after 6 hours collect blood sample for analysis. All chemicals and reagents of analytical grade and were purchased from Merck Chemicals, Mumbai, India

Instrumentation

Samples were analysed on Shimadzu 1800 UV-visible spectrophotometer, equipped with UV spectroscopic detector, with 1 cm quartz cell and slit width 1.0 nm**.** The solvent was prepared freshly filtered through 0.45 µm membrane filter (Millipore, USA) and Sonicated for 30 min [2].

Preparation of standard stock solutions

A) Collection of blood plasma: Blood sample was transferred into purple top EDTA tubes and centrifuged (2000 rpm) at 4°C for 20 minutes. After centrifugation using clean pipette technique place 1.0 ml of plasma into 1.5 ml eppendorf tube labelled with tracking number and plasma and freeze immediately at –80° freezer [3]. After collecting blood plasma it can considered as working stock solution.

B) Preparation of working standard solution: By using the working standard solution it is further diluted by normal patient's blood plasma and prepares concentration up to 20, 40, 60, 80 and 100 µg/m for linearity study.

Correspondence to: Sandip S Chaudhari, Department of Pharmaceutical Chemistry, KYDSCT's College of Pharmacy, Sakegaon Bhusawal, Maharashtra, India, Tel: 9922246400; E-mail: Sandipc246@gmail.com

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Determination of λ_{max} of tablet

The sample of blood plasma containing dapagliflozin drug solution was scanned separately between 200 nm to 400 nm.

Study of beer-lambert's law [linearity study]

A) Linearity Study for DAPA: An accurately measured aliquot portion of working standard solution blood plasma in 10 ml volumetric flask [4]. The volume was made up to the mark using blood plasma to obtain concentrations (20-100 µg/ml). Absorbance of this solution was measured at 224

nm, Calibration curve was plotted, absorbance *vs.* concentration (Table 1 and Figures 2-7).

RESULTS

Application of the proposed method for estimation of drug

Absorbance of drug in stock solution can study by amount of drug estimated. The results are reported in the Table 2

Validation of proposed method

Validation is normally done to assure the reliability of the proposed method and was performed as per the ICH guide lines for the following criteria [5].

1) **Accuracy:** Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). Mean % recovery were found to be 98.33% for absorption method with % RSD less than 2.

2) **Precision:** Precision studies were calculated as SD and % RSD. The results within prescribed limit showed that the method was found to be precise and % RSD less than 2.

3) **Ruggedness:** The method was found to be rugged with no significant changes on test result upon change of analytical conditions like different time (Intraday), different day (Interday) and different analyst [6-8].

Table 1: Linearity study of DAPA at 224 nm.

Sr. No.	Concentration of DAPA [µg/ml]	Absorbance mean ± S.D. [n=5]	% R.S.D.
1	0	0 ± 0	0
2	20	0.229 ± 0.0015	0.152753
3	40	0.502 ± 0.0005	0.057735
4	60	0.636 ± 0.0021	0.216025
5	80	0.885 ± 0.0014	0.141421
6	100	1.078 ± 0.0020	0.206155

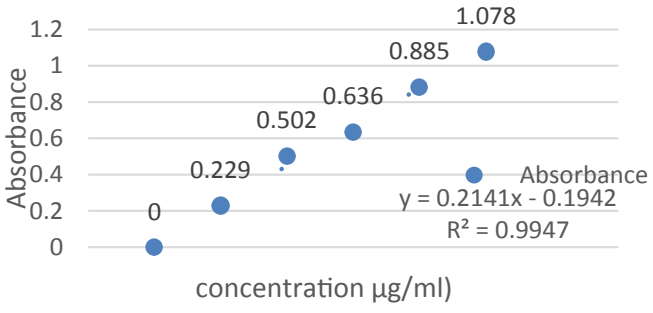

Figure 2: Calibration curve of Dapagliflozin.

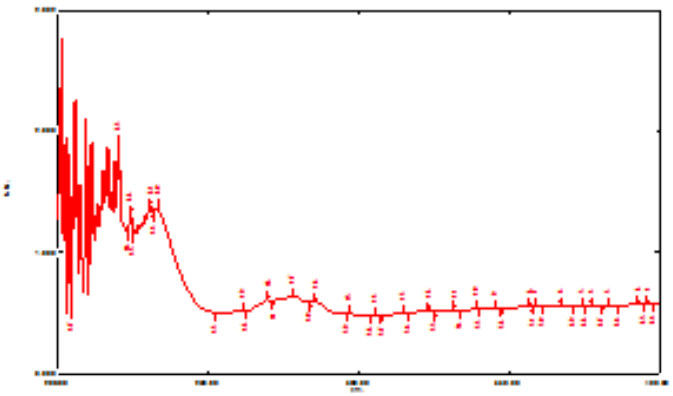

Figure 3: Calibration curve of DAPA (20 µg/ml).

