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Original Article

# A VALIDATED LC-MS/MS METHOD FOR PHARMACOKINETIC STUDY OF CANAGLIFLOZIN IN HEALTHY RABBITS

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**ABSTRACT**

**Objective:** A liquid chromatography-tandem mass spectrophotometric (LC-MS/MS) method was developed for quantification of canagliflozin in rabbit plasma employing Liquid-Liquid extraction technique.

**Methods:** Chromatographic separation was achieved on Inertsil ODS 5 µm C18, 50×4.60 mm with 30:70 v/v of 0.01M ammonium acetate: methanol as an isocratic mobile phase with a flow rate of 0.8 ml/min. The developed LC-MS method was applied to assess Cmax, $t_{1/2}$, $AUC_{0-t}$, and $AUC_{0-inf}$ of canagliflozin tablet after oral administration in healthy rabbits.

**Results:** The developed method was linear over working range of 5ng/ml to 600ng/ml with a coefficient of correction ($r^2$) = 0.999. The % recovery of the method was found to be 102.05%. The mean intraday and inter-day precision of the method was found to be 0.77 to 3.72%. The Canagliflozin showed $T_{max}$ of 1.58±0.2 and mean $C_{max,}$ $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \alpha}$ for Test formulation is 272±13.24, 2571.20±251and 2777.43±276 respectively.

**Conclusion:** The developed method can be applied for routine analysis for quality control and the established LLOQ is sufficiently low to conduct a pharmacokinetic study with any marketing formulation of Canagliflozin in healthy rabbits.

**Keywords:** Canagliflozin, Pharmacokinetics, LC-MS/MS, Diabetes mellitus


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## INTRODUCTION

Canagliflozin, an orally active inhibitor of sodium glucose co-transporter 2 (SGLT2), is currently in development for the treatment of patients with type 2 diabetes mellitus [1-2]. Inhibition of SGLT2 causes inhibition of glucose reabsorption in renal proximal tubular cells, thereby reducing the renal threshold for glucose (RTG) [3-4]. As per the Literature Survey, it is revealed that the Ultraviolet spectroscopy (UV), High-Performance Liquid Chromatography (HPLC) and High-Performance thin layer Chromatography (HPTLC) methods were reported for degradation studies and to estimate the canagliflozin from bulk and Pharmaceutical dosage forms [5-10]. Ultra High Performance/liquid Chromatography-Mass Spectroscopy (UHPLC-MS and LC-MS/MS) methods were reported for quantification of the drug in biological fluids like human and rat plasma [11, 12]. To best of our knowledge, no published LC-MS/MS-based methods for the pharmacokinetic study of canagliflozin in healthy rabbits. Therefore a liquid chromatography-tandem mass spectrophotometric (LC–MS/MS) method was developed, validated and applied for quantification of canagliflozin in rabbit plasma employing liquid-liquid extraction (LLE) technique. The established LLOQ is sufficiently low to conduct a pharmacokinetic study with any marketing formulation of canagliflozin in human volunteers.

## MATERIALS AND METHODS

### Apparatus and software

The HPLC system with an autosampler was a shimadzu LC-20ADvp, shimadzu, japan, coupled with applied bio system sciex, MDS Sciex, Canada, API 4000 tandem mass spectrometer. The autosampler was SIL-HTC from shimadzu, Japan. The solvent delivery module was LC-20AD from shimadzu, Japan. The chromatographic integration was performed by analyst software, version: 1.4.2; applied bio systems.

### Chemicals and reagents

Canagliflozin and empagliflozin (IS) were obtained on request from MSN Life Sciences Pvt. Ltd. Hyderabad and Mylon laboratories, Hyderabad respectively, formic acid were procured from Merck Specialities Pvt. Ltd, Mumbai, India. Water used was collected from water purification systems (Milli Q, Milli Pore, USA) installed in the laboratory. Methanol and acetonitrile were of HPLC grade and were supplied by J. T. Baker, USA. Hyderabad. The plasma was obtained from rabit blood by freeze centrifugation. The study was approved by institutional ethical committee no: VCP/IAEC/2016-48.

### Calibration standard solutions

Stock solutions of canagliflozin and empagliflozin internal standard (IS) were prepared in methanol. Further dilutions were carried out in 70 % methanol. Calibration standards of eight concentration levels were prepared freshly by spiking drug-free plasma with canagliflozin stock solution to give the concentrations of 5.00, 10.00, 20.00, 40.0, 100, 200, 400 and 600ng/ml.

