International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 6, September-October 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Method Development and Validation for Estimation of Oral Hypoglycaemic Drug Dapagliflozinina Tablet Dosage form by the Employment of Rp-HPLC

Junaid Ahmed[1], Himanchal Sharma[2], Shiva Teotia[2]

[1]Research Scholar, [2]Assistant Professor,
[1,2]IIMT College of Medical Sciences, Meerut, Uttar Pradesh, India

ABSTRACT

HPLC is a chromatographic technique employed in active compound chemistry & biochemistry to separate a mixture and substances with the goal of identifying, measuring, & purifying the different components of the mixture. It's a much better variety of column and traditional chromatography. The objective of the research work is to develop and validate a simple and accurate reverse phase chromatographic method to estimate amount of drug in dosage form. The developed method successfully can be applied to estimate the amount of Dapagliflozin in tablet dosage form. After oral administration of dapagliflozin, the maximum plasma concentration (Concentration max) under two hours. High performance liquid chromatographic system was alleviated according to the chromatographic settings. After attaining the steady base line, to verify the system suitability, a single 40 µg/ml of standard solution proportional to 100% test concentration of dapagliflozin was injected into the HPLC system. The gradient mobile phase flow rate programming assisted in optimising the lengthy run duration and resolution of sample analysis, making the approach more cost effective and quick. Validation of the developed and optimized HPLC method was carried out according to ICH guidelines with respect to parameters such as linearity, specificity, precision and accuracy.

How to cite this paper: Junaid Ahmed | Himanchal Sharma | Shiva Teotia "Method Development and Validation for Estimation of Oral Hypoglycaemic Drug Dapagliflozinina Tablet Dosage form by the Employment of Rp-HPLC" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-6, October 2021, pp.323-328, URL: www.ijtsrd.com/papers/ijtsrd46395.pdf

IJTSRD46395

1. INTRODUCTION

HPLC is a chromatographic technique employed in active compound chemistry & biochemistry to separate a mixture and substances with the goal of identifying, measuring, & purifying the different components of the mixture. It's a much better variety of column and traditional chromatography[1]. HPLC stands for high-performance liquid chromatography, that is a more advanced version of column liquid chromatography [2]. Instead of allowing a solvent to flow naturally through a column, it is forced through at high pressures of up to 4ooatmospheres. This makes it a lot easier [2]. The basic premise of all chromatographic separations, including HPLC, is the separation of a sample into its constituent parts due to differences in the relative affinities of distinct molecules for the mobile phase & stationary phase used in the separation A liquid sample is injected into a stream of solvent (mobile phase) flowing through a column packed with a separation medium (stationary phase). Sample components separate from one another by a process of differential migration as they flow through the column. As bands emerge from the column, flow carries them to one or more detectors which deliver a voltage response as a function of time. This is called a chromatogram. For each peak, the time at which it emerges identifies the sample constituent with respect to a standard. The peak's area represents the quantity. The basic assembly of an instruments consist of following equipment (i) solvent Degasser – removes air gases from the solvents as they flow to the HPLC pump (ii)HPLC Pump – provides solvent flow and proportioning (iii)Autosampler – draws samples from vials and injects them into the solvent flow provided by the pump. (iv)Detector – responds to the separated analytes emerging from the HPLC column and

produces a signal output for the software(v)Column Oven – houses the HPLC column and keeps a stable temperature for reproducible separations. Dapagliflozin is a drug of the gliflozin class and it can be used to treat type 2 diabetes [3]. Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 9o% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine [].The molecular formula is C21H25ClO6. The molecular weight is 408.873 g/mol. Dapagliflozin [4] is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. Soluble in Methanol and Dichloromethane. The objective of the research work is to develop and validate a simple and accurate reverse phase chromatographic method to estimate amount of drug in dosage form. The developed method successfully can be applied to estimate the amount of Dapagliflozin in tablet dosage form. After oral administration of dapagliflozin, the maximum plasma concentration (Concentration max) under two hours. The absolute oral bioavailability of dapagliflozin following the administration of a 1o micrograms dose is 78.8%. Administration. It increases Cmax by up to 5o.5 percent and prolongs Tmax by about 1 hour, but it has little effect on AUC when compared to the fasted condition[5]. These modifications were not taken into account in clinical trials, therefore dapagliflozin was given with or without food. Distribution-Dapagliflozin is approximately 91.5% protein bound & protein binding not altered in patients having renal or hepatic impairment. Metabolism-The metabolism of dapagliflozin occurs through UGT-1A9. Dapagliflozin metabolized, primarily to yield dapagliflozin 3-o-glucuronide, that is an inactive metabolite. Dapagliflozin 3-oglucuronide accounted for 61.6% of a 50 mg [14C]-dapagliflozin dose.

