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Research Article

STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ANTIDIABETIC DRUGS, DAPAGLIFLOZIN AND SAXAGLIPTIN

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ABSTRACT

For simultaneous measurement of Dapagliflozin (DAPA) and Saxagliptin (SAXA) in combination, as well as quantification of their major degradation products, a simple reversed-phase high-performance liquid chromatographic technique was devised and validated. The separation was accomplished using an ODS C18 column (250 mm 4.6 mm, i.d.5 m) with isocratic elution at room temperature. The optimum mobile phase consists of an aqueous phase (sodium acetate buffer, pH 4) and an organic phase (acetonitrile: methanol, 40:40) at a ratio of (20:80). The flow rate was set to 1.0 ml min^{-1}, and effluents were measured using a 228 nm diode array detector. This method showed good linearity over a range of 2.0-12 µg ml^{-1} and 1-6 µg ml^{-1} for DAPA and SAXA respectively. The retention times of samples were 2.31 and 2.90 minutes for DAPA and SAXA respectively. According to the International Conference Harmonization (ICH) criteria, the drugs in combination were submitted to different stress degradation studies. The suggested approach is appropriate for stability investigations, according to the results of the stress degradation experiments. The proposed method was simple, rapid and precise for the study of a new formulation that has been approved for the cure of diabetes mellitus.

Keywords: Dapagliflozin, Saxagliptin, RP-HPLC, Stress degradation.

1. INTRODUCTION

Dapagliflozin (DAPA) is chemically described as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxy phenyl) methyl] phenyl]-D-glucitol (Fig. 1A) [1, 2]. It belongs to a new class of oral antidiabetic drugs called sodium-glucose cotransporter 2 (SGLT2) inhibitors. These sodium-glucose cotransporters are responsible for glucose reabsorption in the kidney [3, 4]. DAPA is a first-generation, selective SGLT inhibitor that blocks glucose transport with 100- fold selectivity for SGLT2 over SGLT1 [5]. Saxagliptin (SAXA), chemically, known as (1s,3s,5s)-2-[(2s)-2-amino-2-(3-hydroxyl- tricycle [3.3.1.1]dec-1-yl)acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile (Fig. 1B), is a potent, selective, long-acting, and reversible inhibitor of the enzyme dipeptidyl peptidase 4 (DPP-4) used for the treatment of type 2 diabetes mellitus. It is used as monotherapy or in combination with other drugs [6, 7].

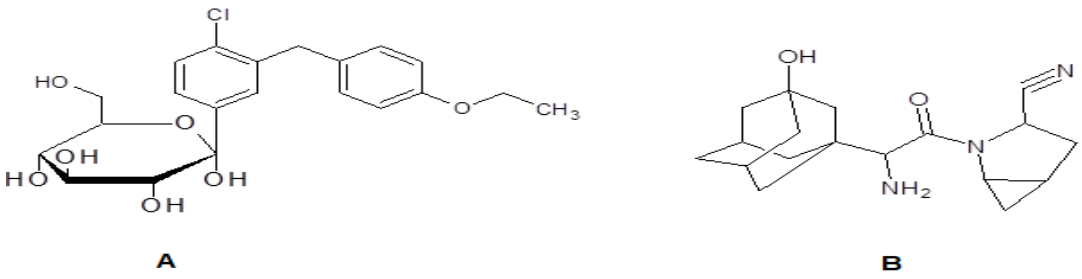

Fig. 1: Chemical structure of (A) Dapagliflozin (B) Saxagliptin

The US FDA has approved a once-daily dose of Qtern® (10mg Dapagliflozin and 5 mg Saxagliptin) for the treatment of type-2 diabetes [8]. An extensive literature survey has revealed that there are few reverse phase high

performance liquid chromatographic (RP-HPLC) method available for individual or simultaneous estimation of DAPA and SAXA in bulk, or pharmaceutical dosage forms but in this study, the retention time is less and forced degradation study experimented thoroughly [9, 10]. A few analytical methods were reported in the literature for the determination of DAPA and SAXA alone [11-16]. A few analytical methods were reported for simultaneous estimation of DAPA and SAXA [17-19]. The newly developed method was validated for accuracy, precision, ruggedness and sensitivity as per ICH guidelines. Stress testing carried out under various conditions such as pH (acid/base), temperature, light, oxidation, humidity, etc [20-22].

