ARTICLE

Bio-analytical Development and Validation of RP-HPLC Liquid Method for Quantification of Dapagliflozin Propanediol in Spiked Human Plasma

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ABSTRACT

The purpose of the present research was to develop a suitable simple and reproducible RP-HPLC method for a quantification of dapagliflozin propanediol in spiked human plasma samples. The liquid-liquid extraction plasma spiked samples of dapagliflozin propanediol were analyzed by using a ODS C18 Prontosil column under isocratic conditions. The extracted plasma spiked samples were carried using methanol, acetonitrile and pH 5.6 acetate buffer in the ratio of 50:20:10 (v/v) with a flow rate of 0.9 mL/min. The detector response was monitored at 228 nm using UV detector. The method was validated as per the ICH guidelines for bio analytical method validation and all the validation parameters were found to be within the acceptance limit The plasma spiked samples shows stability at room temperature over a period of 48 h. Thus, this method would be employed for routine quantification of dapagliflozin in human plasma samples.

KEYWORDS

Dapagliflozin, HPLC, Bio-analytical, Spiked plasma, Stability study.

INTRODUCTION

Dapagliflozin propanediol (DGPD) is a gliflozin class drug used for glycemic control in patients with type 2 diabetes mellitus. Dapagliflozin propanediol prevent glucose re-absorption in kidney by inhibiting sodium-glucose co-transporter 2.

Dapagliflozin propanediol is not recommended for patients with type 1 diabetes mellitus and treatment of diabetic ketoacidosis. Hypoglycemia, urinary tract infections and frequent urination are the major side effect associated with the use of dapagliflozin propanediol. The analytical methods available for analysis of dapagliflozin propanediol includes, four HPLC formulation assay [1-4], two stability indicating methods [5,6], formulation assay with other combination as simultaneous assay method [7-12], UV spectrophotometric [13-16] and LCMS methods [17]. The literature survey indicate that there is no bioanalytical method for the analysis of dapagliflozin propanediol in biological samples like plasma and hence the present study was aimed to develop the bio-analytical method development and validation for dapagliflozin propanediol in plasma.

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EXPERIMENTAL

Dapagliflozin propanediol was kindly gifted by Sun Pharmaceutical Industries Ltd. India and used as reference Standard without further purification. HPLC grade methanol, water and acetonitrile were purchased from Merek Specialties Pvt. Ltd Mumbai, India. Purification of all solvents and solutions was done by using the 0.45 µm Millipore membrane filter and degasses before use by Ultrasonication.

Chromatography was performed using PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), analytical column prontosil ODS C18 (250 mm × 4.6 mm, 5 µm) Colum. Electronic balance-DENVER (S1234), manual rheodyne injector with a 20 µL loop was used for the injection of sample. PEAK LC software was employed.

Preparation of standard stock solution: An accurately weighed 25 mg of dapagliflozin propanediol was weighed accurately and was dissolved in 25 mL methanol. Based on the potency of dapagliflozin propanediol in the standard drug, the concentration of the prepared stock solution was calculated. Then it was filtered and the solution is labeled appropriately and then stored in the refrigerator below 8 °C.

Extraction of drug from plasma: A simple liquid-liquid extraction method was employed for the extract dapagliflozin propanediol drug from plasma. An aliquot of 15 mL of plasma sample and 1.5 mL standard drug was added into a clean glass tube and vortex-mixed for 30 s followed by the addition of 2 mL of diethyl ether into each tube. The vortex-mixing was repeated for further 2 min followed by centrifugation at 4000 rpm for 5 min at 4 °C. The upper organic layer was transferred into a clean tube and evaporated to dryness under a gentle stream of nitrogen at 35 °C. The residue was reconstituted with 200 mL of mobile phase out of which 20 mL was injected into the HPLC for analysis.

Preparation of plasma spiked calibration curve dilutions: The prepared standard dilutions of dapagliflozin propanediol were used to spike the screened blank human plasma matrix to prepare plasma calibration curve standards in the range of 90 to 630 µg/mL. The liquid-liquid extraction method was followed for the extraction of dapagliflozin propanediol from plasma matrix for the preparation of plasma spiked calibration curve dilutions. The extracted plasma spiked calibration curve dilutions were taken in the pre-labeled polypropylene vials, which were then capped tightly and stored in a freezer at -70 °C.

Method validation: The method was validated according to the international council of harmonization (ICH) guidelines for the validation of bio-analytical method.

The selectivity of the method developed for dapagliflozin propanediol was determined by investigating the chromatographic interference of the blank plasma matrix. The human blank plasma samples was extracted using the same extraction procedure and was analyzed in the developed method. The results observed for blank plasma matrix was compared with analyte were at LOQ level.

