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RESEARCH ARTICLE

Validated RP - HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin Hydrochloride in Tablet Dosage Form

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ABSTRACT

A new, precise, rapid, accurate RP – HPLC method was developed and validated for simultaneous estimation of Dapagliflozin and Saxagliptin Hydrochloride in bulk and in tablet dosage form. The samples were isocratically eluted by using Hypersil BDS C_{18} (250 mm × 4.6 mm) 5µm column with a mobile phase mixture of Phosphate buffer (pH 4.5) : Methanol in the ratio of 85:15 v/v at a flow rate of 1 ml/min and detection wavelength of 222 nm. The retention time of Dapagliflozin and Saxagliptin HCl found to be 4.080 min and 5.343 min. A good linear response was obtained in the concentration range of 10 – 30 µg/ml for Dapagliflozin and 5 – 15 for Saxaglitpin HCl. The correlation coefficient R^2 value is found to be 0.998 for Dapagliflozin and 0.993 for Saxaglitpin HCl. The limit of detection (LOD) and limit of quantification (LOQ) of Dapagliflozin were found to be 1.16 µg/ml and 3.52 µg/ml, while those of Saxagliptin HCl were found to be 0.53 µg/ml and 1.62 µg/ml. The method was found to be rapid, sensitive, linear, specific, accurate, precise and economic for estimation of Dapagliflozin and Saxagliptin HCl in marketed tablet dosage forms.

KEYWORDS

RP-HPLC, Dapagliflozin, Saxagliptin HCl, ICH guideline

INTRODUCTION

Chemically Dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol as shown in figure 1. Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney. Use of Dapagliflozin leads to blood glucose to be eliminated through the urine, which can lead to weight loss and tiredness.[1,2] Dapagliflozin was approved by FDA on 8th January 2014.

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While Saxagliptin HCl is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile as shown in figure 2. Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones.[3,4] Saxagliptin HCl was approved by FDA on 31st July 2009.

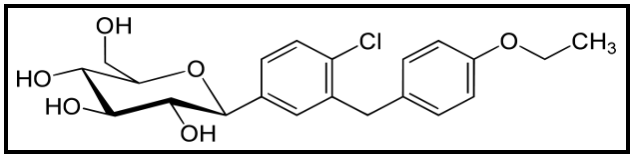

Figure: 1 Structure of Dapagliflozin

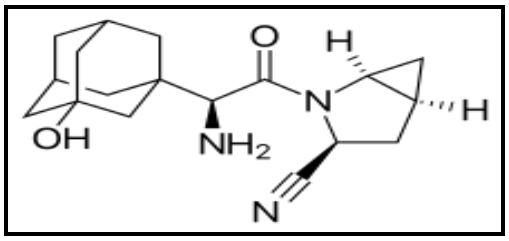

Figure: 2 Structure of Saxagliptin HCl

Both the drugs either alone or in combination therapy are used to treat type 2 Diabetes mellitus. Literature survey revealed estimation of Dapagliflozin and Saxagliptin HCl by HPLC method either in alone or in combination with other drugs.[5-10] Literature survey revealed that there were no any official or reported methods available for the estimation of both the drugs in combination.

In this present study an attempt was made to develop simple, rapid, reliable, accurate and economical RP-HPLC method for estimation of Dapagliflozin and Saxagliptin HCl in combined tablet dosage formulation with better sensitivity, precision and accuracy by using BDS column.

MATERIAL & METHODS

Active pharmaceutical ingredients like Dapagliflozin was procured from Gitar lab, Ahmedabad and Saxagliptin HCl was procured from Neer chemicals, Ahmedabad as gift samples. All solvents used in the work were of HPLC grade and obtained from Rankem. The tablets containing 10 mg of Dapagliflozin and 5 mg of Saxagliptin HCl (QTERN®) were purchased from local markets.

Instrumentation

The analytical method was performed by using the HPLC system Shimadzu (SPD-AT20) equipped with auto sampler, UV and Photo-Diode Array (PDA) detector, Rheodyne injector with 20 µl loop volume, analytical balance (Model AX200), pH analyser (Chemiline CL 180 based pH meter) and Toshcon Ultra Sonicator.

