SIMPLE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS QUANTIFICATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN IN RAT PLASMA

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(Received 18 May 2020) (Accepted 02 November 2020)

ABSTRACT

A simple HPLC method was developed for the simultaneous estimation of saxagliptin and dapagliflozin in rat plasma. The separation has been achieved by C_8 Eclipse plus column (25cm x 5cm x 4.6µ) at 1 mL min^{-1} flow rate. The mobile phase comprises of 0.01 % trimethylamine in water and methanol (40:60 V/V). The effluents were monitored at 228 nm. The retention times were found to be 4.243 min and 11.304 min for saxagliptin and dapagliflozin, respectively. The quantification ranges were found to be linear over 25-175 ng mL^{-1} and 100-700 ng mL^{-1} for saxagliptin and dapagliflozin, respectively. Regression equations of saxagliptin and dapagliflozin were found to be y = 594.84x - 801.14 and y = 128.6x - 348.57 with regression coefficient (R^2) 0.9988 and 0.9963, respectively using weighting factor of $1/x^0$, 1/x, $1/\sqrt{x}$, and $1/x^2$. The percentage recoveries were 81.51±1.276 to 86.23±2.012 and 81.48±2.487 to 85.40±2.145 for saxagliptin and dapagliflozin, respectively. This reported method was extensively validated according to US-FDA guideline.

Keywords: Saxagliptin, dapagliflozin, method development and validation, high performance liquid chromatography (HPLC)

ABBREVIATIONS

HPLC: High performance liquid chromatography, **Rt:** Retention time, **DM**: Diabetes mellitus, **Saxa**: Saxagliptin, **Dapa**: Dapagliflozin, **CC**: Calibration curve standard, **%RE**: Percentage of relative error, **QC Level**: Quality control level, **CV**: coefficient of variation, **SD**: Standard deviation, **LLOQ**: Lower limit of quantification, **LQC**: Low quality control, **MQC**: Mid quality control, **HQC**: High quality control

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by insulin deficiency or insulin resistance as a result of which the body fails to utilize proximate nutrients in the form of carbohydrates, proteins and fats. If it is uncontrolled, it leads to several other complications or secondary diseases or disorders. As per the World Health Organization (WHO), the global incidence of diabetes was 171 million in 2000 and is projected to rise up to 366 million in 2030[1].

USFDA has approved a fixed-dose combination of saxagliptin and dapagliflozin for the treatment of Type 2 diabetes mellitus. It can reduce blood glucose level without the possibility of weight gain[2]. Dapagliflozin is chemically known as (1*S*)-1, 5-anhydro-1-C-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-D-glucitol. It acts by inhibiting sodium-glucose co-transporter 2 and prevents renal reabsorption of glucose[3]. Chemically, saxagliptin is represented as (1*S*, 3*S*, 5*S*)-2-[(2*S*)-2-amino-2-(3 hydroxytricyclo [3.3.1.13, 7] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile. It shows antidiabetic property by inhibiting the activity of the enzyme dipeptidyl peptidase-4 (DPP-4), thereby enhancing insulin production and decreasing hepatic gluconeogenesis[4].

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https://doi.org/10.53879/id.59.07.12537

In preclinical studies, an efficient bioanalytical method like LC-MS/MS can be utilized to determine the safety and efficacy of the drugs, although the availability of this sophisticated instrument is limited to a few well-established research laboratories. Even instrument handling is expensive and needs a skilled person[5]. Few analytical methods have been reported for simultaneous estimation of saxagliptin and dapagliflozin. Vinutha Kommineni et al. provided a RP-HPLC method (stability-indicating) for simultaneous estimation and validation of saxagliptin and dapagliflozin[6]. B. Reddy Padmaja et al, developed a RP-HPLC method for the validation and concurrent estimation of dapagliflozin and saxagliptin in tablet dosage forms[7]. Adluri Phanindra et al. reported a validated LC-ESI-MS/MS method for estimating both the drugs simultaneously using human plasma[8]. Nima Suthar et al. have published a validated HPLC stability-indicating method to determine these drugs simultaneously in a synthetic mixture[9].

