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Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form

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ABSTRACT

A simple, fast, and highly selective RP-HPLC method was developed for the determination of Dapagliflozin (DAP) and Saxagliptin (SAX) in API and tablet dosage form. The separation was done using a Xterra RP18 (4.6×150 mm, 5 µm particle size) column with Acetonitrile: water (60:40). The isocratic elution mode at a flow rate of 1 mL/min, and the analytes were measured at 248 nm. The retention time for DAP and SAX were about 2.091 and 3.249 min, respectively. Calibration curves were found to be linear in the ranges of 100-500 µg/ml for DAP and 50-250 µg/ml for SAX, with correlation coefficients of 0.9998. The detection and quantification values for DAP was 3.0 and 9.98 µg/ml and SAX was 3.02 and 10 µg/ml respectively.

INTRODUCTION

Dapagliflozin is chemically known as (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol (Fig. 1a). Saxagliptin is chemically known as (1S,3S,5S)-2-{(2S)-2Amino-2-[(1r,3R,5R,7S)-3-hydroxyadamantan-1-yl]acetyl}-2-azabicyclo[3.1.0] hexane-3 carbonitrile [1,2] (Fig. 1b). A Literature survey shows that numerous analytical methods are reported for the individual estimation of DAP and SAX or with other pharmaceutical preparations, by various methods such as UV spectrophotometry [3] HPLC [4-8], HPTLC [10,11], UPLC [12], LC MS [13-15]. On the other hand, there is no method reported for dapagliflozin and saxagliptin by HPLC. Hence there is a need for a sensitive HPLC method which is stable and indicating for DAP and SAX. Stability studies was carried out by forcing the drug under variety of stress conditions such as thermal, oxidative, light and hydrolysis (acid and base), The established HPLC method was validated as per ICH guidelines [16].

MATERIALS AND METHODS

Chemicals

DAP and SAX were obtained as a gift from Glenmark Pharma&Piramal healthcare (India). Fixed dose combination of tablet formulation Qtern tablets (AstraZeneca) containing 10 mg/5 mg of DAP and SAX were procured form local market. HPLC grade acetonitrile and water were procured from Merck, India. A membrane filter of 0.45 µm porosity was used to filter and degassed the mobile phase. Chemicals used were of analytical or HPLC grade.

Instrumentation and materials

Waters HPLC 2695 was used for analysis. The separation was done on a UV detector and sampling was done by auto sampler. Data collection for chromatogram was done by empower software 2. The column used was Xterra column (150×4.6 mm) with mobile phase composition of

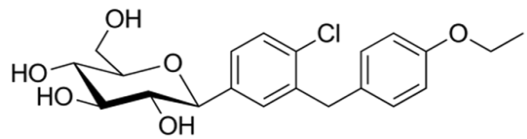

Figure 1a. Structure of Dapagliflozin

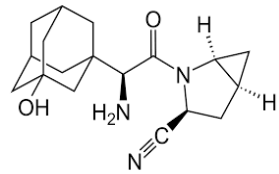

Figure 1b. Structure of Saxagliptin

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acetonitrile: water (60:40). Filtration of mobile phase was carried out by 0.45 µm membrane filter under the isocratic condition with flow rate of 1.0 ml/min, injected volume was 20 µL and elution monitored at 248 nm with run time of 10 min.

PREPARATION OF SOLUTION

Standard preparation

Standard stock solutions were set by dissolving 10 mg of DAP and 5 mg of SAX in acetonitrile: water (60:40) mixture as diluents in 10 ml volumetric flask to achieve concentration of 1000 µg/ml for Dap and 500 µg/ml for SAX respectively. It was sonicated followed by filtration using 0.45 µm porosity filter paper. The stock solution was diluted by pipetted out 3 ml of above solution into 10 ml volumetric flask to produce reference standard solution containing DAP (300 µg/ml) and (SAX 150 µg/ml), respectively.

Sample preparation

Weight equivalent to powder containing 10 mg of DAP and 5 mg of SAX were dissolved in a 10 ml clean dry volumetric flask and diluent was added. It was sonicated, followed by filtration using 0.45 µm porosity filter paper (stock solution). We further pipetted 3 ml of Dapagliflozin and Saxagliptin from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. The solutions were subject to analysis and results shown in Table 5.

DEGRADATION STUDIES

Preparation of stock

The stock solution was prepared by dissolving 10 mg of DAP and 5 mg of SAX in acetonitrile: water (60:40) mixture as diluents in 10 ml volumetric flask and then sonicated for 10 min and finally made up to the volume (Stock solution).

Hydrolytic degradation under acidic condition

The acid hydrolysis was done by pipetted out 3 ml of solution along with 3 ml of 0.1 N HCl into 10 ml volumetric flask. This was kept at 60°C for 24 hours and then neutralized with 0.1 N NaOH, followed by filtration with 0.45 µm syringe filter and placement in vials.