Preparation of Standard Stock Solution

Accurately weighed and transferred 20 mg of Dapagliflozin and 10 mg of Saxagliptin HCl into 100 ml volumetric flask and diluted up to the mark with HPLC grade Methanol to give a stock solution having strength of 0.2 mg/ml of Dapagliflozin and 0.1 mg/ml of Saxagliptin HCl.

Preparation of Working Standard Stock Solution

1 ml of the standard stock solution was pipette out into 10 ml volumetric flask and diluted up to 10 ml with HPLC grade Methanol to produce final approximate concentration of 20 µg/ml of Dapagliflozin and 10 µg/ml of Saxagliptin HCl.

Preparation of Working Sample Solution

Separately accurately weighed 20 tablets and average weight of individual tablets were found out and weight equivalent to 10 mg of Dapagliflozin and 5 mg of Saxagliptin HCl were taken into 100 ml volumetric flask and dissolved in 60 ml HPLC grade Methanol with sonication for 20 minutes. The solution was filtered through 0.45 µ Millipore Nylon filter and the residues were washed thoroughly with HPLC grade Methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with HPLC grade Methanol to get a final concentration of 100 µg/ml of Dapagliflozin and 50 µg/ml of Saxagliptin HCl. Then 1 ml of this solution was pipette out into 10 ml volumetric flask and diluted to 10 ml with HPLC grade Methanol to produce final approximate concentration of 10 µg/ml of Dapagliflzin and 5 µg/ml of Saxagliptin HCl.

Preparation of Buffer Solution

Took about 6.8 gm of Potassium Dihydrogen Ortho Phosphate reagent into a 1000 ml beaker. Add 800 ml Methanol and dissolve. Adjust pH 4.5 of this solution with 1% Orthophosphoric acid. Make up the volume up to 1000 ml with Water.

Preparation of Mobile Phase

Mixed 85 ml of buffer and 15 ml of HPLC grade Methanol. Filtered through 0.45 µ Millipore Nylon filter and degassed.

RESULTS

Method Development

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Water and Acetonitrile as mobile phases, in which the drug did not respond properly. The organic content of the mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes an important factor. Thereafter, Phosphate buffer and Methanol were taken in isocratic ratio 85: 15 and with a flow rate of 1.0 ml/min was employed. Hypersil BDS column C_{18} (250 mm × 4.6 mm) 5µ was selected as the stationary phase to reduce the tailing of the peak. 222 nm was selected as the detection wavelength for PDA detector. The retention time was found to about 4.080 min for Dapagliflozin and 5.343 min for Saxagliptin HCl. The results were shown in table 1 and figure 3.

Table 1: Optimized Chromatographic Conditions

Sr no	Parameter	Condition
1	Mobile Phase	Phosphate buffer: Methanol (85 : 15 v/v)
2	pH	4.5
3	Column	Hypersil BDS C_{18} (250 mm × 4.6 mm) 5µ
4	Column Oven Temperature	25ºC
5	Wavelength	222 nm
6	Injection Volume	20 µl
7	Flow Rate	1.0 ml/min
8	Retention Time	4.080 min for Dapagliflozin and 5.343 min for Saxagliptin HCl

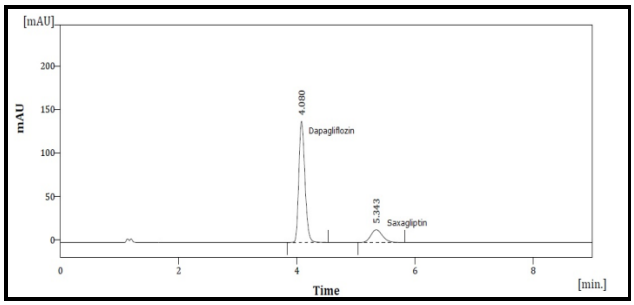

Figure: 3 HPLC Chromatogram of Dapagliflozin (20 µg/ml) and Saxagliptin HCl (10 µg/ml)

Method Validation

The method was validated by determining system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing Dapagliflozin and Saxaglitptin HCl. The analytical method validation was carried out as per ICH method validation guidelines.[11,12]

System Suitability

A system suitability test was performed to evaluate the chromatographic parameters (retention time, number of theoretical plates, capacity factor and asymmetry factor) before the validation runs. The results of system suitability parameters were given in table 2.