The prepared plasma spiked calibration curve dilutions of dapagliflozin propanediol were analyzed in the developed method (Table-1) and calibration curve was constructed using peak area responses observed *vs*. the concentration of dapagliflozin propanediol.

TABLE-1
PLASMA SPIKED CALIBRATION CURVE RESULTS FOR DAPAGLIFLOZIN PROPANEDIOL IN THE OPTIMIZED METHOD

Concentration (ng/mL)	Peak area
90	135487
180	255445
270	393865
360	512569
450	623992
540	775066
630	887573
Slope	1399
Intercept	8338
r^2	0.999

Intraday and inter-day variations of the experimental results was determined by analyzing the plasma extracted samples in HQC, MQC and LQC levels in the calibration range. The % relative standard deviation (RSD) of the replicate experiments of plasma extracted samples of dapagliflozin propanediol was calculated.

The stability of studied drug in human plasma was assessed under different study conditions: *i.e.*, standing at ambient temperature over 24 h (Bench top-stability) using QC samples at low, medium and high concentration levels and storing at -20 °C for one month (long-term stability) QC samples experiencing three freeze-thaw cycles (freeze-thaw stability) were analyzed together. The stability study results of dapagliflozin propanediol in biological plasma matrix were expressed as percentage recoveries.

RESULTS AND DISCUSSION

Dapagliflozin propanediol was chromatographically separated from matrix components under a isocratic elution profile with a prontosil ODS C8 column. The influence of organic modifier concentration and pH were carefully studied. Utilizing methanol alone as organic modifier not only improves peak shape and decrease the run time but also decrease the method specificity due to the interference of the dapagliflozin propanediol peak with endogenous biological substance. Decreasing of organic modifier concentration resulted in high specificity with regard to the separation of the studied drug from endogenous biological substances and more retained of the drug on the column that led to excessive tailing of eluting peaks and long run time. Inclusion of acetonitrile in the mobile phase and decrease the strength of methanol improves the specificity and system suitability.

Variation of pH plays an important role in the separation process pH 5.6 was chosen as the optimum value both for separation of drug from endogenous biological substances and better peak shape and reasonable run time. The optimized separation was achieved using a mobile phase of methanol, acetonitrile and pH 5.6 acetate buffer in the ratio of 50:20:10 (v/v) at a flow rate of 0.9 mL/min. the UV absorbance characters

that show high absorbance at the chosen wave length (228 nm) that increase the sensitivity of the method. Analysis was completed at a run time of 10 min and the optimized chromatogram was given in Fig. 1.

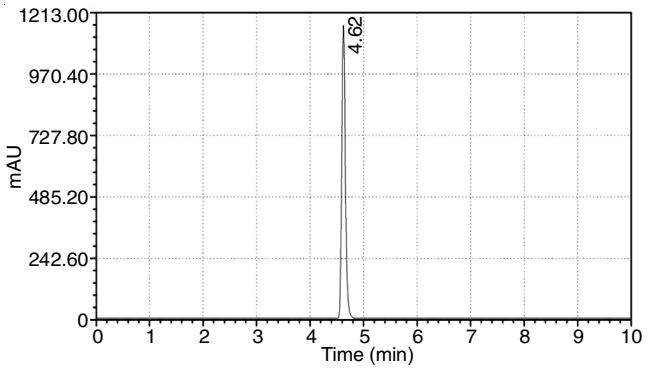

Fig. 1. Standard chromatogram of dapagliflozin propanediol in spiked plasma

Matrix effect is investigated to ensure that selectivity and precision are not compromised within the matrix screened. The matrix effect was not observed in the method developed for the analysis of dapagliflozin propanediol in biological samples. Typical chromatogram obtained from a plasma sample spiked with dapagliflozin propanediol is presented in Fig. 1. Hence, the developed method was found to be selective and specific for dapagliflozin propanediol.

A calibration curve was established on each validation day. Linearity was established by analyzing seven concentrations of dapagliflozin propanediol ranging between 90-630 mg/mL by plotting the peak area against the corresponding concentration. Linearity of the calibration graphs was validated by the high value of the correlation coefficient (> 0.999) and the linear regression equation was found to be y = 1399.1x + 8338.6 (Fig. 2). The calibration range for the proposed method was established through considerations of the practical range required and the concentrations of dapagliflozin propanediol present in the biological samples to give accurate, precise and linear results.

