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Development and Validation RP-HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk and Pharmaceutical Dosage Form

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Abstract

A simple, precise, sensitive, and rapid reverse phase high performance liquid chromatographic method was developed and validated for simultaneous estimation of Dapagliflozin (DAPA) and Saxagliptin (SAXA) in bulk as well as in tablet formulation according to the ICH guidelines. The chromatographic phase consisted by phosphate buffer: Acetonitrile, pH 4.0 adjusted by glacial acetic acid. The flow rate was adjusted to 0.8 ml/min and UV detection was carried out at 220 nm. Retention time was found to be 2.144 and 3.156 min; respectively. The detector was showed that linear responses over the concentration range 10–24 μg/ml for SAXA and 12–40 μg/ml for DAPA a good correlation coefficient of 0.999. This proposed method is highly sensitive, precise, and accurate which reduces cost of analysis; hence, recommended for routine quality analysis in laboratories.

Keywords: Dapagliflozin, Saxagliptin, Simultaneous estimation, Validation

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Introduction

The combination of saxagliptin (SAXA) and dapagliflozin (DAPA) may have a major glycemic regulation advantage without the risk of weight gain and hypoglycemia, which may be linked with other medicines used to treat diabetes of Type 2. SAXA is an oral hypoglycemic (anti-diabetic) dipeptidyl peptidase-4 (DPP-4) inhibitor with a IUPAC name (1S, 3S, 5S)-2[(2S)-2-(3-hydroxy-1-adamantyl)-acetyl]-2-azabicyclo[3.1.2] hexane-3-carbonitrile [Figure 1].[1] It is used for the treatment of Type 2 diabetes either in monotherapy or in combination with other drugs. The drug acts to suppress protein/enzyme, DPP-4, competitively resulting in an increased amount of active augmentin: Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1. DAPA is a sodium-glucose cotransporter-2 inhibitor with a chemical name (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol [Figure 2]. Such sodium-glucose cotransporters are responsible for reabsorbing glucose into the kidney.[2,3] An extensive literature survey has revealed that there are few methods that are reported for estimation of SAXA and DAPA individually or in combination with other drugs by UV method,[4-7] reverse phase high performance liquid chromatographic (RP-HPLC) method,[8-16] tandem mass spectrometry method,[17] or LC-MS.[18,19] Hence, we propose to develop methods that are more reliable, sensitive, and simple for simultaneous estimation of SAXA and DAPA in bulk as well as a pharmaceutical formulation. In the present work, we developed and validated the UV method and RP-HPLC method for the simultaneous estimation of SAXA hydrochloride and DAPA in bulk drug and its formulation as per ICH guidelines Q2(R1).[20]

Materials and Methods

Reference standards for DAPA and SAXA were gifted by CTX Lifesciences (Surat) and Zydus Healthcare (Ahmedabad), HPLC grade methanol, and all chemicals were obtained from LOBA. During the entire study, HPLC grade water was obtained from the Milli-Q. Micro Laboratories Private Limited, Pondicherry. Pharmaceutical preparation for combination of DAPA and SAXA was obtained from local market. Methanol and acetonitrile of analytical reagent grade and HPLC grade, orthophosphoric acid, and water of HPLC grade were purchased from Merck Limited, India.

Chromatographic Conditions

The analysis of drugs was carried out on a Shimadzu LC isocratic system, prominence equipped with an manual sampler and PDA detector was used for the analysis. The data were recorded using LC-solution software. Hypersil C18 (10 um, 150 × 4.6 mm) was used for the analysis. A ultrasonicator was used for degassing of the mobile phase. In this RP-HPLC method, separation was carried out using a mobile phase consisting of phosphate buffer: Acetonitrile, pH 4.0 adjusted by glacial acetic acid The mobile phase was filtered using a 0.45 μm nylon membrane filter. The analysis was carried out room temperature and the flow rate was 0.8 ml/min. Optimized chromatographic conditions was shown in Table 1.

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Preparation of Standard Stock Solution

100 mg of DAPA and 100 mg SAXA were weighed separately and transferred to 100 ml volumetric flasks. Both of them were dissolved in methanol and volume was made up to mark with methanol giving final solution containing 1000 μg/ml, respectively, from this 10 ml transfer to 100 ml to get 100 μg/ml, from this required amount was transferred to 10ml to get the 10–22 μg/ml and 12–40 μg/ml of SAXA and DAPA.