### Quality control standards

Lowest quality control standards, median quality control standards and highest quality control standards were prepared by spiking drug-free plasma with canagliflozin to give a solution containing 10, 300 and 500 ng/ml respectively. They were stored at-20 °C till the time analyzed.

### Chromatographic conditions

Chromatographic separation was performed on inertsil ODS 5 µm C18, 50×4.60 mm with 30:70 v/v of 0.01M ammonium acetate: methanol as an isocratic mobile phase with a flow rate of 0.8 ml/min. Injection volume was 5µl. Total analysis time of single injection was 2.00 min. Column oven temperature and autosampler temperature was set to 40 °C and 5 °C, respectively.

### Mass spectrometric conditions

The LC eluent was split (75%), and approximately 0.25 ml/min was introduced and quantitation was achieved with MS/MS detection in negative ion mode for the analytes and IS using a MDS Sciex API-4000 mass spectrometer (Foster City, CA, USA) equipped with Turboion spray interface at 400 °C. The ion spray voltage was set at 5500 V. The source parameters viz., the nebulizer gas, curtain gas, CAD gas were set at 8, 10 and 6 psi, respectively. The compound

parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) for canagliflozin and canagliflozin-D3 were similar and are 18, 10, 10, 10 V. A turbo ion spray interface (TIS) operated in positive ionization mode was used for the detection. Detection of the ions was carried out in the multiple-reaction monitoring mode (MRM), by monitoring the transition pairs of m transitions of m/z 462.1→267.0 for canagliflozin, and m/z 451.2 →71.0 for empagliflozin. Quadrupoles Q1 and Q3 were set on the unit resolution.

**Study design**

Six male albino rabbits (Weighing about 2.5 kg) procured by vijaya college of pharmacy which was obtained from the approved vendor. The rabbits selected for the study was approved by institutional ethical committee no: VCP/IAEC/2016-48. The age of the rabbits was 8-12 w and had no medication for two weeks prior to the study. Twelve hours before drug administration, food was withdrawn from the rabbits until 24 hr post-dosing, while, water was available for rabbits throughout the study. The tablet with the dose of 13 mg based on the animal surface area was administered to rabbits. T-Blood samples (0.6 ml) were withdrawn from the marginal ear vein before dosing (zero time) and at time intervals of 0.15, 0.25,0.5 0.75, 1,1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 20 and 24 h after administration. For each animal, the total number of blood samples drawn during the study was 18. EDTA disodium salt was used as an anticoagulant. Plasma was separated by centrifugation at 5000 rpm for 10 min and the resulting plasma sample from each blood sample was divided into two aliquots and stored in suitably labelled polypropylene tubes at 20 °C until used. All the plasma samples were analysed under the construction of standard calibration curve of canagliflozin in rabbit's plasma. The canagliflozin concentrations in the rabbit plasma samples were calculated using the calibration curve, obtained after linear regression of the peak area ratio (canagliflozin/empagliflozin) versus the concentration of canagliflozin.

**Sample preparation method**

To 250 µl of plasma, 50 µl of empagliflozin (50ng/ml) was added and vertexed. The drug was extracted with 3 ml of TBME (tertiary butyl methyl ether) followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15 min at 4 °C. The organic phase was withdrawn and dried using lyophiliser. To the residue, 250 µl of mobile phase was added and inject by using HPLC-ESI-MS/MS.

**Pharmacokinetic analysis**

Single dosage pharmacokinetic parameters were calculated using PK Solver tool from plasma drug concentration-time data by non-compartmental methods. The maximum plasma concentration ($C_{max}$) and time to maximum plasma concentration ($T_{max}$) were obtained directly from the observed concentration-time profiles. The linear trapezoidal rule was used to estimate the area under the plasma concentration versus time curve (AUC) from 0 to the last measurable concentration (AUC 0-t). The area under the plasma concentration versus time curve from 0 to infinity (AUC 0-∞) was calculated as AUC 0-t+Ct/ke, where Ct was the last measurable concentration. Ke was the elimination rate constant. The terminal elimination half-life (t1/2) was calculated as 0.693/Ke.

**Validation**

**Specificity**

A solution containing 4.0ng/ml was injected on to the column under optimized chromatographic conditions to show the separation of canagliflozin from impurities and plasma. The specificity of the method was checked for the interference from plasma.