Hydrolytic degradation under alkaline condition

The base hydrolysis was carried out by pipetted out 3 ml of solution along with 3 ml of 0.1 N NaOH into 10 ml volumetric flask. This was kept at 60°C for 24 hours and then neutralized with 0.1 N Hcl, followed by filtration with 0.45 µm syringe filter and placed in vials.

Thermal induced degradation

Thermal degradation was carried out by placing solid samples and tablets in a Petridish and keeping these in a hot air oven at 110°C for 3 hrs, followed by filtration with 0.45 µm syringe filter and placed in vials.

Oxidative degradation

The oxidative degradation was carried out by pipetted out 3 ml of solution along with 1 ml of 12.5% w/v of hydrogen peroxide into a 10 ml volumetric flask. This was then kept at room temperature for 15 min, followed by filtration with 0.45 µm syringe filter and placed in vials.

Photo degradation

The photolytic degradation was carried out by taking solid samples and tablets placing these spread out as a thin layer on a Petri plates. It subsequently exposed to UV light in a chamber for 48 hrs. The stressed sample was filtered through 0.45 µm syringe filter before its analysis.

Validation parameters[16]

Accuracy

The accuracy method was performed by utilizing the standard additional method. The concentration of drug at different levels (50%, 100%, 150%) was evaluated and the mean recovery of DAP and SAX was calculated.

Precision

Intraday precision was performed by taking a concentration of 300 µg/mL for Dapagliflozin and 150 µg/mL for Saxagliptin on the same day. The inter day precision were carried out at a similar concentration on three days by different operators, respectively. The standard solution was injected for six times and the area for all six Injections was measured in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Linearity

The linearity was done by diluting the stock solution with mobile phase to yield a concentration of 100-500 µg/mL for DAP and 50-250 µg/mL for SAX. The linearity was performed by linear regression analysis using least square method.

Robustness

Robustness was done by varying slight changes in the parameters such as the mobile phase composition, flow rate, wavelength and column temperature.

System suitability study

System suitability tests were carried out on a freshly prepared standard solution of the DAP and SAX to analyse the various optimized parameters such as (eg. Theoretical plates, resolution and tailing factor).

RESULTS AND DISCUSSION

The chromatographic method was optimized by varying parameters, such as flow rate, mobile phase, column temperature and detection wavelength. The method was performed with various columns such as the C18 column, Hypersil column, Lichrosorb and Intersil ODS column. Xterra RP18 (4.6×150 mm, 5 mm) were found to be ideal as it gives

good peak shape and resolution at 1.0 ml per min flow. The method was optimized with mobile phase composition of acetonitrile and water 60:40 (v/v) at a flow rate of 1 mL/min and at 248 nm by using a Xterra RP18 (4.6×150 mm, 5 mm) column. The peak was eluted at less than 5 min. The results are seen in Table 1.

Table 1. Chromatographic conditions

Column	Xterra RP18 (4.6×150 mm, 5 µm particle size)
Elution method	Isocratic
Mobile phase	Acetonitrile: water (60:40)
Flow rate	1 ml/min
Column temperature	25c
Volume of injection	20 µL
Detector	UV detector
Detection wavelength	248
Run time	10 min

The retention times obtained for dapagliflozin and saxagliptin were 2.089 and 3.253 min, respectively. The standard and sample chromatogram were shown in Fig. 2 and 3, respectively. Quantitative linearity of drugs was obeyed in the concentration range of 100-500 µg/ml for DAP and 50-250 µg/ml for SAX, respectively. The relevant regression equations were y=2189.9x+32315 for dapagliflozin (r^2=0.9998) and y=2889.6x+10443 for saxagliptin (r^2=0.9998) (where y is the peak area and x is the concentration for dapagliflozin and saxagliptin. The corresponding mean recoveries for dapagliflozin and saxagliptin were 100.72% and 100.02%. This reveals that the method is quite accurate and precise. The %RSD was found to be less than 2 for accuracy and precision, indicating that the method is accurate (Table 2&2a).

Table 2. Accuracy studies (n=6)

Sample	Level (%)	Peak area	Amount recovered (µg/mL)	%RSD
Dapagliflozin	50	671842.3	100.40	0.6
	100	1361348	100.78	0.3
	150	2045898	100.97	0.1
Saxagliptin	50	432916.3	100.43	0.7
	100	861056.3	99.88	0.3
	150	1289755	99.78	0.7

Table 2a. Precision studies (n=6)

Injection	Area for Saxagliptin	Area for Dapagliflozin
Injection-1	111368	852828
Injection-2	112717	852337
Injection-3	112655	858355
Injection-4	113939	852839
Injection-5	1112.513	858513
Injection-6	112282	857582
Average	112662.3	855409.0
Standard Deviation	845.7	12.524.5
%RSD	0.8	0.4