Such methods caanot not be used for the quantification of saxagliptin and dapagliflozin in rat plasma due to less specificity and sensitivity. Thus, advancement in bioanalytical method is required for the quantification of both the drugs simultaneously in rat plasma. The present research illustrates a validated bioanalytical method to quantify both saxagliptin and dapagliflozin concurrently in rat plasma. In this method, various validation parameters like sensitivity, selectivity, precision, accuracy, recovery and stability studies were carried out as per US-FDA guidelines. A simple protein precipitation technique is used to extract drugs from plasma using methanol.

MATERIALS AND METHODS

Materials and reagents

Saxagliptin and dapagliflozin were obtained from Yarrow Chemicals Pvt. Ltd., Mumbai, India. Methanol (HPLC grade) was purchased from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. Acetic acid glacial (AR grade) and sodium acetate anhydrous (AR grade) were purchased from SD Fine Chem Ltd., Mumbai. India. HPLC grade Mili-Q water was used for developing and validating the analytical method.

Experimental animals

Healthy Albino Wistar rats of either sex weighing 140-160 g were housed at 12 h light/dark cycle at room temperature (22°C ± 1 °C) and humidity (55 ± 5%). Animals were provided with free access to food and water *ad libitum.* All the conducted studies were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Adichunchanagiri College of Pharmacy, BG Nagara, with ethical clearance No 377/PO/ReBi/S/01/CPCSEA.

Blood collection and plasma extraction

Six healthy Albino Wistar rats were anaesthetized by administering isoflurane through inhalational route. Approximately 0.5 mL of blood was collected from each rat through a retro orbital route using heparinised capillary tube and subjected to centrifugation at 5000 rpm for 5 min at 4° C to separate plasma layer. Separated plasma layer was collected in Eppendorf tubes and used for the study.

Equipment and chromatographic conditions

Chromatographic development was achieved on Shimadzu high performance liquid chromatography (HPLC) instrument equipped with a binary pump LC-20AD and Controller SPD-M20A along with PDA Detector. LC Real-time Analysis software was utilized for data integration and postern analysis purposes. Chromatographic separation was carried out on Eclipse plus C_8 Column (25cm X 5cm x 4.6µ) with L1 packing. The mobile phase containing a mixture of 0.01 % trimethylamine in water