**Linearity**

Spiked concentrations were plotted against peak area ratios of canagliflozin to the internal standard and the best fit line was calculated. Wide range calibration was determined by solutions containing 5ng/ml to 600ng/ml.

**Recovery**

The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against unextracted Quality control samples at HQC, MQC and LQC.

**Precision and accuracy**

Intraday precision and accuracy was determined by analyzing quality control standards (10, 300and 500ng/ml) and LLOQ Quality control standard (4.00 ng/ml) five times a day randomly, interday precision and accuracy was determined from the analysis of each quality control standards (10, 300and 500ng/ml) and LLOQ quality control standards (4.00 ng/ml) once on each of five different days.

**Matrix effect**

The matrix effect for the intended method was assessed by using chromatographically screened human plasma. Concentrations equivalent to LQC and HQC of canagliflozin were prepared with six different lots of plasma and are injected.

**Stability studies**

The stability of canagliflozin was determined by measuring concentration change in control samples overtime under set conditions. Freeze-thaw stability study (-80 °C) of canagliflozin was carried out by subjecting samples to three freeze and thaw cycles. Samples before the study and after study were analysed by developed method. Similarly stock solution stability study of canagliflozin (Stability after 6 H), Long-term stability (-80 °C, 30days), benchtop stability study of canagliflozin (at ambient temperature, 6h), dry residue stability(4 °C, 48h) and Auto-sampler stability (4 °C, 24h) of canagliflozin were carried out by subjecting samples to study conditions.

**RESULTS AND DISCUSSION**

**Results of method validation**

The chromatography observed during the course of validation was acceptable and representative chromatograms of standard blank, HQC, MQC and LQC samples are shown in (fig. 1-4).