2. Materials and Methods

2.1. Materials

HPLC grade water, Acetonitrile, Methanol, Potassium dihydrogen orthophosphate, ortho phosphoric acid obtained from Sd fine-Chem ltd, Mumbai. Dapagliflozin was provided as a gift sample by SUN Pharmaceuticals limited [Mumbai, India].

2.2. Diluent:

A suitable quantity of water & Methanol in the ratio 1:9 (v/v) used as a diluents.

2.3. Blank Preparation:

Use diluent as blank. Preparation of dilute orthophosphoric Acid Mixture 1o ml of oPA-transferred to 5o ml of volumetric flask & volume make up with water & mixed. Preparation of pH 2.o Buffer Mixture (Mobile phase A 1.4 grams of Potassium di-hydrogen orthophosphate was accurately weighed & transferred into 1litre of water & sonicated for 1o-15 minutes so as to dissolve & proper mixing.

2.4. Standard solution preparation

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well [6,7]. A quantity of powder of Dapagliflozin equivalent to 1oo mg were transferred to clean and dry 1oo ml volumetric flask and 7o ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 1oo ml with same solvent. Then 1o ml of the above solution was diluted to 1oo ml with HPLC grade methanol. One ml (1 ml) of the prepared stock solution diluted to 1oo ml and was filtered through membrane filter (o.45 µm) and finally sonicated to degas.

2.5. Sample solution preparation

15 mg of Dapagliflozin working standard was accurately weighed and transferred into a 15 ml clean dry volumetric flask. Add about 20 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1ooo ppm. Further pipette 1 ml of the above stock solution into a 1o ml volumetric flask was diluted up to the mark with diluents (100 ppm solution) [8]. Further 1 ml of prepared 1oo ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 1o ppm Dapagliflozin working standard solution. The solution was mixed well and filtered through o.45 µm filter.

2.6. Apparatus condition

Shimadzu LC-1oAT (Shimadzu Corporation) HPLC system associated with VWD detector (UV-Visible) and Lab Solutions programming (Version) was utilized for the method development studies and validation. The Agilent 11oo series (Agilent Technologies Inc.,) HPLC framework associated with VWD indicator (UV-Visible) and Chemstation programming was utilized for ruggedness studies to demonstrate the outcomes are independent of system. pH adjustments were done utilizing Metrohm computerized pH meter (Reproducibility ± o.o1 pH), model 78o. Analytical weighing of standards, test samples and chemicals were done utilizing Mettler Toledo XP6 micro analytical balance (Maximum limit of 6.1 g, sensitivity of ± o.o1 mg) and Sartorius BS/BT 2245 model analytical balance (Maximum limit of 22o g, sensitivity of ± o.1 mg).

3. Results and Discussion

Schimadzu C_{18}, 5 µm, 25 cm × 4.6 mm i.d. used as a stationary phase, whereas phosphate buffer and acetonitrile in the ratio of 6o:4o used a mobile phase. pH can be adjusted to 2.9 with orthophosphoric acid and a flow rate of 1.o ml/min with detection wavelength of 237 nm achieved the best separation of Dapagliflozin [9]. Standard solution of Dapagliflozin was prepared by using above procedure and the chromatograms were recorded (**Figure s1-3**). The retention time of Dapagliflozin was found to be 3.461 minutes.