Linearity and Range

The linearity of Dapagliflozin and Saxagliptin HCl were evaluated at five concentration levels by diluting the standard stock solution to give solutions of Dapagliflozin and Saxagliptin HCl in the concentration range from 10 - 30 µg/ml and 5 - 15 µg/ml. The regression analysis was carried out for the slope, intercept and correlation coefficient. The results were given in table 3, 4 and figure 4 - 6.

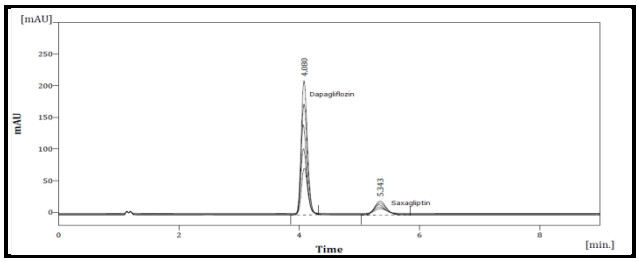

Figure: 4 Linearity Spectra of Dapagliflozin and Saxagliptin HCl at 222 nm

Table 2: System Suitability Parameters (n=3)

Sr. No.	Parameters	Dapagliflozin	%RSD of Dapagliflozin	Saxagliptin HCl	%RSD of Saxagliptin HCl
1	Retention time (min) ± SD	4.087 ± 0.00	0.01	5.348 ± 0.01	0.01
2	Theoretical plate ± SD	7123.33 ± 106.71	1.53	3445.67 ± 55.19	1.60
3	Capacity factor ± SD	0.55 ± 0.00	0.00	1.03 ± 0.00	0.20
4	Asymmetry factor ± SD	1.41 ± 0.02	1.56	1.26 ± 0.00	0.00
5	HETP ± SD	34.74 ± 0.09	0.25	72.56 ± 1.18	1.62
7	Resolution ± SD	-----	----	4.51 ± 0.04	0.88

Table 3: Linearity Data of Dapagliflozin and Saxagliptin HCl

Sr. No.	Concentration (µg/mL) of Dapagliflozin	Average Area of Three Replicates for Dapagliflozin	Concentration (µg/mL) of Saxagliptin HCl	Average Area of Three Replicates for Saxagliptin HCl
1	10	511.350	5	102.378
2	15	734.094	7.5	147.721
3	20	1008.403	10	202.410
4	25	1253.278	12.5	253.218
5	30	1515.813	15	305.502

Table 4: Regression Analysis of Calibration Curve

Sr. No.	Parameters	Dapagliflozin	Saxagliptin HCl
1	Correlation coefficient	0.9992	0.9993
2	Slope	50.562	20.47
3	Intercept	-6.6561	-2.4517

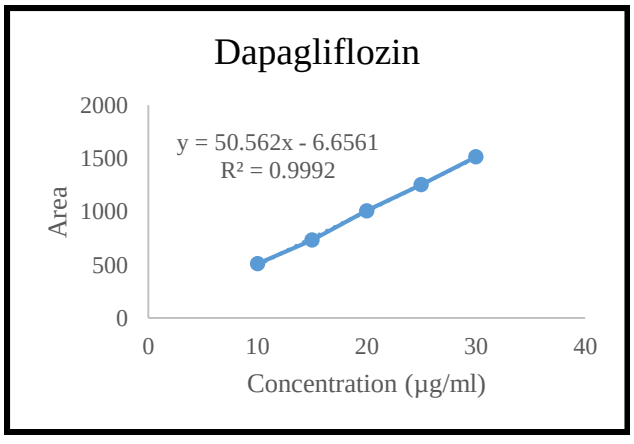

Figure: 5 Calibration Curve of Dapagliflozin

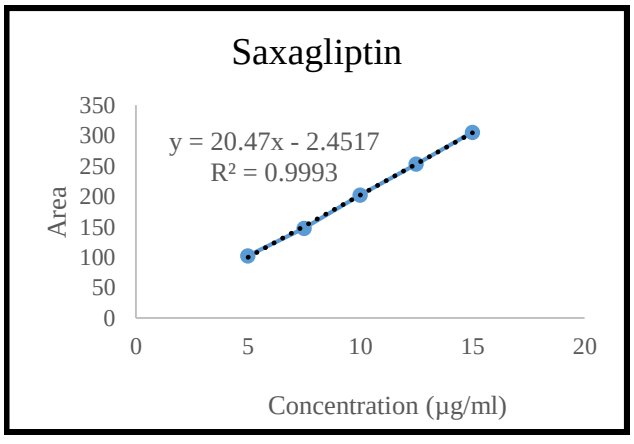

Figure: 6 Calibration Curve of Saxagliptin HCl

Accuracy

The accuracy of the method was determined by the standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 80%, 100% and 120% level of standard solution. The solutions were analyzed in triplicate at each level as per the proposed method. The corresponding results were recorded in table 5.