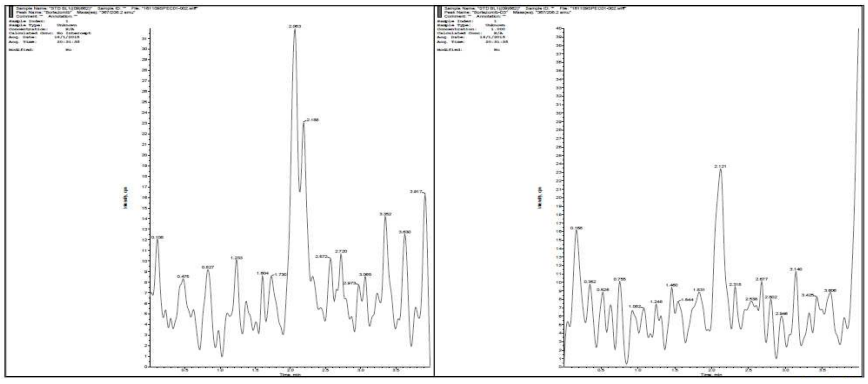

**Fig.** 1: **Representative blank chromatograms of canagliflozin and IS in blank plasma**

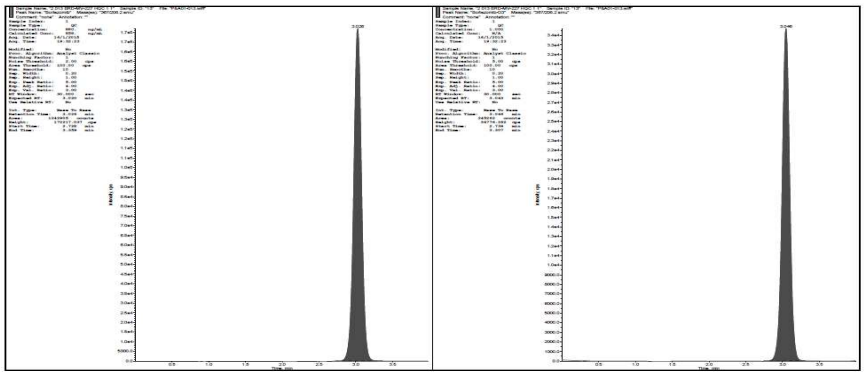

**Fig. 2**: **Representative HQC-chromatograms of canagliflozin in plasma with internal standard**

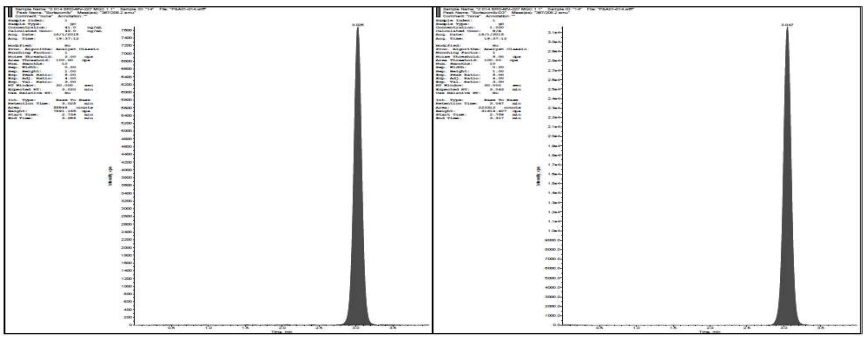

**Fig. 3: Representative MQC-chromatograms of canagliflozin and its internal standard**

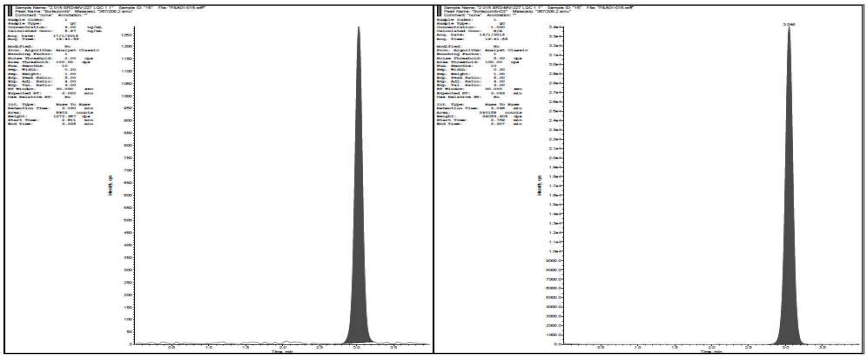

**Fig. 4: Representative chromatograms of canagliflozin and its internal standard at LQC Level**

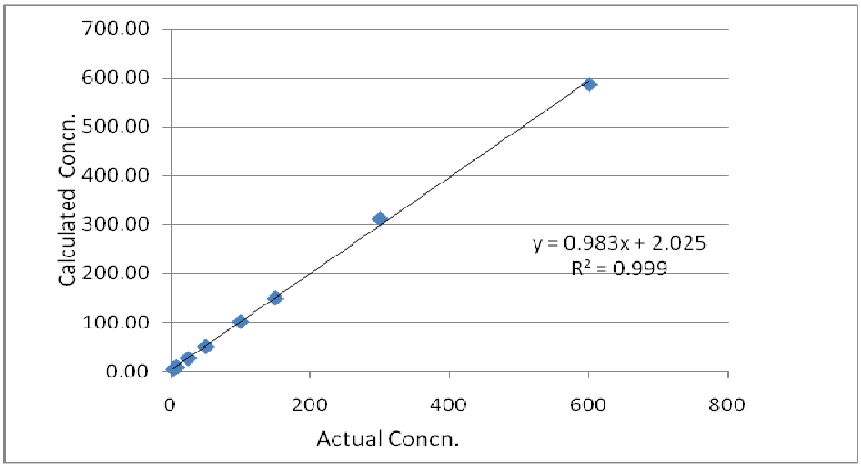


**Fig. 5**: **Calibration curve**

**Table 1: Summary of linearity standards for canagliflozin**

| Actual conc. (ng/ml) | 4 | 8 | 25 | 50 | 100 | 150 | 300 | 600 | Slope | Intercept |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.95 | 7.73 | 25.02 | 50.9 | 102.7 | 150.33 | 309.5 | 586.5 | 0.983 | 2.493 |
| 2 | 3.875 | 7.73 | 25.25 | 49.96 | 100.53 | 148.67 | 315.5 | 588.75 | 0.99 | 1.854 |
| 3 | 3.975 | 7.86 | 25.44 | 49.23 | 98.76 | 147.67 | 307 | 582 | 0.976 | 1.729 |
| Mean | 3.93 | 7.78 | 25.24 | 50.03 | 100.67 | 148.89 | 310.67 | 585.75 | 0.983 | 2.025 |
| ±SD* | 0.052 | 0.077 | 0.208 | 0.835 | 1.970 | 1.347 | 4.368 | 3.436 | 0.007 | 0.409 |
| %CV** | 1.32 | 0.99 | 0.83 | 1.67 | 1.96 | 0.90 | 1.41 | 0.59 | | |
| % Accuracy | 98.33 | 97.22 | 100.96 | 100.06 | 100.66 | 99.25 | 103.55 | 97.625 | | |
| LOD*** | 1.38 | | | | | | | | | |
| LOQ**** | 4.17 | | | | | | | | | |

*Standard deviation, **coefficient of variation, ***limit of detection, ****limit of quantification.