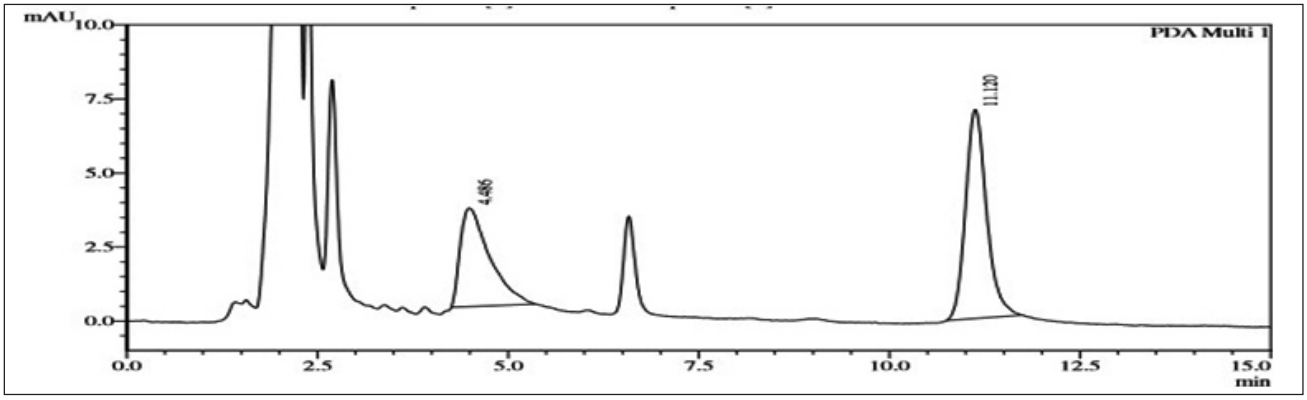

Fig. 1: Chromatogram of saxagliptin and dapagliflozin

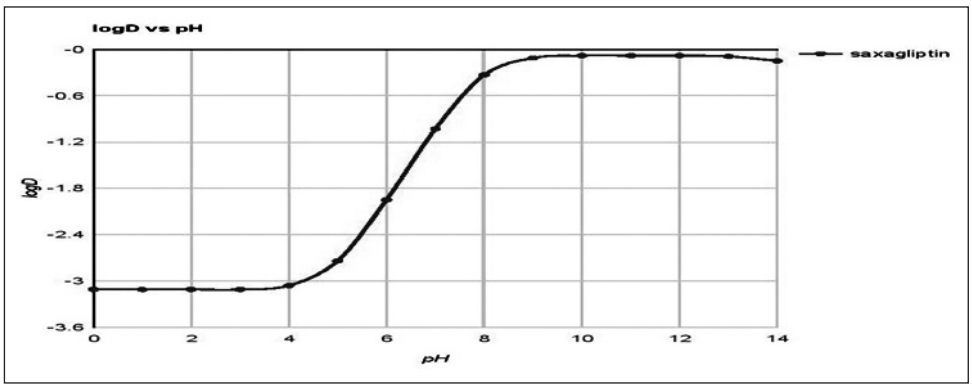

Fig. 2: Log D plot of saxagliptin

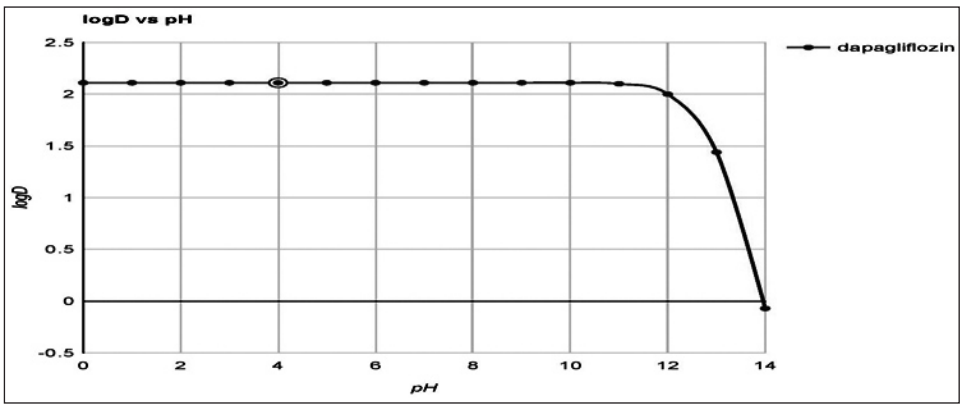

Fig. 3: Log D Plot of dapagliflozin

and methanol (40:60 % V/V) was selected as an optimum composition for symmetrical peak with peak purity index 0.9999 at the flow rate of 1 mL min^{-1} and UV detection was carried out at 228 nm with 15 minutes run time at ambient temperature.

Retention times of saxagliptin and dapagliflozin were found to be 4.486 min and 11.120 min., respectively. A representative chromatogram for sample containing saxagliptin and dapagliflozin is depicted in Fig. 1.

Preparation of standard solutions and quality control samples

Both working standards for calibration curves were made in volumetric flasks to get concentration range 25, 50, 75, 100, 125, 150 and 175 ng mL^{-1} of saxagliptin and 100, 200, 300, 400, 500, 600 and 700 ng mL^{-1} of dapagliflozin by diluting stock solutions with methanol. The same stock solution was used for the preparation of quality control solutions of saxagliptin and dapagliflozin for LLOQ (25 ng mL^{-1} and 100 ng mL^{-1}), LOQ (75 ng mL^{-1} and 300 ng mL^{-1}), MOQ (125 ng mL^{-1} and 500 ng mL^{-1}) and HOQ (175 ng mL^{-1} and 700 ng mL^{-1}). All the solutions were kept at -20 ^{0}C and brought to room temperature before use.

Sample preparation

200 µL blank plasma was spiked by adding 50 µL of methanol and 50 µL of the final drugs solution to get a concentration range of 25-175 ng mL^{-1} for saxagliptin and 100-700 ng mL^{-1} for dapagliflozin. The quality control samples for saxagliptin and dapagliflozin were made similarly. The solutions were vortexed for 5 minutes and centrifuged at 5000 rpm at 4 ^{0}C for 5 min, then filtered and sonicated before use.