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

DAPA and SAXA reference standard was gifted by AKUMS drugs pharmaceutical Ltd, Haridwar, Uttrakhand and Metrochem API Private Limited, Hyderabad, Telangana. Tablets of Qtern® were purchased from the local market of Moradabad, U.P. Sodium acetate buffer, acetonitrile, methanol andwater were of HPLC grade and supplied by Merck Ltd. All other chemicals included in the study were of AR grade. Milli-Q water was used for the preparation of the mobile phase.

2.2. Instrumentation

Chromatography was performed using Shimadzu®LC-20AD pumps, adaptable wavelength programmable SPD-M20A photodiode array detector with CBM-20A system controller and manual injector. The data acquisition was performed by the Shimadzu®LC solution software.

2.3. Chromatographic conditions

The Chromatographic separation of DAPA and SAXA was achieved by the C18 column (hypersil ODS-C_{18}; 4.6 mm x 250 mm,2.5 µm particle size) with a constant flow rate of1 ml ml^{-1}. The analytes were analyzed by a diode array detector at 228 nm. The injection volume was set at 20 µl. The isocratic mobile phase consisted of an organic phase (80%) along with sodium acetate buffer, with pH adjusted to 4.0. The organic phase comprised methanol: acetonitrile in the ratio of 40:40 and the filtration were done with a 0.45 µm membrane filter and degassed before use.

2.4. Preparation of buffer solution

After dissolving 2.86 mL glacial acetic acid with 1 mL (50 % w/v sodium hydroxide) in a 1000 mL volumetric flask, fill the flask with HPLC grade water and adjust the pH to 4.0 using sodium hydroxide. The buffer solution was then filtered through a membrane filter of 0.45 µm porosity and degassed the mobile phase with the help of a sonicator.

2.5. Preparation of stock and standard solutions

Stock solutions of DAPA and SAXA were prepared separately by dissolving accurately weighed 10 mg of each drug in 100 mL of methanol to obtain a stock solution of an individual drug of 100µg/mL. The stock solutions were further diluted with the same mobile phase as appropriate to obtain the working standard solution of 2-12 µg ml^{-1} and 1-6 µg $ml^{-1}$of DAPA and SAXA, respectively for linearity and other analytical methods. The final solution was filtered through a 0.45µm Millipore membrane filter.

2.6. Selection of UV wavelength

DAPA has a λmax at 224 nm and SAXA has λmax at 235 nm in a water and methanol mixture (80:20) [23]. An acceptable response was obtained upon detection of both drugs at 228 nm either individually or in combination.

2.7. Method validation

The optimized chromatographic method was validated according to the procedures described in the ICH guidelines Q2 (R1) for evaluating system suitability, specificity, precision, accuracy, linearity, the limit of detection (LOD), the limit of quantitation (LOQ) and robustness [24].

2.7.1. System suitability

System suitability parameters for tailing factor, repeatability, number of theoretical plates and resolution between DAPA and SAXA peaks were assessed by injecting a blank mobile phase followed by six replicates of DAPA (5µg/mL) and SAXA (2.5µg/ml) mixture.

2.7.2. Specificity

The specificity study was performed to demonstrate the effective separation of title analyte peaks from placebo, biological matrix and all related degradation peaks. The placebo solution consisted of all the excipients commonly used for manufacturing of dosage form.

2.7.3. Linearity

Aliquots of working solutions of the drugs were transferred to 10mL volumetric flasks and diluted with the mobile phase to obtain 6 standard concentrations (Table 1). Linearity was evaluated by linear regression analysis, calculated by the least-squares regression method.

2.7.4. Precision

The intra-day precision analysed on the same day and inter-day precision analysed for three consecutive days. The results were indicated by a calculated per cent relative standard deviation.

2.7.5. Accuracy

Accuracy was carried out at three different levels 50%, 100% and 150%. The percentage of accuracy was calculated as mean ± standard deviation.

2.7.6. Robustness

Deliberate minute variations in the chromatographic conditions such as flow rate, mobile phase ratio, wavelength, pH of the buffer component have been made. These variations were also evaluated for tailing factor, % RSD and percentage recovery of DAPA and SAXA peaks.

2.7.7. Limit of detection and limit of quantification

LOD and LOQ for metformin and gliclazide were calculated from the linear regression equation based on a standard deviation of the intercept and the slope using the formula.

LOD = 3.3 Q/S and LOQ = 10 Q/S

where Q: the standard deviation of the intercept, S: the slope of the calibration curve.