Preparation of Sample Solution

20 tablets was weighed and its average weight was determined. Accurately weigh and transfer the quantity of tablet contents equivalent to about 5 mg of SAXA and 10 mg of DAPA transfer into a 10 ml volumetric flask and add methanol and sonicated for about 20 min. Dilute it up to mark with methanol and mix to get a solution containing 500 μg/ml of SAXA and 1000 μg/ml of DAPA. Take 10 ml aliquot in a separate 100 ml volumetric flask. Dilute it up to mark with methanol to get a solution containing 50 μg/ml of SAXA and 100 μg/ml of DAPA. Take 3.2 ml of an aliquot in separate 10 ml volumetric flask. Dilute it up to mark with methanol to get a solution containing 16 μg/ml of SAXA and 32 μg/ml of DAPA. The analysis procedure was repeated 3 times.

System Suitability

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition at a 100% level to verify the precision of the chromatographic system. The purposed RP-HPLC method permits the determination of DAPA and SAXA in sample drug that have different retention times. System suitability data are given in Table 2.

Determination of Purity

The amounts of DAPA and SAXA per tablet were calculated by extrapolating the value of area from the calibration curve using UV detection at 220 nm Procedure was repeated 3 times with the same tablet formulation. Moreover, obtained results are tabulated in Table 3.

Method Validation

The present method of analysis was validated according to the recommendations of ICH-1996 and USP-30 for the parameters such as specificity, system suitability, accuracy, linearity, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).

Table 1: Optimized chromatographic conditions

Stationary phase	C18 (250 mm×4.6 mm I.D, 5 μ)
Mobile phase	Phosphate buffer: Acetonitrile, pH 4.0 adjusted by glacial acetic acid
Mobile phase ratio	55:45
Detection wavelength	220 nm
Flow rate	0.8 ml/min
Sample size	10 μl
Temperature	Room temperature

Table 2: System suitability parameters

S. No.	*System Suitability Parameters*	*Dapagliflozin*	*Saxagliptin*
1.	Retention Time (Min)	3	7
2.	Theoretical Plates	5302.5	3545.4
3.	Area Under Curve	659.13	690.34
4.	Tailing Factor	0.96	1.16

Specificity

It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resoluted from any other peak by resolution of minimum 2. This could be done injecting placebo and compare it with that of standard and placebo spiked with standard and sample, then peak purity was ascertained by use of PDA.

Linearity

The response for the detector was determined to be linear over the range of 10–24 μg/ml for SAXA and 12–40 μg/ml for DAPA, linearity curves were constructed using relative peak area to avoid very high value for intercept.

Precision

The precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was determined by repeatability, inter-day, and intra-day experiments.

Accuracy

Accuracy was calculated by addition of standard drugs to pre-analyzed sample at three different concentration levels (50%, 100%, and 150%) and computing percentage recoveries. Standard limit of % recovery study is 98–102% as per ICH guideline. From the studies, it was concluded that % recovery study of DAPA and SAXA complies with standard limit of ICH guideline.

Robustness

The robustness is the capacity of method to remain unaffected by small but deliberate changes in chromatographic conditions. Robustness was studied by testing the influence of small changes in column temperature (±5°C), change in flow rate (±10%), and changes in mobile phase composition (70:30).

Quantification limit

LOD and LOQ for the optimized method were performed as per ICH guidelines. LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQ was considered as the lowest concentration of analytes in standards

Table 3: Assay of DAPA and SAXA sodium

Drug	*Label Claim (mg)*	*Sample Conc. (μg/ml)*	*Peak Area*
Saxagliptin	5	16	344754
			344960
			344759
%Assay±SD			99.68±117.5174
%RSD			0.03408
Dapagliflozin	10	32	1843832
			1843736
			1843941
%Assay±SD			99.97±102.5687
%RSD			0.005563

*All the results were average of 6 readings, (*n*=3). DAPA: Dapagliflozin, SAXA: Saxagliptin