The method developed was validated for linearity, accuracy and precision, and stability as per ICH guidance [13-19]. The results of validating parameters are given below.

**Linearity**

The three calibration curves (peak area ratio Vs concentration) were linear over working range of 5ng/ml to 600ng/ml with eight-point calibration used for quantification by linear regression (fig 5 and table 1). The regression equation for the analysis was 0.983x-2.025with coefficient of correction ($r^2$) = 0.999.

**Recovery**

The % mean recovery for canagliflozin in LQC (10 ng/ml), MQC(300 ng/ml) and HQC (500ng/ml) was 95.83%, 103.97% and 106.35% respectively (table 2).

**Intraday and inter-day precision**

The mean intraday and inter-day precision of the method was found to be 0.77 to 3.72% for the quality control samples. This is within the acceptance limits of precision is 15% (table 3).

**Table 2**: **The % mean recovery of canagliflozin for LQC, MQC and HQC**

| ID | LQC | | | MQC | | | HQC | | |
|---|---|---|---|---|---|---|---|---|---|
| | Un extracted (area ratio) | Extracted (area ratio) | % recovery | Un extracted (area ratio) | Extracted (area ratio) | % recovery | Un extracted (area ratio) | Extracted (area ratio) | % recovery |
| 1 | 0.164 | 0.151 | 92.073 | 0.956 | 0.984 | 102.93 | 2.077 | 2.158 | 103.90 |
| 2 | 0.161 | 0.146 | 90.683 | 0.97 | 0.992 | 102.27 | 2.117 | 2.16 | 102.03 |
| 3 | 0.147 | 0.144 | 97.959 | 0.977 | 0.977 | 100.00 | 1.914 | 2.144 | 112.02 |
| 4 | 0.157 | 0.153 | 97.452 | 0.922 | 0.98 | 106.29 | 2.048 | 2.151 | 105.03 |
| 5 | 0.146 | 0.145 | 99.315 | 0.927 | 0.985 | 106.26 | 1.989 | 2.096 | 105.38 |
| 6 | 0.163 | 0.159 | 97.546 | 0.932 | 0.989 | 106.12 | 1.973 | 2.166 | 109.78 |
| Mean | 0.156 | 0.150 | 95.838 | 0.947 | 0.985 | 103.977 | 2.020 | 2.146 | 106.356 |
| ±SD* | 0.008 | 0.006 | 3.546 | 0.023 | 0.006 | 2.644 | 0.075 | 0.026 | 3.774 |
| %CV** | 5.11 | 3.87 | 3.70 | 2.48 | 0.56 | 2.54 | 3.70 | 1.19 | 3.55 |

*Standard deviation, **coefficient of variation.

**Matrix effect**

The % CV for HQC and LQC samples was observed 3.39% and 2.39% respectively (table 4), which are within 15% as per the acceptance criteria.

**Stability studies**

The Stability studies were assessed using quality control samples (HQC and LQC) and the %mean stability for HQC and LQC were presented which is within the acceptance limits of 85 to 115% (table 5).

**Pharmacokinetic studies**

The Pharmacokinetic parameter of canagliflozin was calculated from the plasma concentration-time curves using pk solver software. Also, the area under the plasma concentration-time curve from 0 to 24hr ($AUC_{0\text{-}24}$) was calculated using trapezoidal rule. Canagliflozin showed $T_{max}$ of 1.58±0.2 and mean $C_{max,}$ $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \alpha}$ for Test formulation is 272±13.24, 2571.20±251and 2777.43±276 respectively. The results were presented in table 6, table 7 and fig. 6.