2.7.8. Solution stability

The stability of the DAPA(50 µg/ml) and SAXA(25 µg/ml) mixed solutionwas assessed after 24 h at room temperature, 25°C (light protected to minimize possible light degradation) and after 1 week in fridge when maintained at 2-8°C.

2.7.9. Forced degradation studies

Forced degradation studies were performed on DAPA and SAXA to prove stability, indicating a property of the method. The stress conditions employed for the degradation study include acid hydrolysis (0.5 N HCl), base hydrolysis (0.5 N NaOH), oxidation (3% H_2O_2), and thermal and photolytic degradation.

2.7.9.1. Acid and base degradation

Acid and base degradation studies were carried out by taking 1 ml aliquot each of DAPA and SAXA from the sample stock solution in a 10ml standard volumetric flask and mixing with 0.5 N hydrochloric acids. The flask was kept in a controlled temperature bath at 80°C±1°C for four hours. Similarly, forced degradation in basic media was performed using 0.5 N sodium hydroxide instead of 0.5 N hydrochloric acid. Both the samples were neutralized, and the final solution was injected in triplicate under optimized chromatographic conditions.

2.7.9.2. Oxidative degradation

Oxidative degradation was performed by transferring 1 ml aliquot of sample solution of an individual drug into a 10ml standard volumetric flask and mixing with 1 ml of 4% v/v of hydrogen peroxide. The flask was kept in a controlled temperature bath at 80°C±1°C for four hours. The final solution was injected in triplicate under optimized chromatographic conditions.

2.7.9.3. Thermal degradation

For thermal stress, 1 ml aliquot each of a sample solution of DAPA and SAXA was transferred to a 10ml standard volumetric flask and then placed in a controlled temperature oven and heated at 80°C±1°C for four hours. This solution was further diluted with mobile phase, and the final solution was injected in triplicate to obtain the chromatogram.

2.7.9.4. Photolytic degradation

Photolytic degradation was conducted by transferring 1 ml aliquot each of a sample solution of DAPA and SAXA into a 10ml standard volumetric flask and exposing it to direct sunlight for four hours. This solution was further diluted with mobile phase and injected in triplicates under an optimized chromatographic condition.

3. RESULTS AND DISCUSSION

3.1. Method optimization

The main objective of this study was to develop and validate an optimized method by changing different parameters like sodium acetate buffer system with varied pH and organic solvent (methanol and acetonitrile) composition, wavelength, pH and flow rate for simultaneous detection of DAPA and SAXA with a sharp peak, maximum theoretical plates, less tailing factor and short separation time. The best result was obtained with the mobile phase consisting of sodium

acetate buffer (pH 4) and an organic phase (acetonitrile: methanol, 40:40) at a ratio of (20:80) with a flow rate of 1 $ml^{-1}$and effluents were measured using a 228 nm diode array detector. The typical chromatogram represented the different chromatograms of a placebo, DAPA, SAXA and both DAPA and SAXA mixture is illustrated in Fig. 2.

3.2. System suitability

The testing findings revealed that all the parameters evaluated were within acceptable limits, suggesting that the system is adequate for the study at hand (Table 1).