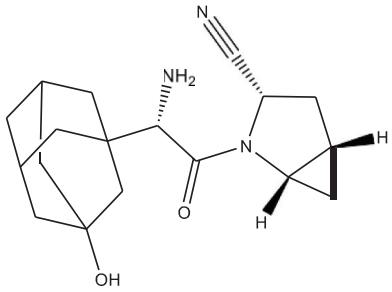

Figure 1: Structure of Saxagliptin

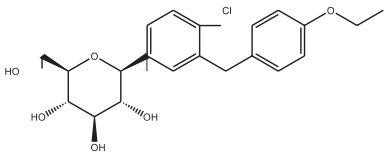

Figure 2: Structure of Dapagliflozin

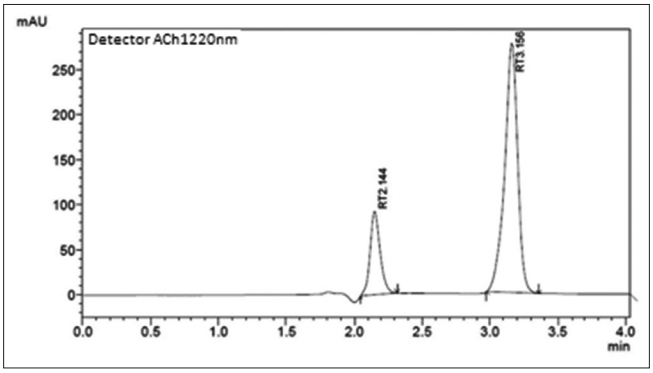

Figure 3: RP-HPLC chromatogram of Dapagliflozin and Saxagliptin standard drug

that can be reproducibly measured with acceptable accuracy and precision. LOD and LOQ were separately determined at a signal to noise ratio (S: N) of 3:10 and which was based on calibration curve. The standard deviation of y intercept and slope of the regression line were used. The LOD and LOQ were calculated using following formula as LOD = 3.3 × D/S and LOQ = 10 × D/S

Where, S = Slope of regression line, D = Standard deviation of y-intercept on the regression line.

Results and Discussion

The proposed RP-HPLC method required fewer reagents and materials, standard solution of DAPA and SAXA was injected to get a chromatogram. The chromatogram of SAXA and DAPA is shown in Figure 3. There was clear resolution between SAXA and DAPA with retention time of 2.144 and 3.156 min, respectively.

Linearity (Calibration Curve)

The response for the detector was determined to be linear over the range of 10–24 µg/ml for SAXA as shown in Figure 4 and data are shown in Table 4. The response for the detector was determined to be linear over the range of 12–40 µg/ml for DAPA as shown in Figure 5 and data are shown in Table 5. Each of the concentration was injected in triplicate to get reproducible response calibration curves that were constructed by plotting peak area verses concentration in µg/ml. Each reading was average of three determinations. They were represented by the linear regression equation. For SAXA Y = 25112x – 51688, r^2 = 0.9984 and for DAPA Y= 51715x + 23427, r^2 = 0.997.

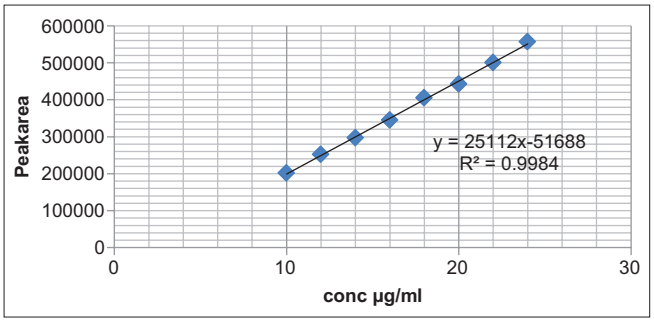

Figure 4: Calibration curve of Saxagliptin

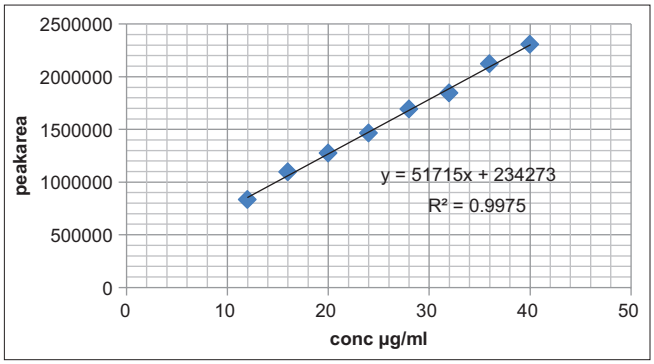

Figure 5: Calibration curve of Dapagliflozin

Table 4: Linearity data SAXA (*n*=3)

S. No.	*SAXA*		
	Conc. (µg/mL)	*Peak area Mean±SD*	*%RSD*
1.	10	202156±4410	1.18
2.	12	252329±1830	0.72
3.	14	297284±5386	1.81
4.	16	344760±1398	0.40
5.	18	405429±2821	0.69
6.	20	442463±1163	1.62
7.	22	500817±1233	0.24
8.	24	556461±4073	0.73

Linearity Equation: Y=25112x–51688, r^2=0.9984. SAXA: Saxagliptin

Table 5: Linearity data DAPA (*n*=3)

DAPA		
Conc. (µg/mL)	*Peak area Mean±SD*	*%RSD*
12	834613±3958	0.47
16	1095381±638	0.05
20	1273055±9887	0.77
24	1463528±2191	0.14
28	1691868±30634	1.81
32	1843934±3596	0.19
36	2122818±7747	0.36
40	2305784±8416	0.36