**Table 3: Intra-day and inter-day quality control samples for canagliflozin**

| QC | Canagliflozin (ng/ml)(n=6) | | | |
|---|---|---|---|---|
| Intra-batch | LLOQ QC (5ng/ml) | LQC (10ng/ml) | MQC (300ng/ml) | HQC (600 ng/ml) |
| Mean* | 4.93 | 10.59 | 293.70 | 618.53 |
| SD** | 0.0520416 | 0.33 | 6.04 | 4.79 |
| %CV*** | 1.05 | 3.16 | 2.06 | 0.77 |
| Mean | 5.183333 | 9.10 | 301.13 | 635.24 |
| SD | 0.052042 | 0.34 | 6.03 | 7.38 |
| %CV | 1.004019 | 3.72 | 2.00 | 1.16 |
| Mean | 4.703333 | 10.15 | 295.82 | 620.89 |
| SD | 0.052042 | 0.37 | 5.62 | 9.27 |
| %CV | 1.106484 | 3.64 | 1.90 | 1.49 |
| Inter-batch | LLOQ QC (5ng/ml) | LQC (10ng/ml) | MQC (300ng/ml) | HQC (600 ng/ml) |
| Mean | 4.783333 | 0.25 | 2.66 | 5.47 |
| SD | 0.052042 | 0.00 | 0.08 | 0.08 |
| %CV | 1.087979 | 3.22 | 3.06 | 1.47 |

*Average of six determinations, **standard deviation, ***Coefficient of variation.

**Table 4: Matrix effect obtained with six different lots of plasma**

| QCID | LQC | HQC |
|---|---|---|
| **Actual conc. (ng/ml)** | **10** | **600** |
| 1 | 11.08 | 612.5 |
| 2 | 10.95 | 608.9 |
| 3 | 10.5 | 603.3 |
| 4 | 10.83 | 604.8 |
| 5 | 11.54 | 586.5 |
| 6 | 10.62 | 631.3 |
| Mean | 10.92 | 607.8 |
| ±SD* | 0.37 | 14.55 |
| % CV** | 3.39 | 2.39 |
| % accuracy | 109.24 | 101.31 |

*Standard deviation, **coefficient of variation

**Table 5: Results of stability studies**

| Stability condition | Nominal | Calculated concentration | |
|---|---|---|---|
| | Concentration (ng/ml) | Mean*±SD** | % Bias |
| Freeze-thaw stability | 10 | 10.77±0.211 | 7.7 |
| (-80 oC) | 600 | 594±7.86 | -1 |
| Long-term stability | 10 | 9.63±0.169 | -3.7 |
| (-80 oC, 30days) | 600 | 594±20.8 | -1 |
| Autosampler Stability | 10 | 9.69±0.204 | -3.1 |
| (4 oC, 24h) | 600 | 591±12.0 | -1.5 |
| Bench Top stability (6h) | 10 | 9.22±0.411 | -7.8 |
| | 600 | 582±6.56 | -3 |
| Dry residue stability (4 oC, 48h) | 10 | 9.83±0.121 | -1.7 |
| | 600 | 591±10.4 | -1.5 |
| Autosampler Stability | 10 | 9.44±0.221 | 5.5 |
| (4 oC, 24h) | 600 | 589±8.0 | -1.833333 |
| N=6 | | | |

*Average of six determinations, **standard deviation

**Table 6: Calculated plasma concentrations in rabbits at each time point**

| Time points (h) | Calculated concentrations (ng/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rabbit 1 | Rabbit 2 | Rabbit 3 | Rabbit 4 | Rabbit 5 | Rabbit 6 | Mean | SD* |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| 0.15 | 24 | 15 | 30 | 21 | 18 | 12 | 20 | 6.48 |
| 0.25 | 48 | 54 | 57 | 36 | 45 | 54 | 49 | 7.75 |
| 0.5 | 102 | 87 | 96 | 93 | 111 | 102 | 98.5 | 8.36 |
| 0.75 | 156 | 174 | 180 | 174 | 156 | 168 | 168 | 10.04 |
| 1 | 213 | 243 | 237 | 207 | 195 | 207 | 217 | 18.85 |
| 1.5 | 252 | 288 | 273 | 261 | 279 | 267 | 270 | 12.87 |
| 2 | 231 | 249 | 267 | 246 | 264 | 279 | 256 | 17.25 |
| 2.5 | 204 | 225 | 228 | 210 | 228 | 243 | 223 | 14.03 |
| 3 | 189 | 174 | 219 | 198 | 207 | 234 | 203.5 | 21.42 |
| 4 | 174 | 162 | 192 | 186 | 186 | 201 | 183.5 | 13.74 |
| 5 | 165 | 183 | 183 | 171 | 162 | 147 | 168.5 | 13.74 |
| 6 | 156 | 168 | 171 | 147 | 141 | 123 | 151 | 17.97 |
| 8 | 147 | 159 | 150 | 126 | 108 | 105 | 132.5 | 22.88 |
| 12 | 138 | 147 | 135 | 120 | 99 | 96 | 122.5 | 21.25 |
| 16 | 102 | 114 | 114 | 93 | 75 | 66 | 94 | 20.05 |
| 20 | 27 | 18 | 24 | 15 | 18 | 27 | 21.5 | 5.17 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |

*Standard deviation.

