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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP- HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN IN BULK AND TABLET DOSAGE FORM

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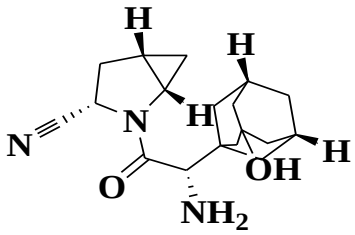
ABSTRACT

A novel rapid, sensitive and reproducible stability-indicating RP-HPLC method has been developed and validated for quantitative analysis of Saxagliptin and Dapagliflozin in bulk drug and pharmaceutical dosage form. Chromatographic separation was achieved on Thermo C_{18} analytical column (250mm×4.60 mm, 5.0µm) with 20mM KH_2PO_4: acetonitrile 35:65 v/v as isocratic mobile phase enabled separation of the drug from its degradation products. UV detection was performed at 252 nm. The method was validated for linearity, accuracy (recovery), precision, specificity and robustness. The linearity of the method was satisfactory over the range 1-5µg/ml for SAXA and 2-10 µg/ml for DAPA with correlation coefficient 0.998, 0.999 for SAXA & DAPA respectively. The limits of detection and quantification of SAXA and DAPA were 0.45,1.20 and 0.52 ,1.53 µg/ml respectively. Recovery of SAXA and DAPA from the pharmaceutical dosage form ranged from 98.13-99.75 and 98.57-99.26% respectively. SAXA and DAPA were subjected to stress conditions (hydrolysis (acid, base), oxidative, photolytic, and thermal degradation) and the samples were analyzed by this method.

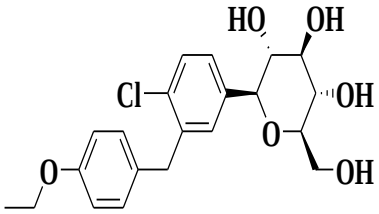
Keywords: Saxagliptin and Dapagliflozin, RP-HPLC, Forced degradation, Method validation

INTRODUCTION

Dapagliflozin (DAPA), (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl) phenyl]-6- (hydroxymethyl) tetrahydro-2H-pyran-3,4,5- triol is indicated for the management of diabetes mellitus Type-2 and functions to improve glycemic control in adults when combined with diet and exercise. DAPA is an inhibitor of sodium-glucose co-transporter 2 (SGLT2) responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. By inhibiting SGLT2, DAPA reduces reabsorption of filtered glucose and lowers the renal threshold for glucose and thereby increases urinary glucose excretion. Saxagliptin (SAXA) (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1- adamantyl)acetyl]-2 azabicyclo[3.1.0]hexane-3-carbonitrile) is an orally active, potent and selective inhibitor of dipeptidyl peptidase- IV (DPP-4) for the treatment of Type-2 diabetes, is marketed in a fixed dose combination SAXA/ DAPA as tablet in USA, Europe and some other countries [1-2]. DPP-4 inhibitors enhance the body's own ability to control blood glucose by increasing the active levels of incretin hormones in the body. Their mechanism of action is distinct from any existing class of oral glucose-lowering agents. They control elevated blood glucose by triggering pancreatic insulin secretion, suppressing pancreatic glucagon secretion and signaling the liver to reduce glucose production [3-5]. The structures of SAXA and DAPA are shown in **Figure 1A &1B** respectively. As literature review reveled that there are few reported methods available for SAXA alone and in combination with other drugs [6-10]. some analytical methods have been reported for the estimation of SAXA and DAPA in combination; all are being HPLC [11-13]. Literature survey also revealed that there are some methods reported for estimation of SAXA and DAPA either in single or in combination and stability studies [14-17]. The developed method can be applied successfully to quality control and for other analytical purposes. To access the reproducibility and wide applicability of the developed method, it was validated as recommended by the international Conference on Harmonization (Q2R1 and Q2R2). [18] Which is mandatory also.



(A)



(B)

Figure 1: Chemical structure of (A) Saxagliptin and (B) Dapagliflozin

EXPERIMENTAL

Instrument:

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 pump for constant flow and constant pressure delivery and UV-Visible detector (detection limit 170nm to 700nm) connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Reagents and chemicals:

Working standards of pharmaceutical grade SAXA and DAPA were obtained as a gift samples from Scan Research Laboratories, Bhopal. The tablet dosage form QTERN manufactured by Astra Zeneca (Label Claim: SAXA 5 mg and DAPA 10 mg) was procured from the local pharmacy. HPLC grade acetonitrile was obtained from Merck (India) limited. Potassium Dihydrogen orthophosphate and Ortho-phosphoric acid (AR grade) was obtained from Merck India. All other chemical used were of HPLC grade.