Linearity Equation: Y=51715x+23427, r^2=0.997. DAPA: Dapagliflozin

Accuracy

Accuracy was performed and % recovery was found to be within 98–102% at all three levels. This indicates that the SAXA Table 6

Table 6: Accuracy data of Saxagliptin (*n*=3)

Level of recovery	*Sample Concentration (µg/ml)*	*Concentration of Std added (µg/ml)*	*Total Concentration (µg/ml)*	*Peak Area*	*Mean Peak Area*[1]	*Amt. Recovered (µg/ml)*	*% Recovery*
80%	10	8	18	405327 405228 404431	404995	17.88	99.33
100%	10	10	20	442467 442364 441459	442096	19.98	99.9
120%	10	12	22	500816 500812 500714	500780	22.21	100.95

[1]Mean area of *n*=3

Table 7: Accuracy data of Dapagliflozin (*n*=3)

Level of recovery	*Sample Concentration. (µg/ml)*	*Concentration of Std added (µg/ml)*	*Total Concentration (µg/ml)*	*Peak Area*	*Mean Peak Area*[1]	*Amt Recovered (µg/ml)*	*% Recovery*
80%	20	16	36	2120817 2115816 2127814	2121482	35.97	99.91
100%	20	20	40	2305672 2304984 2305832	2305496	39.79	99.47
120%	20	22	44	2543210 2543223 2543212	2543215	44.12	100.27

[1]Mean area of *n*=3

Table 8: Intra-day precision data of SAXA and DAPA

S. No.	*SAXA Peak area*			*DAPA Peak area*		
	Sample-1	*Sample-2*	*Sample-3*	*Sample-1*	*Sample-2*	*Sample-3*
1.	202146	344960	501827	834614	1463532	2121314
2.	202151	344657	500813	834604	1463518	2122716
3.	212243	343766	501816	834620	1363425	2021814
Average	205513.33	344461	501485.33	834612.66	1430158	2088614.67
SD	5828.062	620.661	582.283	8.08290	57792.76	57855.3213
% RSD	2.8358	0.18018	0.11611	0.00096	4.041004	2.77003

DAPA: Dapagliflozin, SAXA: Saxagliptin

and DAPA Table 7 can be recovered successfully in presence of excipients.

Intra-day Precision

Sample stock solution containing 10, 16, and 22 µg/ml of SAXA and 12, 24, and 36 µg/ml of DAPA was prepared from their respective solution. Analysis was performed in triplicate; the result of intra-day precision studies was shown in Table 8.

Inter-day Reproducibility (Method Ruggedness)

Three replicates of a different concentration of sample solution are used for each determination. First day: Three replicates, on a 2nd day: Three replicates, then on 3rd day: Three replicates of freshly prepared test from the same sample are analyzed, under the same conditions. The result of inter-day reproducibility and percentage amount obtained is shown in Table 9.

Quantification Limit

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of SAXA and DAPA found to be 3.99 µg/ml and 0.98 µg/ml, respectively. The LOQ of SAXA and DAPA found to be 12.109 µg/ml and 2.994 µg/ml, respectively.

Table 9: Inter-day reproducibility data of SAXA and DAPA

Assay (% labeled amount)						
Sample No	*SAXA*			*DAPA*		
	DAY-1	*DAY-2*	*DAY-3*	*DAY-1*	*DAY-2*	*DAY-3*
Sample-1	99.32	99.4	99.46	99.85	101.76	100.02
Sample-2	99.89	99.92	98.43	100.11	99.52	100.43
Sample-3	99.92	100.32	100.42	99.1	99.69	99.28
Average	99.71	99.88	99.436	99.686	100.32	99.91
SD	0.3380	0.4613	0.9952	0.52443	1.2470	0.5828
%RSD	0.3390	0.4618	1.0008	0.52608	1.2430	0.5833

DAPA: Dapagliflozin, SAXA: Saxagliptin

Discussion

In his study reported HPLC method for simultaneous estimation of SAXA and DAPA showing linearity range at 10–24 µg/ml and

12–40 µg/ml, 0.8 ml/min flow rate, etc., this indicating scope to minimize total cost of analysis.

Conclusion

Proposed study describes HPLC method for the simultaneous estimation of DAPA and SAXA in bulk as well as in tablet formulation. This method showed good separation of two compounds with less retention time than any method listed in reference. This method is useful to minimize total cost of analysis. The method is validated and found to be simple, sensitive, accurate, precise, and robust. Hence, proposed method is suggested for routine quality analysis of DAPA and SAXA in laboratories.

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