**Table 7: Calculated mean values of PK parameters for test animals**

| Parameter | Mean* (n=6) | SD** |
|---|---|---|
| Lambda_z | 0.11 | 0.01 |
| t1/2 | 6.56 | 0.74 |
| Tmax | 1.58 | 0.20 |
| Cmax | 272.00 | 13.24 |
| AUC 0-t | 2571.20 | 251.54 |
| AUC 0-inf_obs | 2777.43 | 275.93 |
| Vz/F_obs | 0.04 | 0.00 |
| Cl/F_obs | 0.00 | 0.00 |

*Average of six determinations, **standard deviation

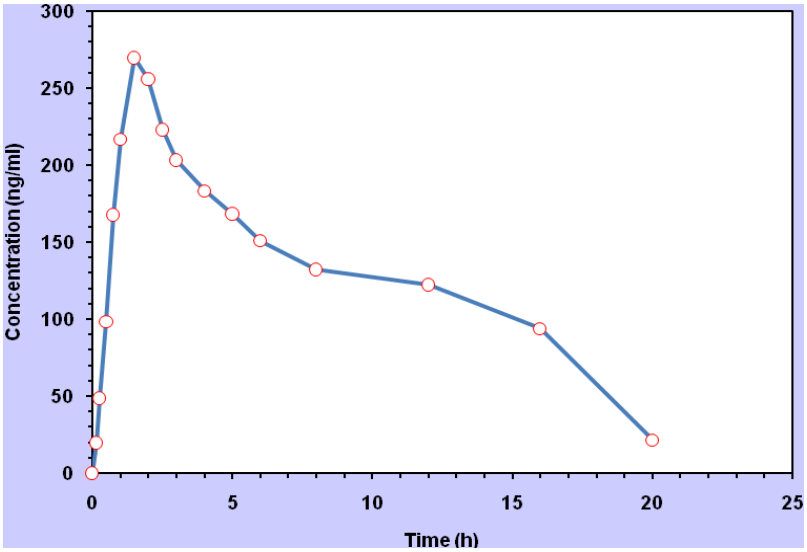


**Fig. 6: Plasma concentration-time profile of test animals**

## DISCUSSION

The established LC-MS/MS method has high selectivity, sensitivity and linear with least LLOQ (5ng/ml) concentration and have good % mean recovery (102.05%) when compared to other reported method for the estimation of canagliflozin metabolites from human plasma and another biological matrix. [11-12]. Liquid-liquid extraction technique was employed to reduce the interference from plasma. As per literature review, no LC-MS/MS method was available for determination of canagliflozin alone from rabit plasma, the validated method has great importance to determine the pharmacokinetic parameters of canagliflozin.

## CONCLUSION

The bio-analytical methodology for determination of canagliflozin described in this manuscript is highly specific, rugged and rapid for therapeutic drug monitoring both for analysis of routine samples of a single dose or multiple dose pharmacokinetics and also for clinical trial samples with desired sensitivity, precision, accuracy and high throughput. The method involved a simple and specific sample preparation by liquid-liquid extraction followed by isocratic chromatographic separation in 2.0 min. The overall analysis time is promising compared to other reported procedures for canagliflozin. The established LLOQ is sufficiently low to conduct a pharmacokinetic study with any marketing formulation of canagliflozin in healthy rabbits.