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Dapagliflozin and Saxagliptin HCl test solution in the equipment. Record the chromatogram. The results were shown in table 6 and 7.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions.

It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined from the standard deviation of y-intercept of the regression line and slope method as per ICH guidelines. Results were shown in table 8.

Analysis of Marketed Formulation by Developed Method

Assay of marketed tablet formulation containing 10 mg of Dapagliflozin and 5 mg of Saxagliptin HCl was carried out by using this validated RP-HPLC method. Three injections of prepared sample and standard solutions were injected. The assay of the commercial sample was calculated by comparing the areas of standard and sample peaks. The assay of marketed formulation QTERN was found within the limit.

Table 5: Results of Recovery Study (n=3)

Drug	Amount of Sample (µg/ml)	Amount of Standard Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery ± SD (n=3)	%RSD
Dapagliflozin	10	8	17.937	8.025	100.31 ± 1.19	1.19
		10	20.001	10.09	100.89 ± 0.95	0.94
		12	22.068	12.16	101.30 ± 0.66	0.65
Saxagliptin HCl	5	4	8.981	4.015	100.38 ± 0.97	0.97
		5	10.012	5.047	100.94 ± 1.2	1.19
		6	10.989	6.023	100.39 ± 0.59	0.59

Table 6: Results of Repeatability

Sample No.	Retention Time (min)		Peak Area	
	Dapagliflozin	Saxagliptin HCl	Dapagliflozin	Saxagliptin HCl
1	4.080	5.343	1011.478	203.113
2	4.087	5.353	1009.442	202.628
3	4.080	5.343	996.977	202.811
4	4.083	5.347	1009.389	202.615
5	4.090	5.353	1007.360	202.80
6	4.097	5.363	1017.485	203.242
Mean	4.086	5.350	1008.689	202.872
SD	0.006	0.008	6.710	0.261
%RSD	0.16	0.14	0.67	0.13

Table 7: Results of Intermediate Precision (n=3)

Concentration (µg/ml)		Intra-day Precision		Inter-day Precision	
		Measured Mean Area ± %RSD		Measured Mean Area ± %RSD	
Dapagliflozin	Saxagliptin HCl	Dapagliflozin	Saxagliptin HCl	Dapagliflozin	Saxagliptin HCl
10	5	507.460 ± 0.48	101.769 ± 0.17	509.291 ± 0.86	101.345 ± 1.89
20	10	1020.596 ± 0.43	203.844 ± 0.71	990.636 ± 1.23	199.057 ± 1.06
30	15	1510.421 ± 0.31	301.321 ± 1.27	1516.091 ± 0.47	304.791 ± 0.30

Table 8: LOD and LOQ of Dapagliflozin and Saxagliptin HCl

	Dapagliflozin	Saxagliptin HCl
LOD (µg/ml)	1.16	0.53
LOQ (µg/ml)	3.52	1.62

Table 9: Analysis of Formulation by Proposed RP-HPLC Method (n=3)

Name of Tablet	Label claim (mg/Tab)		Amount found (mg/Tab) Mean ± SD		% Label claim (%W/W) Mean ± SD	
QTERN	Dapagliflozin	Saxagliptin HCl	Dapagliflozin	Saxagliptin HCl	Dapagliflozin	Saxagliptin HCl
	10 mg	5 mg	9.92 ± 0.04	4.95 ± 0.04	99.24 ± 0.44	98.99 ± 0.85

CONCLUSIONS

The observations and result obtained from this study, including system suitability, linearity and range, accuracy, precision, robustness lie well within acceptable criteria.

From the experimental studies, it can be concluded that the proposed method can be adopted for the routine analysis of Dapagliflozin and Saxagliptin HCl in their combined dosage form without interference of excipients and impurities.

ACKNOWLEDGEMENT

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