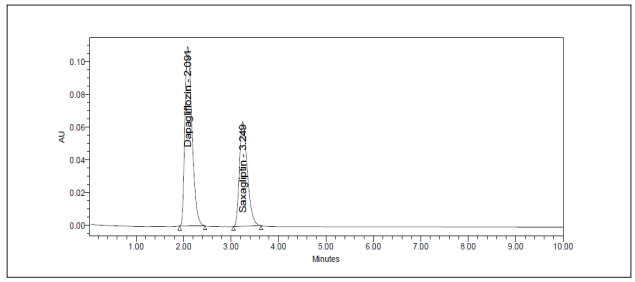

Figure 2. Standard chromatogram of DAP and SAX

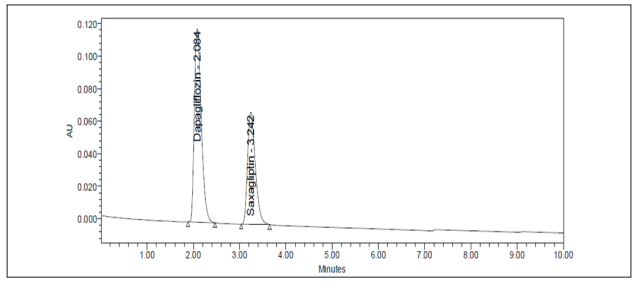

Figure 3. Sample chromatogram of DAP and SAX

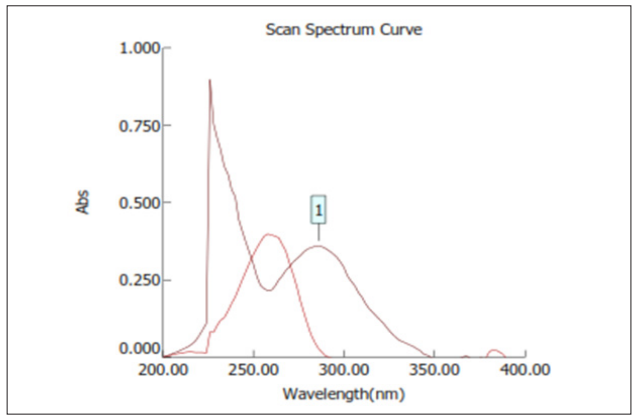

Figure 4. Detection wavelength of DAP and SAX

The experimental LOD and LOQ were 3.00 µg/mL and 9.98 µg/mL for DAP, 3.02 µg/mL and 10.01 µg/mL for SAX, respectively. The validation parameters and the assay results for tablet formulation were shown in (Tables 4 and 5). System suitability parameters such as, column efficiency, resolution and tailing factor of the peaks were calculated. The tailing factor for DAP and SAX was found to be 1.40 and 1.37, respectively. Theoretical plates for DAP and SAX was 2913 and 3772. Resolution was found to be 3.96. The system suitability results are shown in (Table 6). The results of robustness studies are shown in (Table 7).

Table 3. Degradation studies

S.No	Conditions	DAP		SAX	
		R_t(min)	% degraded	R_t(min)	% degraded
1	Acid	2.075	6.7	3.235	4.3
2	Base	2.090	2.3	3.268	6.8
3	Oxidation	2.080	5.1	3.245	2.6
4	Thermal	2.081	4.9	3.219	3.4
5	UV exposure	2.074	4.3	3.240	2.5

Table 4. Summary of validation parameters

Parameter	DAP	SAX
Linearity range(µg/mL)	100-500	50-250
Regression equation	Y=2189.9x+32135	Y=2889.6x+10443
Correlation coefficient	0.9998	0.9998
LOD(µg/mL)	3.00	3.02
LOQ(µg/mL)	9.98	10.01
Interday precision(%RSD)	0.1	0.7
Intraday precision(%RSD)	0.8	0.4

Table 5. Results of assay of marketed formulation

Brand	Drug	Sample peak area	Standard peak area	Labelled amount (mg/tab)	% Assay	RSD
Qtern	DAP	1343348	1355109	10	100.27	0.7
	SAX	857190	863399	5	100.03	0.5

Table 6. System suitability results

S. No	System suitability parameters	Results	
		DAP	SAX
1	USP tailing	1.40	1.37
2	USP plate count(Rs)	2.913	3.772
3	Rt min	2.091	3.249
4	USP resolution	3.96	

Table 7. Robustness study

S.No	Parameter	Change level	DAP			SAX		
			Rt	Peak area	Tailing factor	Rt	Peak area	Tailing factor
1	Flow rate	0.9	2.318	689155	1.43	3.616	449077	1.39
		1.1	1.879	549133	1.39	2.923	360430	1.35
2	Mob. organic phase composition	50:50	2.132	568190	1.43	3.851	373590	5.70
		70:30	2.049	1653142	1.38	2.877	1076066	2.95