Chromatographic conditions:

Chromatographic conditions performed at ambient temperature, the isocratic mobile phase consisted of 20mM KH_2PO_4: acetonitrile (pH 3.0) in the ratio of 35:65v/v, flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered and was degassed before use (20 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic Parameter, sensitivity and selectivity of method for drugs, 252 nm was selected as the detection wavelength for UV-Visible detector.

Selection of Diluent:

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention time and resolution of analyte. After various trials Acetonitrile was used as diluents.

Preparation of standard stock solution:

Accurately weighed 10 mg of API SAXA and 10 mg of DAPA was transferred into 10 ml volumetric flasks separately and dissolved in 5 ml of acetonitrile and sonicate for 10 min., then volume was made up to 10 ml with acetonitrile. Concentration of saxagliptin and dapagliflozin in acetonitrile was 1000µg/ml. **(stock- A)**

Preparation of sub stock solution:

Then 1 ml of solution was taken from stock-A of SAXA and DAPA and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 µg/ml **(Stock-B).**

Preparation of Different Solution

Then 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of **Stock-B (SAXA)** was taken in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml of SAXA. In similar manner 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml of DAPA were also prepared.

Assay of Tablet Formulation

Mixed Blends of SAXA and DAPA were weighed and ground to a fine powder; amount equal to 5mg of SAXA (10mg of DAPA) was taken in 100ml volumetric flask. Then 50ml of acetonitrile was added and the flask was sonicated for about 20 min to solubilizing the drug present in tablet formulation and the volume was made up to the mark with acetonitrile. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with acetonitrile to get the final concentrations of both drugs in the working range. The amounts of SAXA and DAPA in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated three times with formulation

METHOD VALIDATION PARAMETERS:

The method was validated for linearity, specificity, limits of detection (LOD) and limits of quantification (LOQ), system suitability, accuracy, precision, robustness and stability in accordance with ICH guidelines.

Specificity:

Specificity and peak purity was determined by use of the UV- Vis. detector. To check linearity, standard solutions of SAXA and DAPA were prepared in five concentration 1- 5µg/ ml for SAXA and 2-10 µg/ml for DAPA. Each solution was injected in triplicate and calibration graphs were obtained by plotting peak area against concentration.

Linearity:

It was checked over the same concentration range on three consecutive days. % RSD of the slope and Y-intercept of the calibration plot were also calculated.

Limits of Detection and Limits of Quantification:

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Precision:

The **accuracy** of the method was studied by measurement of recovery after adding known amounts of the drug (80, 100 and 120% of the label claim of known amount of SAXA and DAPA per injection) to the placebo. Three samples were prepared for each recovery level and results were calculated by use of the calibration plot.

Robustness:

The **robustness** of the method was assessed by deliberate alteration of the experimental conditions. The change was made by altering the pH or concentration of the mobile phase to check the method capacity to remain unaffected. The effect of changes in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of SAXA and DAPA was studied.

Forced degradation studies:

According to ICH guidelines (Q1A) stability testing is necessary to classify the stability characteristics of active ingredients. In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried Out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml round bottom flask separately then 50 ml of 0.1 N HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 40^{0}C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

50 mg of both the drug sample was taken into a 50 ml round bottom flask separately then, 50 ml of 0.1 N NaOH solution was added and contents were mixed well and kept for constant stirring for 2 h at 40^{0}C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

Oxidative degradation:

50 mg of both the drug sample was taken into a 50 ml round bottom flask separately then, 50 ml of 0.2% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr. at 40^{0}C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

50 mg of the drug sample was taken in to a petri dish and kept in oven at 75^{0}C for 2 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

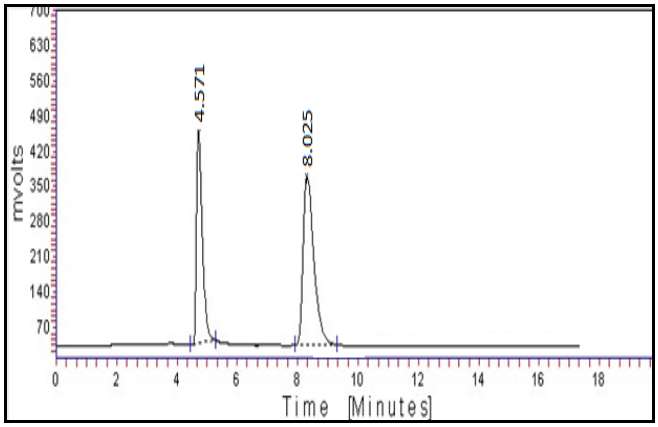


Figure 2: Standard chromatogram of SAXA and DAPA

RESULTS & DISCUSSION

Optimization of chromatographic conditions

To optimize the RP-HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation, good peak symmetry and best resolution was obtained with a mobile phase consisting of 20mM KH_2PO_4: acetonitrile (pH 3.0) in the ratio of 35:65v/v, mobile phase was attempted for quantitation of SAXA & DAPA with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates and HETP) at 252 nm as detection wavelength. The column temperature was 25°C. The tailing factor for SAXA & DAPA was < 2 and retention times were approximately 4.571 ± 0.2 and 8.025± 0.4min for SAXA & DAPA and less than 10 min for the degradation products. This low total runs time resulted in high productivity and low cost of analysis as per sample.