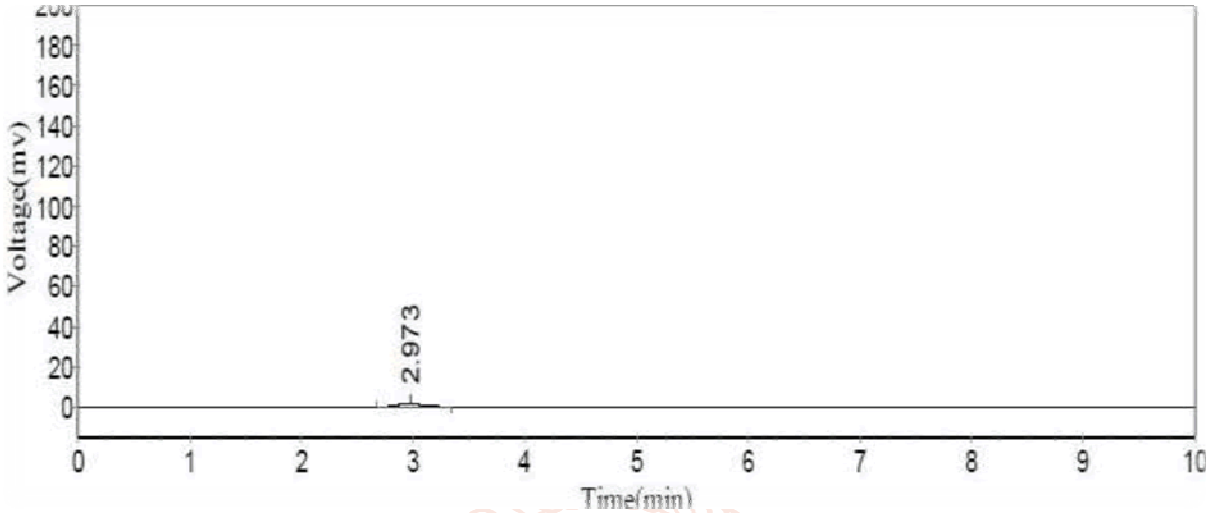

Figure 3.1 Chromatogram of blank

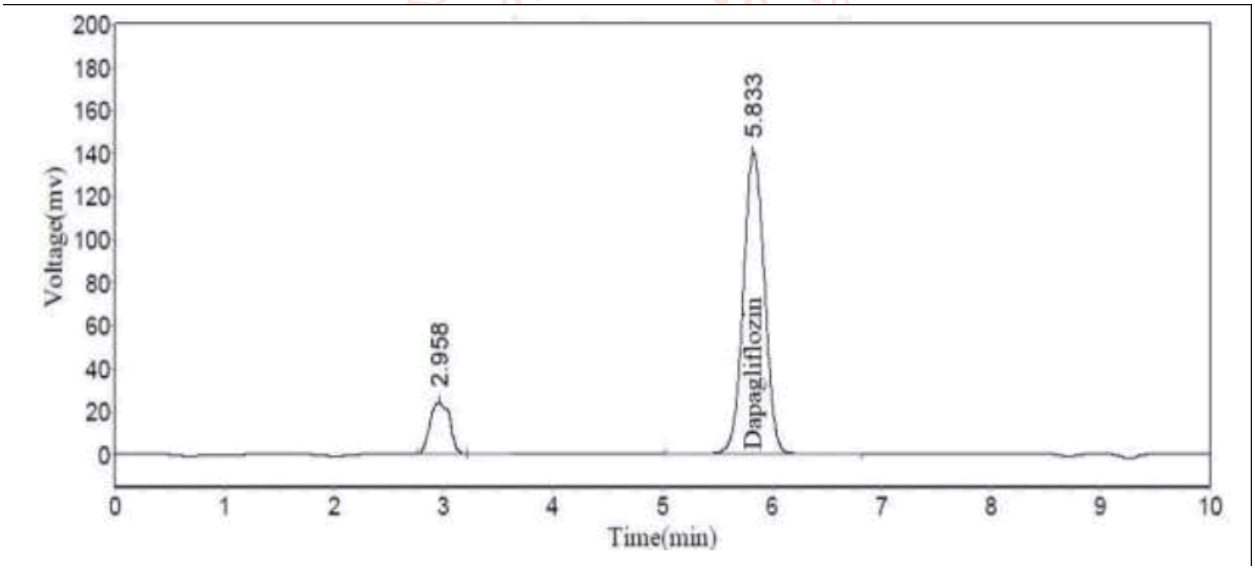

Figure 3.2 Chromatogram of dapagliflozin standard solution at 203 nm

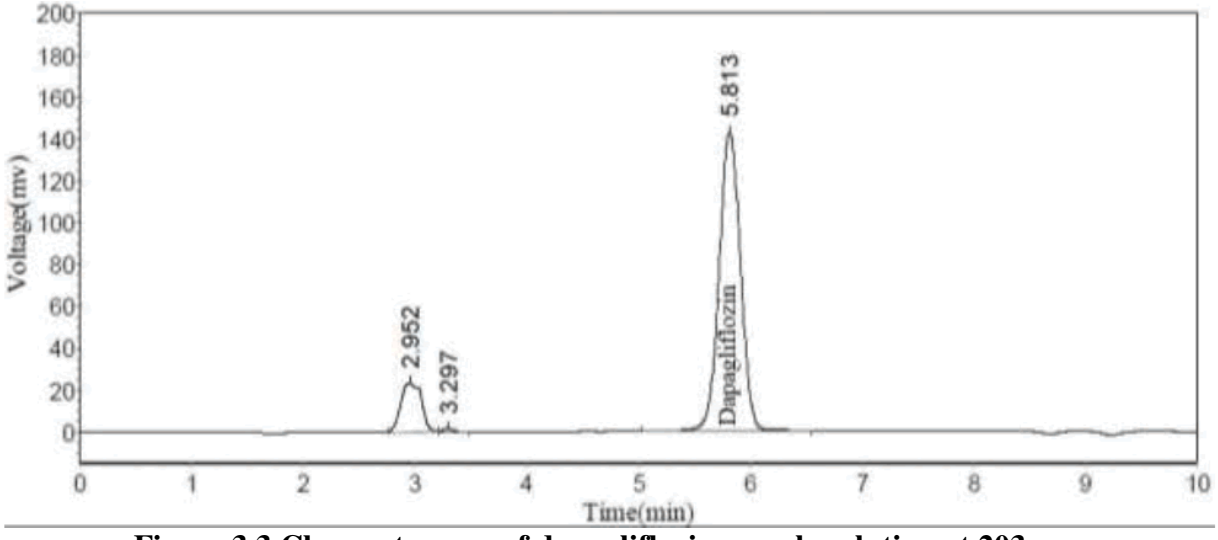