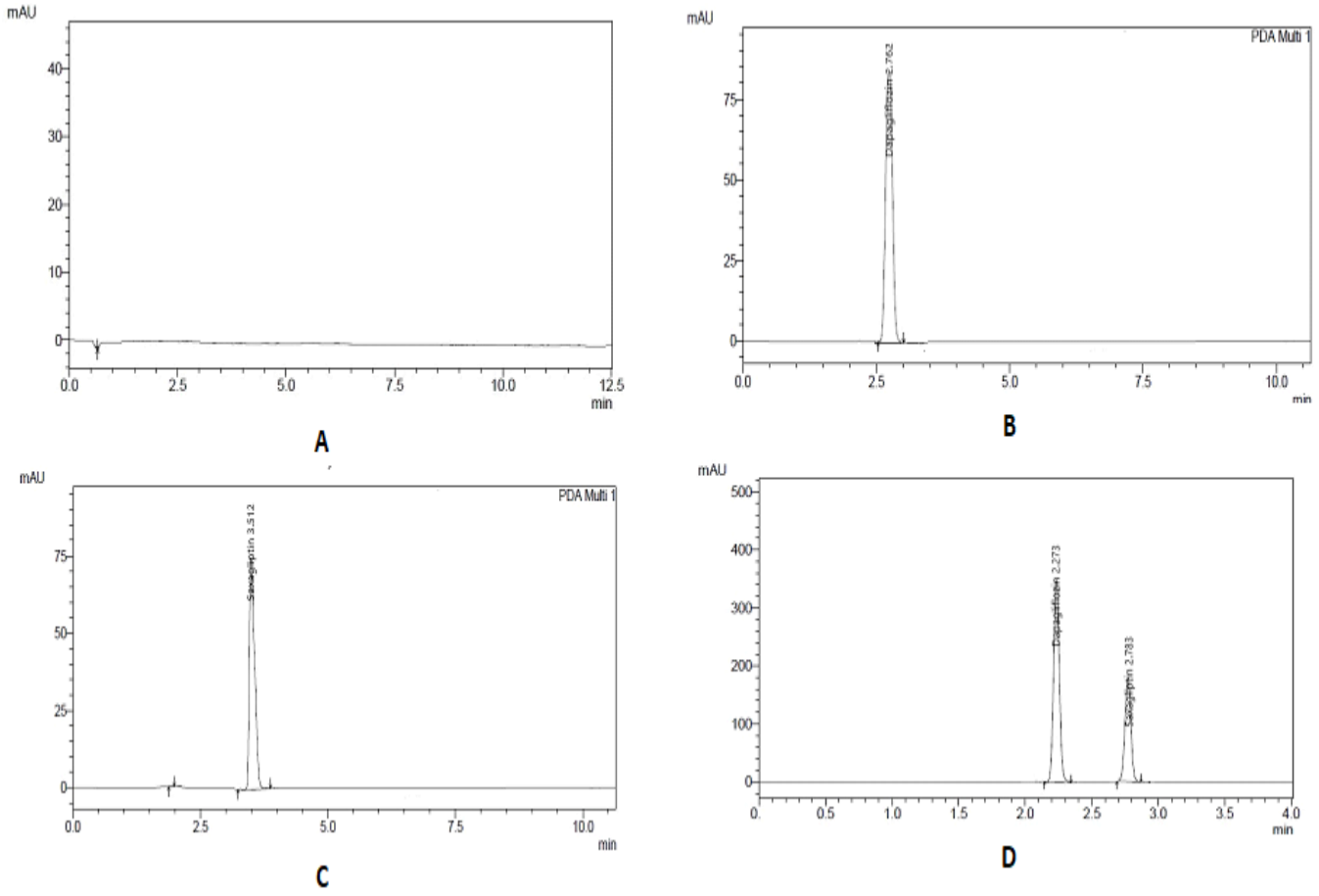

Fig. 2: Chromatograms corresponding to A) Placebo matrix B) Dapagliflozin C) Saxagliptin D) Dapagliflozin and Saxagliptin mixture

Table 1: System suitability parameters (n=6)

Injection Number	Retention time (min)DAPA	Peak Area of DAPA	Retention Time (min) SAXA	Peak Area of SAXA
1	2.273	74271	2.754	54553
2	2.321	74528	2.732	54330
3	2.287	75429	2.683	53224
4	2.278	74472	2.711	54132
5	2.345	76929	2.783	52320
6	2.273	76293	2.683	53465
Mean	2.296166667	75320.33333	2.723	53670.66667
SD	0.029962755	1094.920941	0.039938286	835.7237981
%RSD	1.304903302	1.453685734	1.466701642	1.557133254
USP Tailing Factor	1.34		1.43	
USP Plate Count	5832		4392	

3.3. Method Validation

3.3.1. Specificity

This approach is particular for simultaneous assessment of both drugs since it has good resolution and no interference from blanks or excipients. The explanatory chromatogram revealed no additional peak, validating the method's specificity. The representative chromatogram of, placebo, metformin standard, gliclazide standard and typical chromatogram of metformin and gliclazide mixture is shown in Fig. 2.

3.3.2. Linearity

Both DAPA and SAXA's analytical calibration curves were linear in the required ranges, as evidenced by the closeness of the correlation coefficient R^2 to 1 (R^2 = 0.9999). The linear regression equations for DAPA and SAXA are (Y = 15108x + 1385.7, R^2 = 0.9998) and (Y = 17071x + 550.47, R^2 = 0.9997), respectively. Representative chromatograms of linearity are shown in Fig. 3A and 3B.

3.3.3. Accuracy

The accuracy of proposed analytical method was assessed by measuring the added analytes in the placebo matrix in triplicates at three different levels (50%, 100%, and 150%) and expressing the results in percent recovery of metformin and gliclazide from the spiked matrix. The proximity of the discovered analytes' values to the claimed theoretical concentrations at different levels showed the accuracy of the proposed technique, with DAPA and SAXA recovering >99% of their concentrations from the spiked excipients. Table 2 and Fig. 4 show the DAPA and SAXA recovery results.