Method validation

Peak purity was >99.9% for drug substance and drug degradation products at 252 nm which showed that the analyte peaks were pure and that formulation excipients and degradation products were not interfering with analyte peaks. LOD and LOQ for SAXA and DAPA were 0.45, 1.20 and 0.52, 1.53µg/ml respectively for 20µl injection volumes. Results from regression analysis are listed in **table 1** with system suitability data. When precision was determined by six fold analysis of drug injection, the RSD of SAXA and DAPA peak area was less than 2% indicating that the method is reliable. Results from assessment of precision are listed in **table 2**. Results obtained from recovery study are listed in **table 3** and results from robustness and analysis of formulation are shown in **table 4 & 5** respectively.

Table 1 Results from regression analysis and system suitability data

PARAMETERS	SAXA	DAPA
Retention time*	4.623±00.052	8.037±0.020
Tailing factor*	1.273±00.049	1.195±0.059
Theoretical plate*	3255.500±34.057	3403.333±109.876
Linear range (µg/ml)	1-5	2-10
LOD (µg/ml)	0.45	0.52
LOQ (µg/ml)	1.20	1.53
Linear equation	AUC=158.1conc.+ 10.024	AUC=102.5conc. + 4.425
Slope	158.1	102.5
Intercept	10.024	4.425
Correlation coefficient (r^2)	0.998	0.999

*Mean of six readings

Table 2: Results of precision

PARAMETERS	% MEAN±SD*	
	SAXA	DAPA
Repeatability	98.881±0.033	98.936±0.047
Intermediate precision		
Day to day precision	98.420±0.020	99.273±0.035

***** Value of five replicate and five concentrations

Table 3: Results of recovery study

% LEVEL	% MEAN±SD*	
	SAXA	DAPA
80%	99.75±1.379	99.19±0.452
100%	98.70±00.064	98.57±0.357
120%	99.10±0.566	99.26±0.212

***** Value of three replicate and three concentrations.

Table 4: Results of robustness

PARAMETERS	% MEAN±SD*	
	SAXA	DAPA
Robustness	98.803±0.045	98.269±0.111

***** Value of five replicate and five concentrations

Table 5: Results of formulation analysis

	SAXA*	DAPA*
Label Claim (mg)	**5mg**	**10mg**
% Found (mg)	4.98	9.96
% Assay	99.60	99.60
% RSD	0.145	0.156

*Average of three determination

Forced degradation study:

Forced degradation studies were performed in various stress conditions. Peak purity test results confirmed that the SAXA and DAPA peak was homogeneous under all the stress conditions tested. The mass balance of SAXA and DAPA in stress samples was close to 100% and moreover, assay of unaffected SAXA and DAPA in the injection confirmed the stability-indicating nature of the method. Chromatographic peak-purity data were obtained from the spectral analysis report, peak purity greater than 99% is indicative of a homogeneous peak. The peak purity for SAXA and DAPA from degradation studies was in the range 99.9-100.0.No degradation peaks co-eluted with the SAXA and DAPA peak, suggesting the method enabled specific analysis of SAXA and DAPA the presence of its degradation products. The results of forced degradation studies are summarized in table 6 & 7.

Table 6: Results of Forced degradation studies of SAXA

STRESS CONDITIONS	DRUG RECOVERED (%)	DRUG DECOMPOSED(%)
Standard drug	99.45	0
Acidic hydrolysis	83.56	16.44
Alkaline hydrolysis	82.23	17.77
Oxidative degradation	94.45	5.55
Photolytic degradation	95.56	4.44

Table 7: Results of Forced degradation studies of DAPA

STRESS CONDITIONS	DRUG RECOVERED (%)	DRUG DECOMPOSED (%)
Standard drug	99.65	0
Acidic hydrolysis	82.32	17.68
Alkaline hydrolysis	85.56	14.44
Oxidative degradation	90.12	9.88
Photolytic degradation	**93.32**	**6.68**

CONCLUSION:

The method developed for quantitative analysis of SAXA and DAPA is rapid, precise, accurate and selective. Peak purity studies under all the stress conditions showed the drug peak to be pure and hence the method is stability indicating. The developed method can be utilized for the successful quantification of the drug in presence of its degradation product and excipients. The method was completely validated and satisfactory results were obtained for all the characteristics tested. The method can be conveniently used for routine analysis of SAXA and DAPA as bulk drug and in respective pharmaceutical dosage forms.

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