Figure 3.3 Chromatogram of dapagliflozin sample solution at 203 nm

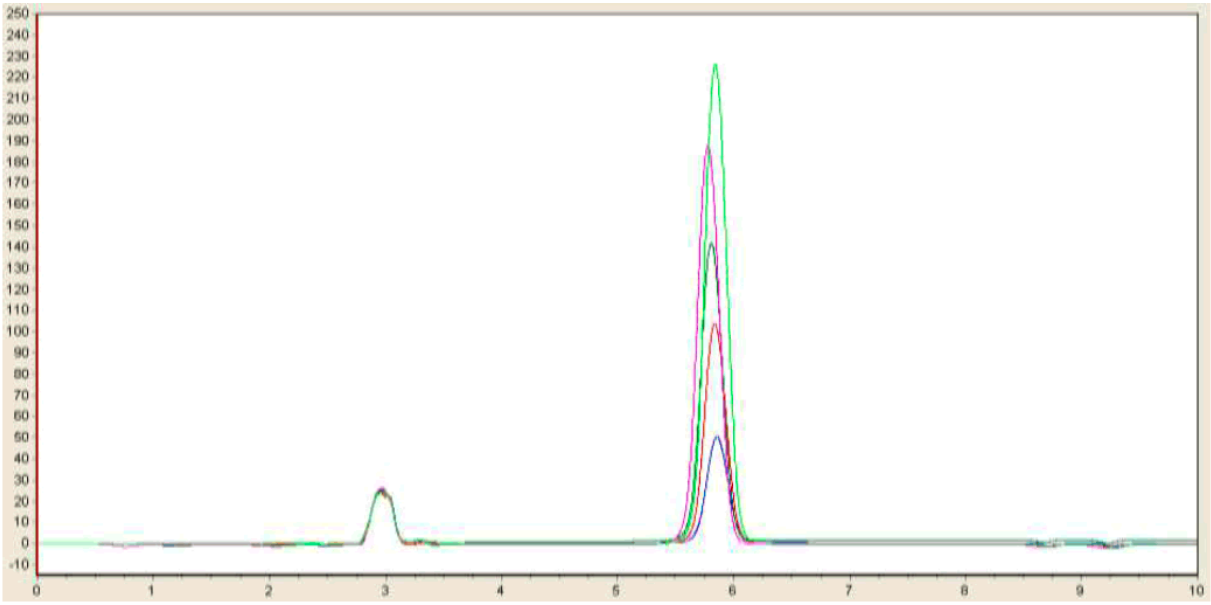

Figure 3.4 Overlay chromatogram dapagliflozin (Aliquots 10 to 40 ug/ml) at 203 nm