Forced degradation studies were carried out in acid, base, thermal, photolytic and peroxide conditions; DAP was degraded more (6.7%) in acidic conditions than in other conditions. In basic conditions, SAX was degraded more (6.8%) than other conditions (Fig. 5). For DAP and SAX, the basic conditions resulted in a significant increase in the area with the additional peaks. Under the basic conditions, a significant decrease of the peak area of DAP and SAX was observed within 5 min, with one additional peak detected at 1.459 min, respectively. This is shown in (Fig. 6). Under oxidative condition, a significant decrease of the area of DAP and SAX was detected and small degradation peaks were seen approximately at 1.458 min. This is shown in (Fig. 7). Under photo degradation, a small additional peak was detected at 6.631 min. This is seen in (Fig. 8). Under thermal condition, a slight decrease of the area was detected and one small degradation peaks were seen approximately at 1.446 min – as shown in (Fig. 9). The stressed samples were, respectively, analysed for dapagliflozin and the saxagliptin results are shown in (Table 3).

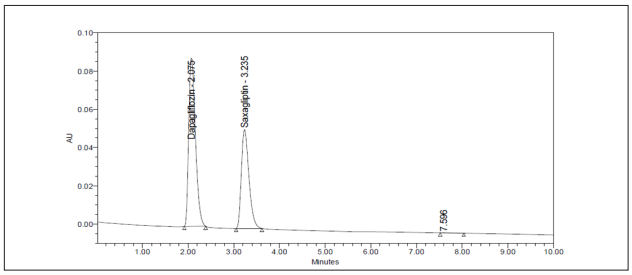

Figure 5. Chromatogram of Acid degradation (24 hrs)

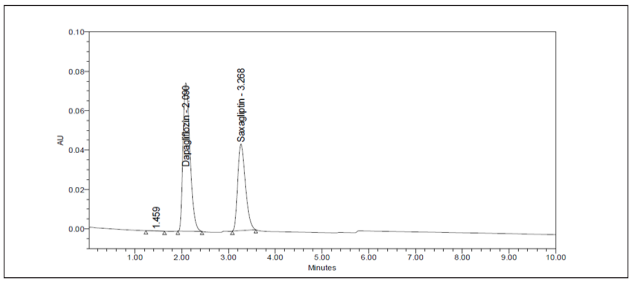

Figure 6. Chromatogram of base degradation (24 hrs)

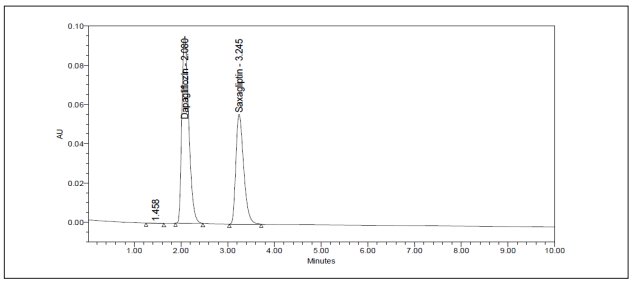

Figure 7. Chromatogram of peroxide degradation (15 min)

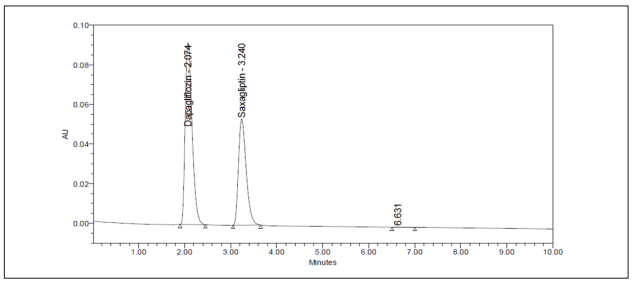

Figure 8. Chromatogram of photo degradation (48 hrs)

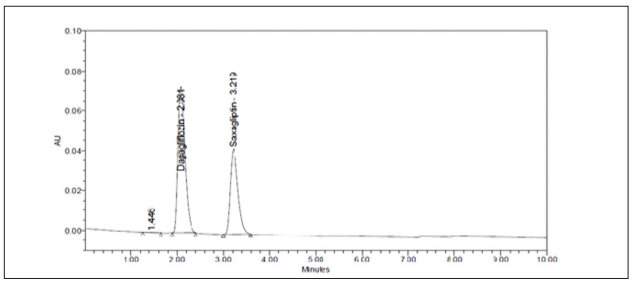

Figure 9. Chromatogram of thermal degradation (3 hrs)

CONCLUSION

The validated method shows that the stability-indicating RP-HPLC method is simple, fast and high selective, accurate and specific without any interference from the excipients and degradation products. The method was successfully applied for the quantitative analysis of DAP and SAX in marketed formulations.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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