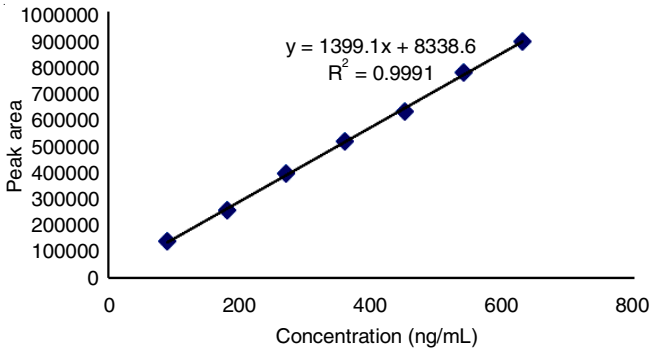

Fig. 2. Plasma spiked calibration curve for dapagliflozin propanediol in the optimized method

HQC, MQC and LQC levels in the calibration range of dapagliflozin propanediol was studied for intraday and interlay precision studies. The % recoveries and the % RSD was calculated and the % mean recoveries were found to be 86.98,87.70 and 85.28 % and the % RSD of recovery was found to be 0.222,0.802 and 1.941 in HQC, MQC and LQC respectively. High % recoveries were obtained for dapagliflozin propanediol in the developed method.

The accuracy of the method was determined by calculating relative error (RE) and the precision by calculating RSD. Table-2 summarizes the precision and accuracy on each of dapagliflozin propanediol in human plasma. The % RSD was found to be 0.903, 1.215 and 1.719 in HQC, MQC and LQC respectively. Since the % RSD was found to be < 2, hence method was found to be precise and accurate.

TABLE-2
PRECISION AND ACCURACY RESULTS

Concentration (ng/mL)	HQC	MQC	LQC
630	898179	511719	136974
630	876573	524326	132439
630	884188	520573	132184
630	883369	511729	130637
630	892800	512593	132935
630	880840	507871	135212
RSD (%)	0.903	1.215	1.719

The HPLC method developed for the estimation of dapagliflozin propanediol was found to be robustness because when we tried to induce minor deliberate changes in the organic strength (± 5 %), pH (± 0.1 unit) of the mobile phase and detector wavelength there is no significant changes was observed in the results. In all the changed conditions, the retention time of dapagliflozin propanediol peak was not significantly affected (± 0.05 min) and the % change in the peak area of dapagliflozin propanediol was found to be very less (less than 2 %). Hence there is no significant change in results was observed when change in the method conditions.

In bench term top stability studies, the measured concentrations of the drugs in these QC samples incubating at room temperature for 24 h were compared with that obtained with the corresponding QC sample freshly prepared and proceed immediately. The % RSD was found to be 0.845, 1.703 and 0.881 in HQC, LQC and MQC levels respectively (Table-3). The results indicate that the studied drugs were stable for at least 24 h in human plasma when stored at ambient temperature. On the other hand, QC samples experiencing three freeze-thaw cycles (Freeze-thaw stability) were analyzed together. The % RSD was found to be 1.834, 1.492 and 1.946 in HQC, LQC and MQC levels respectively in freeze-thaw stability study (Table-3). Also the studied drug showed the stability in human plasma when stored at -20 °C for one month as long term stability when compared with the freshly prepared sample.

Conclusion

The present HPLC method for identification and quantification of dapagliflozin propanediol in human plasma proved to be simple, sensitive, precise, accurate, robust and reproducible in accordance with the ICH guidelines. The drug dapagliflozin propanediol was found to be stable in the sample placed at room temperature over a period of 48 h and freez thaw studies. As there is no method was reported for the estimation of dapagliflozin propanediol in biological samples, the method reported here was found to be best choice for the routine analysis of dapagliflozin propanediol in biological samples.

TABLE-3
STABILITY RESULTS

S. No.	Long term stability			Freeze thaw stability			Bench top stability		
	HQC at 630 ng/mL	MQC at 360 ng/mL	LQC at 90 ng/mL	HQC at 630 ng/mL	MQC at 360 ng/mL	LQC at 90 ng/mL	HQC at 630 ng/mL	MQC at 360 ng/mL	LQC at 90 ng/mL
1	875260	516314	130158	897153	516438	142775	886823	527895	143573
2	885362	510106	136270	896676	513220	147953	895215	515782	143038
3	906952	513466	133100	893188	508033	147417	899582	512900	145516
4	897153	511235	135282	899523	521833	141567	887756	527447	143462
5	882075	511242	136228	857239	517876	146625	886558	504932	145680
6	885714	513260	134889	898024	500566	142554	878064	518266	145765
RSD	1.282	0.435	1.746	1.834	1.492	1.946	0.845	1.703	0.881

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