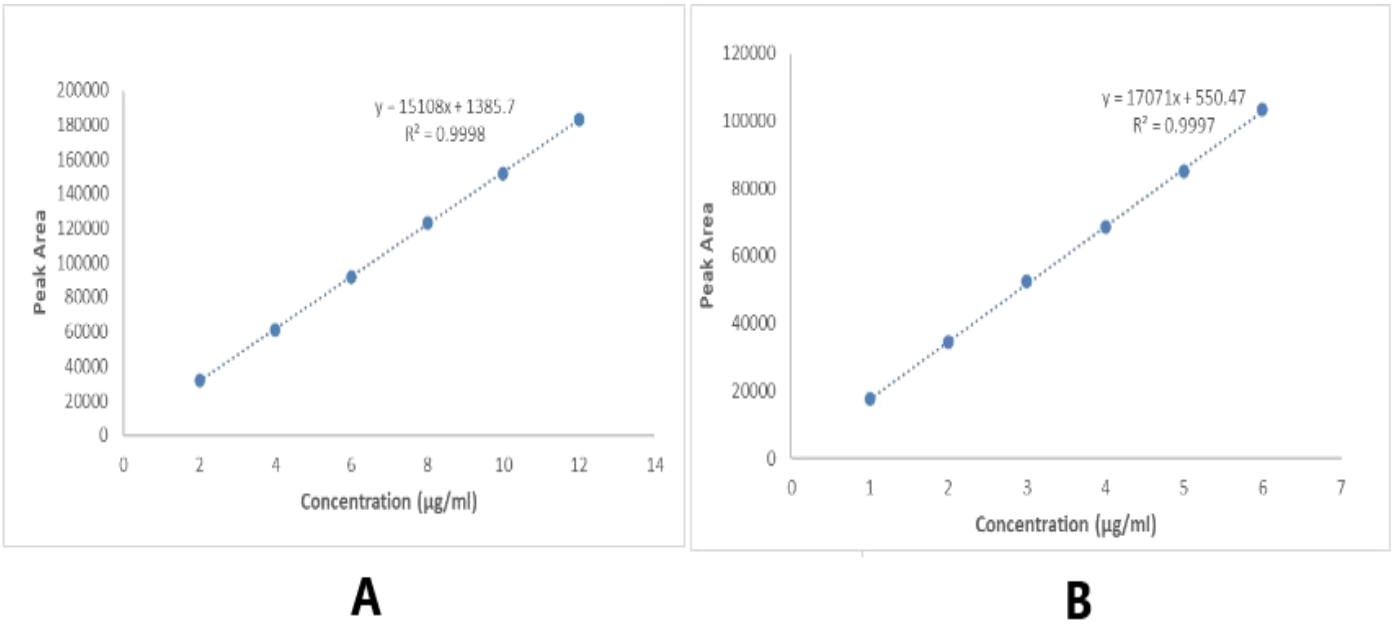

Fig. 3: Calibration curve of DAPA (A) and SAXA (B)

Table 2: Accuracy and recovery data (n=6)

Sample name	% Level Spiking	Amount of drug (Tablet) µg	Amount of drug (Standard) µg	Total Drug (µg)	Total Found (µg) Mean ± SD	% RSD	% Recovery
DAPA	50%	4	2	6	6.02 ± 0.102	1.70	100.33
	100%	4	4	8	8.03 ± 0.071	0.89	100.37
	150%	4	6	10	9.98 ± 0.06	0.60	99.87
SAXA	50%	2	1	3	3.02 ± 0.058	1.92	100.67
	100%	2	2	4	4.03 ± 0.074	1.85	100.83
	150%	2	3	5	5.01 ± 0.09	1.80	100.37