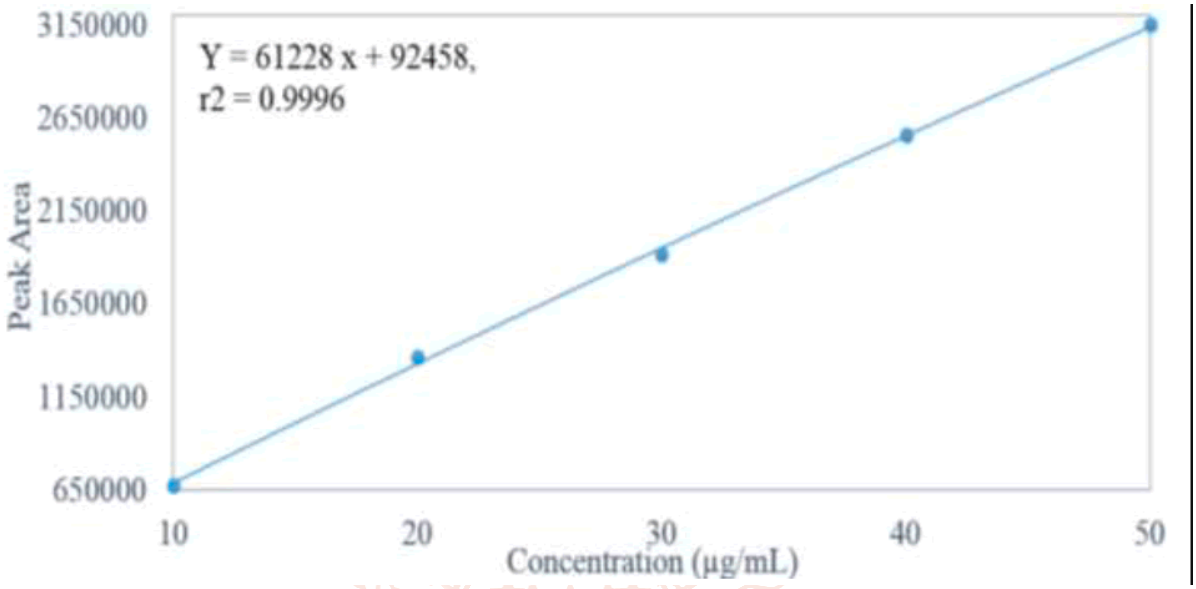

Figure 3.5: Calibration curve of Dapagliflozin at 203 nm

3.1. System suitability

High performance liquid chromatographic system was alleviated according to the chromatographic settings. After attaining the steady base line, to verify the system suitability, a single 40 µg/ml of standard solution proportional to 100% test concentration of dapagliflozin was injected into the HPLC system. The system suitability parameters (Retention Time (Rt), capacity factor, theoretical plates and peak tailing factor) were taken to establish the suitability for the planned method and the acquired outcomes were tabularised in **Table 4.1**.

Table 3.1: System suitability parameters for dapagliflozin

Parameter (n=6)	System suitability results
Retention Time (Rt) (min)	5.5 ± 0.5
Capacity factor (k)	6.8
Theoretical plates (N)	4229
Peak tailing factor	1.03

3.2. Precision

Precision is the assessment of the confidence of the obtained results in a sequence of estimations of a multiple injections of a similar specimen. Precision of the established method is considered by computing % relative standard deviation of six replicates of a single calibration standard solution (40 µg/ml). The precision and intermediate precision were calculated by measuring the resultant responses of principle peak on the same day and on another day for same solution concentration. The obtained results are tabularized (**Table 4.2**) in terms of Percentage Relative Standard Deviation (%RSD) of RT, peak area and height.