Table I: Accuracy data of saxagliptin and dapagliflozin (n = 6)

	Saxagliptin (ng mL^{-1})			Dapagliflozin (ng mL^{-1})		
S. No.	**Nominal concentration**	**Mean concentration**	**%RE**	**Nominal concentration**	**Mean concentration**	**%RE**
CC1	25	24.29	-2.921	100	99.05	-0.954
CC2	50	47.10	-6.134	200	189.64	-5.459
CC3	75	69.25	-8.292	300	303.49	1.151
CC4	100	98.47	-1.548	400	389.91	-2.585
CC5	125	120.45	-3.774	500	472.41	-5.839
CC6	150	148.77	-0.821	600	614.85	2.416
CC7	175	172.77	-1.285	700	692.57	-1.071

CC: Calibration curve standard *%RE: Percentage of relative error*

Table II: Intra and Inter day precision data of saxagliptin and dapagliflozin (n = 6)

Analyte	QC Level	Nominal concentration (ng mL^{-1})	Interday precision		Intraday precision	
			Measured concentration (Mean ± SD)	% CV	Measured concentration (Mean ± SD)	% CV
Saxa	LLOQ	25	23.12±0.122	2.480	23.01± 1.190	1.099
	LQC	75	72.33±0.080	1.103	72.98± 1.793	1.019
	MQC	125	119.10±0.122	1.966	121.06± 2.324	0.785
	HQC	175	169.05±0.142	1.635	170.78± 3.045	1.734
Dapa	LLOQ	100	97.32±0.525	2.413	93.38±0.219	0.751
	LQC	300	288.88±0.707	1.245	284.68±1.363	2.412
	MQC	500	474.29±0.14	0.489	479.85±1.339	2.514
	HQC	700	691.59±1.01	1.141	676.86±1.553	2.228

QC Level: Quality control level, *CV: Coefficient of variation,* *SD: Standard deviation,*

LLOQ: Lower limit of quantification, *LQC: Low quality control,* *MQC: Mid quality control,*