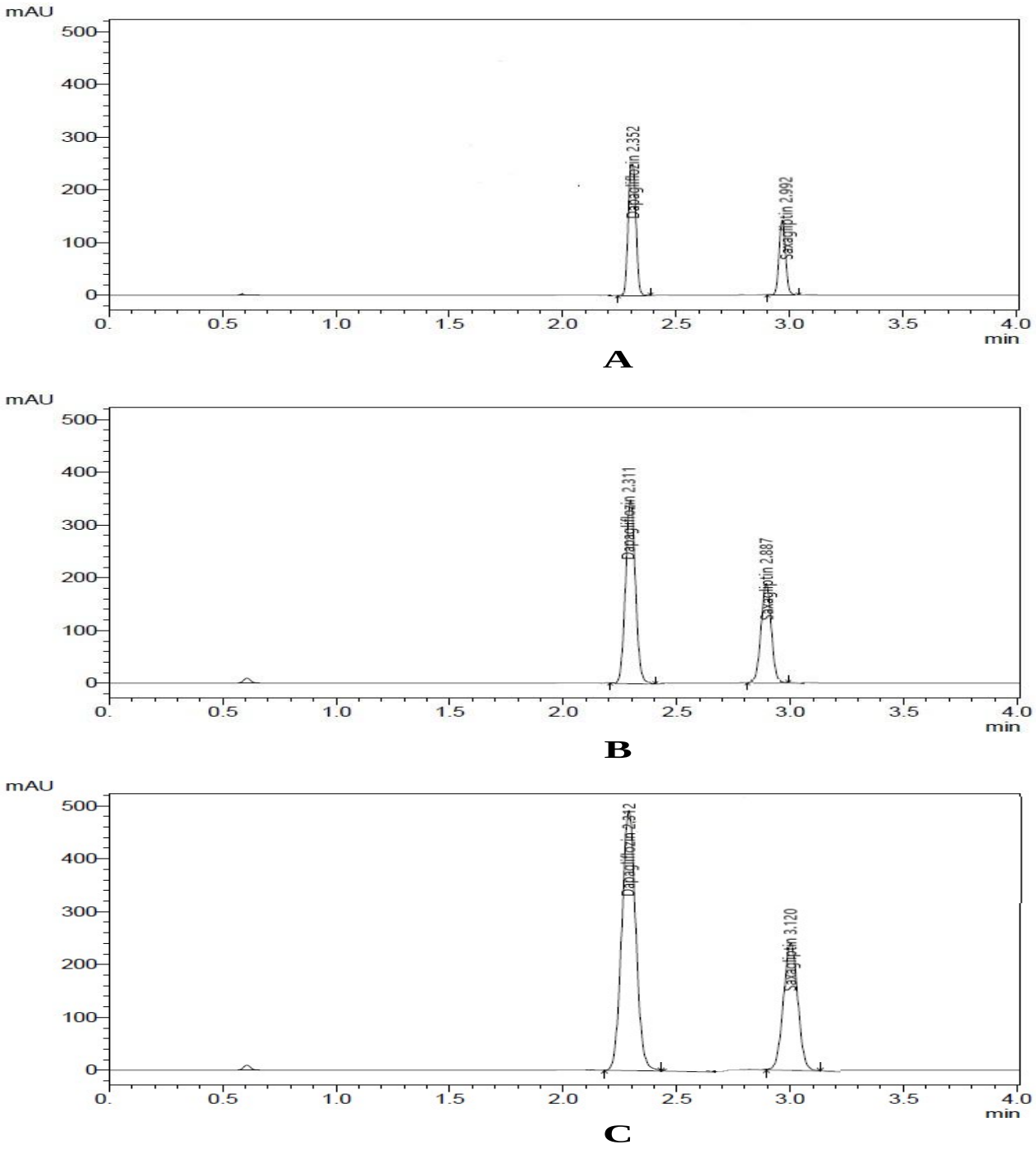

Fig. 3: Accuracy study of DAPA and SAXA at 50% (A), 100% (B) and 150% (C)

3.3.4. Precision

The peak areas obtained after injecting 6 separate combined DAPA and SAXA samples over three days at three different concentrations of both drugs were reproducible and accurate. The findings for both intra-day and inter-day determinations show that the developed technique has excellent accuracy and repeatability, with RSD percent never exceeding 0.90 % for DAPA and 1.36 % for SAXA (accepted limit RSD %< 2). Table 3 shows the results for intra-day and inter-day accuracy.

3.3.5. Robustness

The technique is robust to modest purposeful modifications in terms of flow rate, wave length, pH of the buffer employed, or various mobile phase ratios

since no significant changes were found when minor variations to the chromatographic conditions were applied. DAPA and SAXA peaks were symmetric (tailing factor <2) in all cases, and the RSD percent of DAPA and SAXA retention timewere < 2, indicating that the suggested analytical technique was resistant to modest alterations. Table 3 shows the results for robustness.

Table 5: Robustnessresults for both DAPA and SAXA (acceptance limit RSD% <2).