## AUTHORS CONTRIBUTIONS

The corresponding author Mr. Darshan Bhatt, Research Scholar, Mewar University, Chittorgarh, Rajasthan, India, who completed the intended research work under the guidance of his guide Dr. B. Rajkamal,Research Supervisor, Mewar University, Chittorgarh, Rajasthan, India

## CONFLICT OF INTERESTS

Declared none

## REFERENCES

1. Nomura S, Sakamaki S, Hongu M. Discovery of canagliflozin, a novel C-glucoside with thiophene ring, as sodium-dependent glucose cotransporter 2 inhibitor for the treatment of type 2 diabetes mellitus. J Med Chem 2010;53:6355-60.
2. Sha S, Devineni D, Ghosh A. Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, dose-dependently reduces calculated the renal threshold for glucose excretion and increases urinary glucose excretion in healthy subjects. Diabetes Obes Metab 2011;13:669–72.
3. Lee YJ, Lee YJ, Han HJ. Regulatory mechanisms of Na(þ)/glucose cotransporters in renal proximal tubule cells. Kidney Int Suppl 2007;106:S27-S35.
4. Nair S, Wilding JP. Sodium-glucose cotransporter 2 inhibitors as a new treatment for diabetes mellitus. J Clin Endocrinol Metab 2010;95:34-42.
5. Suneetha, D Sharmila. A validated stability indicating an RP-HPLC method for estimation of canagliflozin in the dosage form. Res J Pharm Biol Chem Sci 2015;6:1186-94.
6. Maddu S, Manasa K, Rajakumari Ch, Lakshmaiah B. RP-HPLC method development and validation for the estimation of canagliflozin in tablet dosage form. Int J Pharm 2014;5:1288-92.
7. Ishpreet K, Sharad W, Harsharan PS, Satish M. Development and validation of a stability-indicating reverse phase HPLC-PDA method for determination of canagliflozin in bulk and pharmaceutical dosage form. Pharm Methods 2016;7:54-62.
8. Sonia D, Muddu K, Gude SS, Vasantharaju SG. Stability indicating assay method development and validation to simultaneously estimate metformin hydrochloride and canagliflozin by RP-HPLC. Curr Trends Biotechnol Pharm 2016;10:334-42.
9. Ishpreet K, Sharad W, Harsharan PS, Satish M. Development and validation of UV spectroscopic method for determination of canagliflozin in bulk and pharmaceutical dosage form. Pharm Methods 2015;6:82-6.
10. Ishpreet K, Sharad W, Harsharan PS. Development and validation of a stability-indicating high-performance thin layer chromatography (HPTLC) method for estimation of canagliflozin in bulk and pharmaceutical dosage form. J Appl Pharm Sci 2016;6:51-7.
11. Iqbal M, Ezzeldin E, Al-Rashood KA, Asiri YA, Rezk NL. Rapid determination of canagliflozin in rat plasma by UHPLC–MS/MS using negative ionization mode to avoid adduct-ions formation. Talanta 2015;132:29-36.
12. Kobuchi S, Yano K, Ito Y, Sakaeda T. A validated LC-MS/MS method for the determination of canagliflozin, a sodium-glucose cotransporter 2(SGLT-2) inhibitor, in a lower volume of rat plasma: application to pharmacokinetic studies in rats. Biomed Chromatogr 2016;30:1549-55.
13. ICH guidelines for the stability of new drug substances and products. Q1A(R2) ICH, Geneva; 2005. p. 1-13.
14. ICH guidelines for validation of analytical procedures: text and methodology. Q2(R1) ICH, Geneva; 2005. p. 1-14.
15. Rambabu M, Nageswara P, Vasu Babu R, Adireddy V, Ajitha M. Simultaneous determination of artesunate and amodiaquine in human plasma using LC-MS/MS and its application to a pharmacokinetic study. Int J Pharm Pharm Sci 2015;7:105-12.

16. Sindhusri M, Swetha T, Ramadevi A, Ashok Kumar A. A novel rapid RP-HPLC method development and validation for the quantitative estimation of balofloxacin in tablets. Int J Pharm Pharm Sci 2014;7:319-22.
17. Raveendra Babu G, Lakshmana Rao A, Venkateswara Rao J. A rapid RP-HPLC method development and validation for the quantitative estimation ribavirin in tablets. Int J Pharm Pharm Sci 2014;7:60-3.
18. Darshan B, Rajkamal B. A UPLC-MS/MS method development and validation for the estimation of sofosbuvir from human plasma. Int J Appl Pharm 2017;9:30-6.
19. Srinidhi M, Mushabbar Basha MD, V Raj Kumar, Rajendra Kumar J. Stability indicating RP-HPLC method development and validation for the estimation of sumatriptan in bulk and pharmaceutical dosage form. J Appl Pharm Sci 2016;6:20-5.