HQC: High quality control

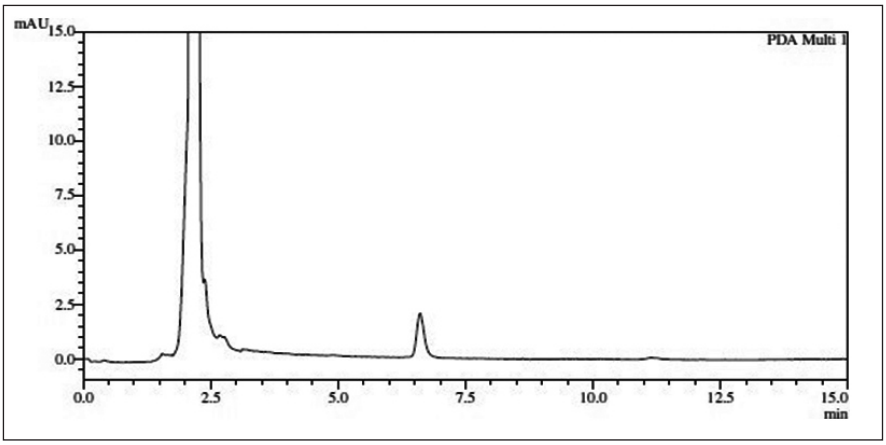

Fig. 4: Blank plasma chromatogram

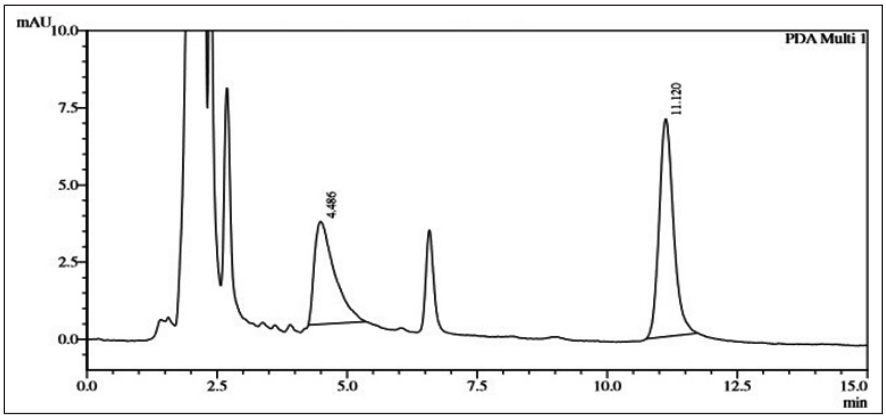

Fig. 5: Chromatogram of saxagliptin and dapagliflozin

Method validation

The proposed method was validated as per US-FDA bioanalytical method validation guideline[10].

Selectivity

The selectivity of the developed bioanalytical method was evaluated by comparing the chromatograms of blank plasma samples (n=6) spiked with saxagliptin and dapagliflozin at LLOQ level with chromatograms of blank plasma sample from six different randomly selected Albino rats. The results are considered acceptable if at least 5 of the 6 tested lots of blank matrix have an instrument response (peak area) of ≤20 % of the response of the analytes at the LLOQ level.

Calibration curve

The calibration curves of saxagliptin and dapagliflozin were constructed by taking seven calibration standards, a double blank plasma sample (unspiked plasma) and a blank sample. The plot was drawn using peak area on Y-axis versus plasma concentra-

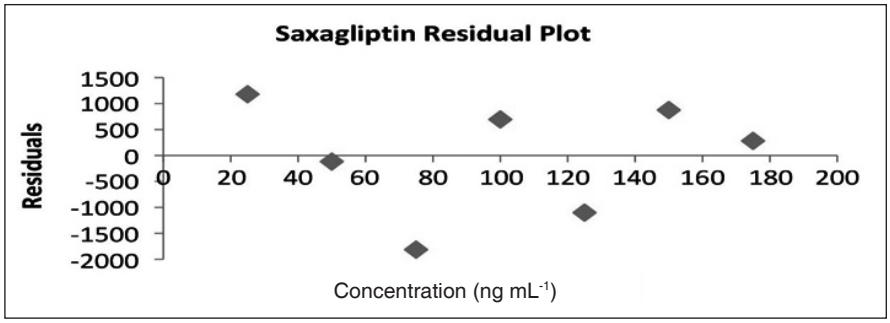

Fig. 6: Saxagliptin residual plot

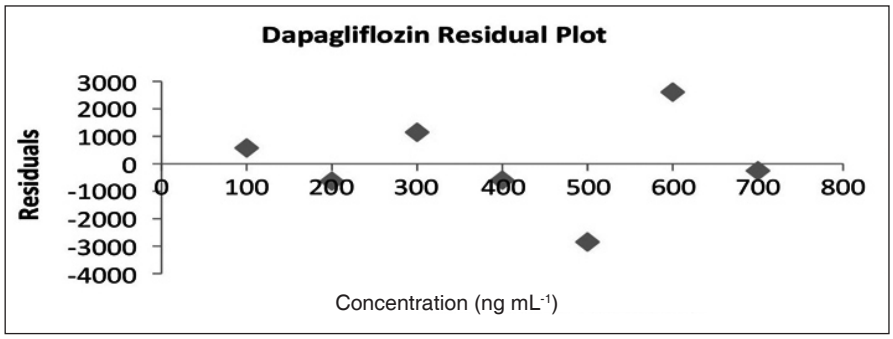

Fig. 7: Dapagliflozin residual plot

Table III: Weighting least square linear regression of saxagliptin

Weight factor (w)	$1/X^0$	1/X	$1/\sqrt{x}$	$1/x^2$
R^2	0.9988	0.7162	0.8214	0.5425
% RE	-24.778	-5324.67	-14.5	-86332.9

%RE: Percentage of relative error R^2: Correlation coefficient

Table IV: Weighting least square linear regression of dapagliflozin

Weight factor (w)	$1/X^0$	1/X	$1/\sqrt{x}$	$1/x^2$
R^2	0.9963	0.7226	0.8258	0.5507
% RE	-12.343	-739.107	-3.3224	-4492

%RE: Percentage of relative error,
R^2: Correlation coefficient

tions on X-axis. Calibration curve fitting was performed by applying the simplest model that adequately describes the concentration-response relationship using appropriate statistical tests for goodness-of-fit. A calibration curve with correlation coefficient (r^2) value and accuracy of ±15% (except for LLOQ where ±20% was applied) has been considered to be acceptable.