Drugs		DAPA			SAXA		
Condition		Retention Time	Tailing Factor	% Recovery	Retention Time	Tailing Factor	% Recovery
Change in Flow rate	Normal Condition (1.0 ml per minute)	2.343	1.09	100.25	2.752	1.03	100.09
	Flow rate (0.8 ml per minute)	2.372	1.12	102.89	2.762	1.06	98.78
	Flow rate (1.2 ml per minute)	2.373	1.11	101.45	2.744	1.05	100.77
	Mean	2.362	1.11	101.53	2.753	1.05	99.88
	Standard deviation	0.017	0.015	1.322	0.009	0.015	1.011
	RSD%	0.721	1.380	1.302	0.328	1.46	1.013
Change in pH	Normal Condition (4.5)	2.341	1.07	100.34	2.755	1.11	99.98
	pH (4.0)	2.345	1.11	101.23	2.672	1.14	98.67
	pH (5.0)	2.403	1.08	102.58	2.763	1.15	101.45
	Mean	2.363	1.09	101.38	2.730	1.13	100.03
	Standard deviation	0.035	0.021	1.128	0.050	0.021	1.391
	RSD%	1.468	1.916	1.112	1.846	1.837	1.390
Change in Wave Length	Normal: Wave Length 228nm	2.346	1.15	99.78	2.61	1.02	99.99
	Wave Length 222 nm	2.382	1.16	101.45	2.638	1.05	100.88
	Wave Length 231 nm	2.373	1.18	100.56	2.652	1.04	101.55
	Mean	2.367	1.16	100.60	2.633	1.04	100.81
	Standard deviation	0.019	0.015	0.836	0.021	0.015	0.782
	RSD%	0.792	1.313	0.831	0.812	1.473	0.776
Change in organic ratio in the mobile phase	Normal Condition (ACN: Methanol: Buffer) (40:40:20)	2.368	1.09	99.87	2.688	1.14	100.2
	(ACN: Methanol: Buffer) (40:30:30)	2.372	1.11	102.23	2.789	1.16	101.87
	(ACN: Methanol: Buffer) (40:50:10)	2.388	1.11	100.29	2.712	1.16	98.76
	Mean	2.376	1.103	100.80	2.729	1.15	100.28
	Standard deviation	0.011	0.012	1.259	0.053	0.012	1.556
	RSD%	0.445	1.046	1.249	1.933	1.001	1.552

3.3.6. Detection and quantitation limits

The estimated LOD and LOQ for DAPA were 0.44µg/ml and 1.33µg/ml, respectively, while for SAXA were 0.26µg/ml and 0.78 µg/ml. The sensitivity of the technique was tested in practice, with experimental LODs of 0.25µg/ml for both gliclazide and metformin and experimental LOQs of 0.75µg/ml for both drugs.

3.3.7. Solution stability

The DAPA and SAXA sample solution remained stable for 24 hours at 25°C (room temperature) and one week

in the fridge at 2-8°C. The % difference between the stability findings was calculated, and no deterioration in the peak regions of either DAPA or SAXA was seen in the conditions stated.

3.3.8. Forced degradation studies

The chromatograms of DAPA and SAXA samples exposed to various forced degradation conditions revealed well-separated active and degradation product peaks at varying retention times. The degradation products' peaks were detected and compared to those of the reference solution, which revealed that they were stable. Degradation tests indicated that the combined sample of DAPA and SAXA was more resistant to oxidation, photolysis, and thermal degradation than acid and base.

3.3.8.1. Acid and base degradation

In the case of DAPA and SAXA, acid degradation studies revealed the presence of one extra peak at 3.012 minutes, while base degradation studies revealed the presence of two additional peaks at 1.842 and 1.982 minutes, but only a modest change in peak area and a drop in peak high. The formation of degradation products is shown by this additional peak. Fig.s 3A and 3B show the chromatograms for acidic and basic degradation of DAPA and SAXA.