Table 3.2: Precision data of intraday and inter-day for dapagliflozin

Parameter	Intraday ,1st day, 1st system, 1st column	Inter day 2nd day, 2nd system, 2nd column and
Capacity factor (k)	6.7	6.7
Theoretical plates (N)	4229	3845
Peak tailing factor	1.03	.98
%RSD of Retention Time (Rt) (min) (n=6)	0.21	0.82
%RSD of area (n=6)	0.76	0.90
%RSD of height (n=6)	0.69	0.73

The present study was aimed at stability indicating RP HPLC method development and validation for simultaneous estimation of, Dapagliflozinand their degradation products. A non-polar C-18 analytical chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of Dapagliflozinand their degradation products. Mixtures of commonly used solvents like water and methanol in different combinations were tested as mobile phases[10,11]. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of methanol and 0.1 % o-phosphoric acid in the ratio of 40:60 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention times of the and Dapagliflozinwere found to be 5.41 min and 7.30 min respectively. The forced degradation study was conducted for determining the stability indicating power of an analytical procedure. The result of the stress studies shown in (Table 4.1 to 4.2 and figures 4.1 to 4.5). Therefore, the proposed method was simple, specific and sensitive and can be used for simultaneous analysis of, Dapagliflozinand their degradation products in bulk samples and its tablet dosage form [13,14].

4. Conclusion

For the determination of Drug Dapagliflozin with good resolution in optimum run time and high sensitivity, the devised technique is simple, rapid, accurate, precise, economical, specific, and reproducible. The peaks were well separated from the primary peak in a single analytical run in the current study. The method's optimization revealed that, in addition to the pH and composition of the mobile phase, flow rate was a crucial factor[15]. The gradient mobile phase flow rate programming assisted in optimising the lengthy run duration and resolution of sample analysis, making the approach more cost effective and quick. Validation of the developed and optimized HPLC method was carried out according to ICH guidelines with respect to parameters such as linearity, specificity, precision and accuracy.

5. References

[1] M. D. Game, NaglaxmiBopudi. Development and Validation of Stability Indicating HPLC Method for Estimation of Dapagliflozin in Marketed Formulation, international journal of pharmacy and pharmaceutical research, 2018; Vol. 12 (3): 123-144. 2. Olokoba AB, Obateru OA, and OlokobaLB. Type

[2] Diabetes Mellitus: A Review of Current Trends. Oman Medical Journal. 2012; 27(4): 269-273.

[3] Hassan AR. Overview on Diabetes Mellitus (Type 2). J Chromat. Separation Technique. 2013: 4(3).

[4] Manasa S, Dhanalakshmi K, Reddy NG and Srinivasa S. Method development and validation of Dapagliflozin in API by RP-HPLC and UV-Spectroscopy. International Journal of Pharmaceutical Science and Drug Research. 2014; 6(3): 250-252.

[5] White JR. Sodium glucose co transporter 2 inhibitors. Medical Clinics of North America 2015; 99(1): 131-143.

[6] Augeri DJ, Robl JA, Betebenner DA, Magnin DR and Khanna A. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type II diabetes. J Med Chem. 2005; 48: 5025-5037

[7] Kulasa K and Edelman S. Saxagliptin: The evidence for its place in the treatment of type 2 diabetes mellitus. Core Evid. 2010; 5: 23-37.

[8] Dave DJ: Saxagliptin. A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus J PharmacolPharmacoTher. 2011; 2(4): 230-235.

[9] Anderson R, Hayes J and Stephens JW. Pharmacokinetic, Pharmaco dynamic and clinical evaluation of Saxagliptin in type 2 diabetes. Journal Expert opinion on Drug Metabolism & Toxicology. 2016; 12(4): 467-473.

[10] N. D. Patel, A. D. Captain, Extractive spectrophotometric method for simultaneous determination of losartan potassium and atenolol in bulk and in pharmaceutical dosage form, International Journal of PharmTech Research, 2013, Vol-5, 629-40.

[11] ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: methodology (Q2B),

[12] Manasa S, Dhanalakshmi K, Nagarjuna Reddy G and KavithaB. Method development and validation of Dapagliflozin API by UV-Spectroscopy. International journal of pharmaceutical sciences review and research. 2014; 27(1): 270-272.

[13] Jani BR, Shah KV and Kapupara PP. Development and Validation of UV-Spectroscopic first derivative method for simultaneous estimation of Dapagliflozin and Metformin Hydrochloride in synthetic mixture. Journal of bioequivalence studies. 2015; 1(1): 1-8.

[14] Chitra KP, Eswaraiah CM and RaoBMV. Unique UV Spectrophotometric method for reckoning of Dapagliflozin in bulk and Pharmaceutical Dosage forms. Journal of Chemical and Pharmaceutical Research. 2015; 7(9): 45-49.

[15] Mante GV, Gupta KR and HemkeAT. Estimation of Dapagliflozin from its Tablet Formulation by UVSpectrophotometry. Pharm Methods. 2017; 8(2): 102-107.