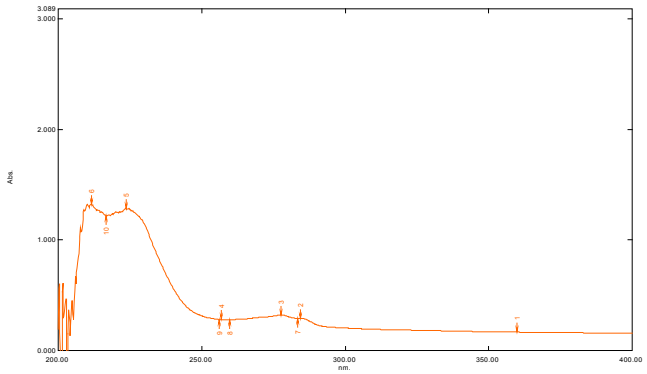

Figure 4: Calibration curve of DAPA (40 µg/ml).

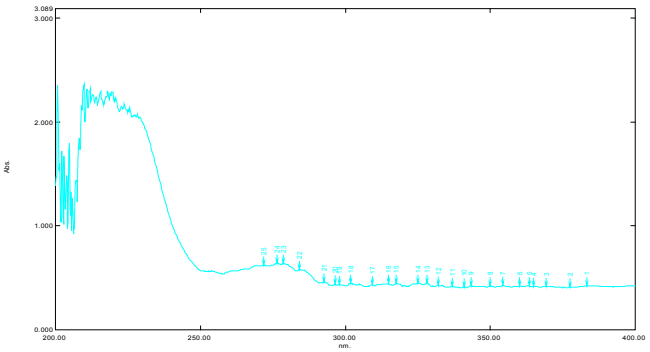

Figure 5: Calibration curve of DAPA (60 µg/ml).

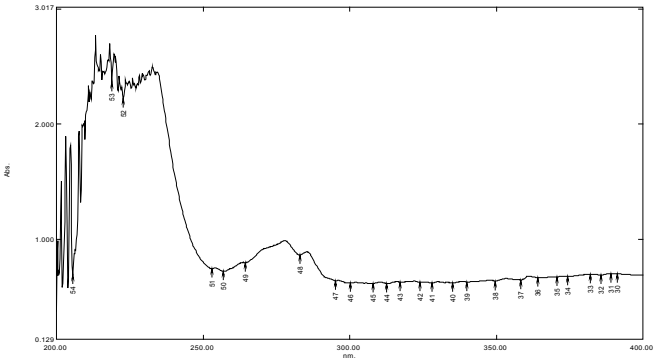

Figure 6: Calibration curve of DAPA (80 µg/ml).

4) **Linearity and range:** The study of linearity and range was performed as per the USP/ICH recommendation. DAPA marketed formulation was found to be linear in the range of 20 to 100 µg/ml, with r^2=0.994 for all drugs at selected wavelength for the methods [9,10].

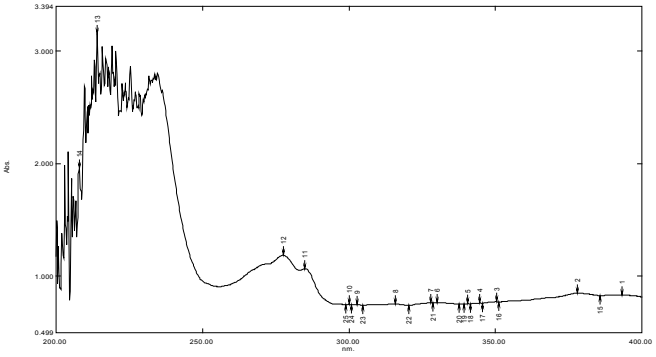

Figure 7: Calibration curve of DAPA (100 µg/ml).

Table 2: Results of estimation of dapagliflozin in sample.

Sr. No.	Concentration of drug sample	Absorbance of mixture at 224 nm	% of Drug estimated
			DAPA
1	20	0.229	98.32
2	40	0.502	98.31
3	60	0.636	98.33
4	80	0.885	98.35
5	100	1.078	98.34
Mean			98.33
SD			0.015811
% RSD			1.581139

5) **LOD:** Limit of detection of Dapagliflozin was found to be 1.09743 µg/ml.

6) **LOQ:** Limit of Quantitation of Dapagliflozin was found to be 0.01233 µg/ml.

DISCUSSION

The management of various diseases and disorders are done by the use of multiple therapeutic agents which acts at different sites. It is necessary to measurement of amount of drug estimate in blood. So that blood plasma of patient given by dapagliflozin tablet is used as sample.

On extensive literature survey it was found that very few quantitative estimation of dapagliflozin by using UV spectroscopy method were used and it seems that this method is accurate, precise and linear.

CONCLUSION

It was made to develop a quantitative estimation of Dapagliflozin tablet (Forxiga) by using UV Spectroscopy. The developed method was validated for linearity, Accuracy, precision, ruggedness and results were within the limits according to ICH guidelines. The proposed method was cost effective, simple, rapid, economic, cheap, Precise and robust.

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