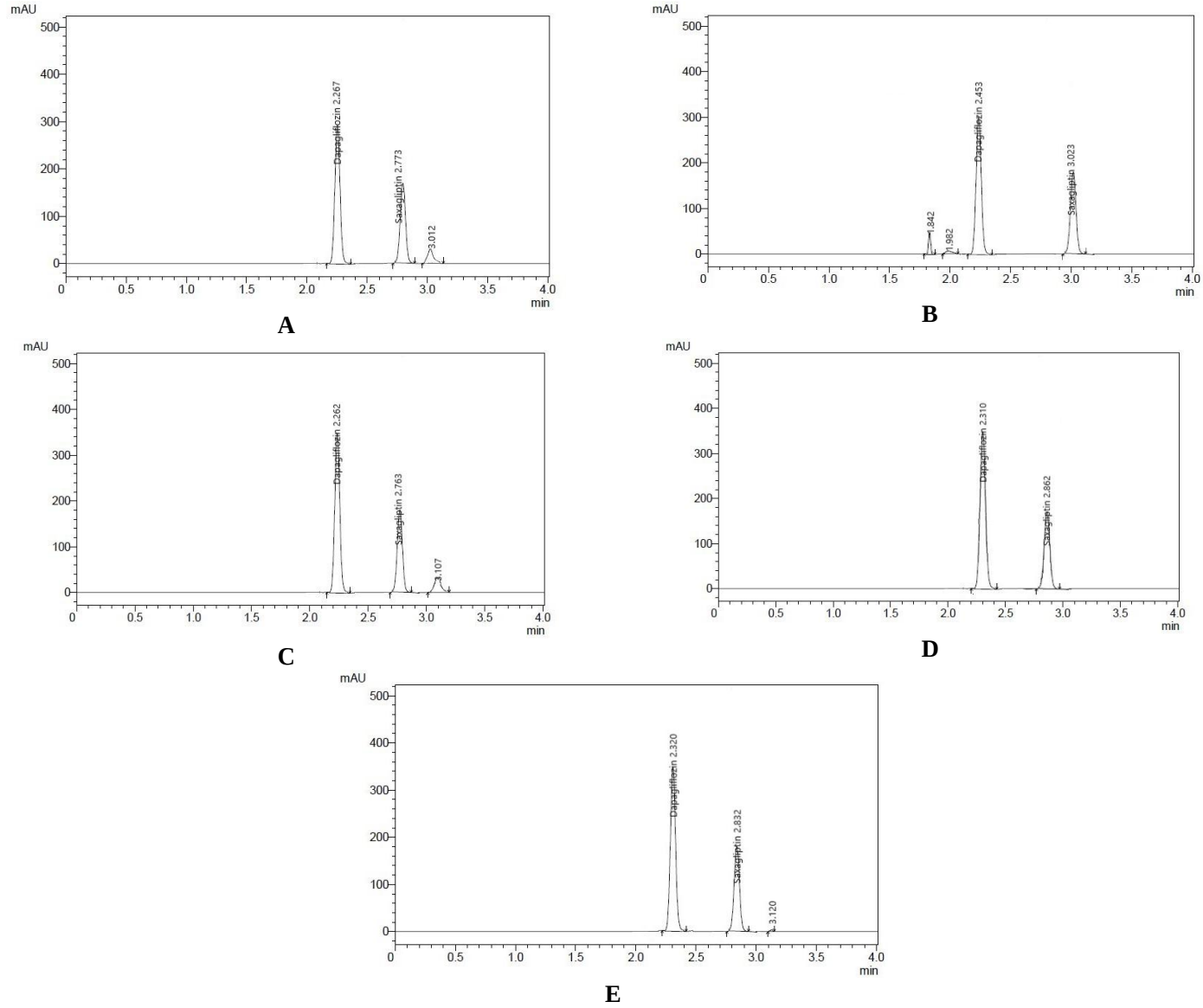

Fig. 3: Chromatograms of forced degradation A) Acid hydrolysis; B) Base hydrolysis; C) Oxidative hydrolysis; D) Thermal degradation; E)Photolytic Degradation

3.3.8.2. Oxidative degradation

A significant decrease in the area with the additional peaks was observed during oxidative stress conditions. One extra peak was observed at 3.107 minutes.

3.3.8.3. Thermal degradation

The peak area was reduced by a non-significant amount under thermal conditions, and no new peak was found.

3.3.8.4. Photolytic degradation

The photolytic conditions resulted in a non-significant reduction of the peak area with no additional peak detected.

4. CONCLUSION

The stability-indicating RP-HPLC technique is simple, economic, accurate, precise, robust, and specific, according to the validation studies, with no interference from excipients or degradation products.Forced degradation studies were performed on DAPA and SAXA to prove stability-indicating a property of the method. The proposed approach was effectively used to analyse DAPA and SAXA in tablets quantitatively. As a result, the method may be utilised for regular analysis, quality control, and stability investigations of pharmaceutical tablets that include these medicines.

5. ACKNOWLEDGEMENTS

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Conflict of Interest

We declare that we have no conflicting interests in this research.

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