Recovery

The percentage recovery of saxagliptin and dapagliflozin were considered as detector response, obtained from an amount of the analyte added to and extracted from the rat plasma, compared to the detector response obtained for the nominal concentration of the pure authentic standard. Recovery experiments were performed by comparing the analytical results for extracted samples at three QC sample (LQC, MQC, HQC) with unextracted sample concentration in the mobile phase. It is expressed as the mean ±SD.

Accuracy and precision

The intra-day and inter-day assay precision and accuracy were performed by evaluating five replicates at three different QC levels (LQC, MQC, HQC) and LLOQ. For intra-day precision and accuracy, samples were analysed on the same day, while inter-day assay precision and accuracy were determined by analysing samples on three consecutive days.

Stability studies

Stability of saxagliptin and dapagliflozin in plasma matrix was tested under different conditions viz., 0 h, Bench Top (24 h) Rt, Freeze and Thaw (- 80 ^{0}C for 3 three cycles), Long term (-80 ^{0}C 45 days). At different QC levels (LQC, MQC and HQC), all stability studies were repeated three times for each concentration. Freeze thaw stability was conducted for three consecutive cycles within 2 days. Bench top stability (24 h) Rt and long term stability (–80 ± 10 °C for 45 days) were evaluated for both the drugs in plasma. Extraction and quantification of all QC samples were tested against fresh QC samples. The acceptance criteria of accuracy and precision for all stability samples should be within±15 %[10].

RESULT AND DISCUSSION

Optimization of chromatographic condition

A systemic RP-HPLC method has been developed by scientific approach in order to obtain a suitable chromatographic conditions, along with easy and quick sample preparation technique. Considering this objective, initially liquid chromatographic conditions such

as choice of mobile phase and its composition, column selection, flow rate and injection volume have been optimized accordingly. Final optimization was carried out by making a deliberate variations in chromatographic conditions such as flow rate, mobile phase composition and column temperature and different system suitability parameters including retention time (Rt), peak tailing (10 %) and resolution (Rs)[11]. The selection of column and best pH working range for mobile phase buffer have been deduced from log D diagrams (Figs. 2 and 3) depicted by the ChemAxon log D predictor (demo version) based on molecular structure. As seen in Figs. 2 and 3 the value of log D for both the drugs is relatively robust (flat slope) with regards to the pH 2-4 in the regions of the diagram. This would suggest that retention times for both molecules should remain constant with regards to the pH in these ranges. In the pH range of 4-8 (saxagliptin) and 9-12 (dapagliflozin), log D curves for the individual compounds change quite drastically with pH, indicating that in this region peak retention values would be more sensitive to small changes in the pH. Therefore, pH range of 2-4 has been selected for method development. Considering these factors, an Eclipse plus C_8 Column (25 cm X 5 cm X 4.6 µm) with L1 packing suitable for the chromatographic separation, was selected. A mixture of 0.01 % trimethylamine in water and methanol (40:60 %) in isocratic mode and 1 mL min^{-1} flow rate was found to be the most suitable for the quantification of saxagliptin and dapagliflozin with best peak shape and peak intensities along with selectivity. Using the optimized chromatographic conditions, retention times have been found to be 4.243 min and 11.304 min for saxagliptin and dapagliflozin, respectively with an overall run time of 15 min.

Table V: Percentage recovery studies of saxagliptin and dapagliflozin (n = 3)

Analyte	QC Level	Nominal Concentration (ng mL^{-1})	% Recovery	
			Mean ± SD	%CV
Saxa	LLOQ	25	81.51±1.276	2.364
	LQC	75	80.06±1.823	3.111
	MQC	125	83.26±1.111	2.175
	HQC	175	86.23±2.012	2.021
Dapa	LLOQ	100	85.40±2.145	1.703
	LQC	300	81.48±2.487	1.452
	MQC	500	83.99±1.256	0.241
	HQC	700	83.05±2.221	2.215

CV: coefficient of variation, SD: Standard deviation

Table VI: Stability of saxagliptin and dapagliflozin in rat plasma in three QC levels (n = 6)

Stability	Measured concentration (Mean ± SD)		% CV	
	Saxa(ng mL^{-1})	Dapa(ng mL^{-1})	Saxa	Dapa
0 h	71.48±5.524	291.47±7.451	2.56	3.75
	121.91±9.369	484.19±9.123	2.41	3.48
	172.14±9.856	683.82±18.214	3.86	1.37
Bench top (24 h) Rt	69.99±8.232	283.89±9.636	3.20	2.60
	122.01±7.526	477.49±15.369	4.99	3.22
	171.29±16.265	680.82±19.67	2.41	5.99
Freeze and thaw (- 80^0 C for 3 three cycles)	70.32±10.555	285.11±5.367	2.04	4.96
	122.35±6.741	481.75±24.78	5.35	3.74
	172.88±13.263	677.73±12.363	2.11	1.96
Long term (-80^0 45 days)	65.29±9.741	279.94±4.290	1.45	5.19
	117.46±6.123	476.62±12.781	3.56	1.83
	167.17±11.159	672.24±25.176	3.01	2.36

CV: coefficient of variation SD: Standard deviation

Because of the presence of chromophores and auxochromes in saxagliptin and dapagliflozin, PDA detector was chosen for detector response at 228 nm. As log P of saxagliptin and dapagliflozin are – 0.08 and 2.11 (predicted by Chem Axon demo version) and the drugs are moderately hydrophilic in nature, polar organic solvents were considered to be suitable for extraction purpose. Extraction with methanol was found to be the best method for recovery study by protein precipitation technique.

METHOD VALIDATION

Selectivity

The selectivity of the method has been verified as interference peaks from blank rat plasma samples successively were resolved and separated from the peaks of saxagliptin and dapagliflozin (Figs. 4 and 5). Therefore, the current method enables the determination of both the drugs in rat plasma selectively.

Accuracy and precision

Accuracy and intra and inter day precision, expressed as RSD, respectively, are shown in Tables I and II. The values of all QC samples (LQC, MQC & HQC) are within the acceptable range (RSD $\leq$ 15 %), assuring that the developed method is fit for quantification of both the drugs with accuracy and precision.

Linearity and sensitivity

In calibration runs, all calibration curve standards were analysed in six replicates using an optimized protein precipitation extraction experiment and chromatographic conditions. At the end of calibration runs, the six-point calibration curve was constructed from the peak area vs. respective concentration of calibration curve standard. The calibration curve standard data were studied using unweighted linear regression and weighted linear regression with a weighting factor of 1/x, 1/ $\sqrt{x}$, and $1/x^2$ and the best weighting factors were chosen according to the R^2 and percentage relative error (% Σ RE) in Tables III and IV. The plot of residuals versus concentration obtained from chromatographic linearity data is shown in Fig. 6 and Fig. 7 for saxagliptin as well as dapagliflozin respectively. The residual plot clearly shows that the errors are randomly distributed around the concentration axis. The linearity of the calibration curves was established over the range of 25 – 175 ng mL^{-1} and 100 – 700 ng mL^{-1} for saxagliptin and dapagliflozin with the correlation coefficient (r^2) 0.9988 and 0.9963, respectively. All the points of the calibration curves are within the criteria of RSD $\leq$ 15 %. As the accuracy and precision have passed with the LLOQ 25 ng mL^{-1} and 100 ng mL^{-1} for saxagliptin and dapagliflozin, respectively. Thus the method is sufficiently sensitive to estimate the drugs concentration in rat plasma[12].

Recovery

The absolute percentage recoveries were 81.51 %- 86.23 % (saxagliptin) and 81.48 % – 85.40 % (dapagliflozin) for LQC, MQC and HQC samples respectively, in both the drugs from Rat plasma (Table V). Such high recoveries were reached by optimizing the extraction solvent i.e. methanol. Thus the recoveries were adequate to obtain precise and accurate determination in the specified assay range for each analytes.

Stability studies

The stability of saxagliptin and dapagliflozin was investigated under different conditions and it is summarized in Table VI. The result describes that both drugs are stable in rat blood plasma. Therefore, this method can be used for the simultaneous estimation of saxagliptin and dapagliflozin in pre-clinical studies.

CONCLUSION

In summary, we have developed a simple, rapid and cost-effective HPLC-UV method successfully for simultaneous quantification of saxagliptin and dapagliflozin in rat plasma. Advantages of the reported method are less sample volume, simple extraction method and simultaneous estimation of combination drugs. Due to high sensitivity, the developed method will support to conduct preclinical studies such as pharmacokinetic, bioavailability and bioequivalence studies. The developed HPLC-UV method was validated for selectivity, linearity, precision, accuracy, recovery and stability. The validated method will also be used in assaying the analytes in pharmaceutical formulations in routine quality analysis.

REFERENCES

1. Kanivalan N., Rajakumar R. and Mani P.: Anti-diabetic and hypolipidamic effects of *Costusigneus* leaves extracts against streptozotocin induced diabetic albino rats, **Acta Sci. Biol. Sci**., 2014, 1(2), 74-79.
2. Donepudi S. and Achanta S.: Simultaneous Estimation of saxagliptin and dapagliflozin in human Plasma by Validated High Performance Liquid Chromatography-Ultraviolet Method, **Turk. J. Pharm. Sci.**, 2019, 16(2), 227.
3. Pal N., Mahtab T., Reddy P.P. and Rao A.S.: A New HPLC Method Development and Validation for the Determination

of Dapagliflozin in Tablet Dosage Form, **World. J. Pharm. Res.**, 2019, 8(9), 1156-1165.

4. Raveendra B. G., Kumar S. S., Greeshma A. and Satyanarayana M.: A Novel Stability-Indicating Method for the Simultaneous Estimation of saxagliptin and dapagliflozin in Rat Serum by Using UV Spectroscopy, **Pharm. Anal. Acta**, 2018, 9(3), 2-5.
5. Italiya Kishan S., Saurabh S., Ishit K., Deepak C. and Anupama M.: Simultaneous estimation of lisofylline and pentoxifylline in rat plasma by high performance liquid chromatography-photodiode array detector and its application to pharmacokinetics in rat, **J. Chromatogr. B.,** 2017, 1061-1062, 49-56.
6. Kommineni V., Chowdary K.P. and Prasad S.V. : Development of a New Stability Indicating RP-HPLC Method for Simultaneous Estimation of saxagliptine and dapagliflozin and its Validation as per ICH Guidelines, **Indo Am. J. Pharm.**, 2017, 4(09), 2920-2932.
7. Padmaja B.R., Sivagami B. and Chandrasekar R.: A Highly Validated RP-HPLC Method Development for the Simultaneous Estimation of dapagliflozin and saxagliptin in Tablet Dosage Forms, **Int. J. Pharm.**, 2018, 10(5), 372-380.
8. Phanindra, A. and Kumar Y.S. : Development and Validation of Sensitive LC-ESI-MS/MS Method for the Simultaneous Estimation of dapagliflozin and saxagliptin in Human Plasma, **Int. J. Pharm.,** 2019, 11(4), 55-59.
9. Suthar N., Marvaniya V. and Patni P.: Stability Indicating Method Development and Validation for the Simultaneous Estimation of dapagliflozin and saxagliptin in Synthetic Mixture by HPLC, **Pharma Sci.**, 2018, 9(2), 112-128.
10. US-FDA, F. (2018). Drug Administration, FDA Guidance for Industry: Bioanalytical Method Validation, DRAFT GUIDANCE. US Department of Health and Human Services, FDA. Center for Drug Evaluation and Research, Rockville, MD, USA, in: https://www. fda. gov/downloads/drugs/guidances/ucm070107. Pdf.
11. Lee J. B., Zgair A., Kim T. H., Kim M. G., Yoo S. D., Fischer P. M. and Gershkovich P.: Simple and sensitive HPLC-UV method for determination of bexarotene in rat plasma, **J. Chromatogr. B.**, 2017, 1040, 73-80.
12. Almeida A. M. D., Castel-Branco M. M. and Falcao A. C.: Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods. **J. Chromatogr. B.**, 2002, 774(2), 215-222.