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A COST EFFECTIVE RP-HPLC METHOD FOR SIMULTANEOUS QUANTITATIVE ANALYSIS OF SAXAGLIPTIN AND METFORMIN HYDROCHLORIDE

Amit Kaushal, Sukhbir Singh*, Sandeep Arora and Neelam Sharma
Chitkara College of Pharmacy, Chitkara University, Punjab, India
*Author for Correspondence: sukhbir.singh@chitkara.edu.in; singh.sukhbir12@gmail.com

Abstract

A cost effective RP-HPLC technique was validated for concurrent quantitative analysis of metformin hydrochloride/Saxagliptin. The separation was carried out by isocratic elution using C18 column and a mobile phase containing acetonitrile: buffer: water. The flow rate was kept at 0.8 ml/min and elute was detected at 220 nm. Several validation parameters were assessed as per ICH guidelines. The study has showed that reported method is accurate, linear, precise sensitive and specific for simultaneous quantitative analysis of saxagliptin and metformin hydrochloride.

***Keywords*:** Metformin hydrochloride; Saxagliptin; RP-HPLC; Isocratic elution; Validation

Introduction

Dipeptidyl peptidase 4 (DPP-4) inhibitor is a newer group of hypoglycaemic agent that inhibits dipeptidyl peptidase 4 (DPP-4) and stops breakdown of incretin hormone, which raises levels of incretin (Harashima and Inagaki, 2015). This step-up insulin release and bring down release of glucagon, thereby reducing plasma glucose levels. The most commonly used DPP-4 inhibitors are saxagliptin, allogliptin, teneligliptin sitagliptin, linagliptin and vildagliptin. Now a day's combination therapy DPP 4 inhibitor with biguanides is used to better glycemic control in T2DM patients (Ahrén, 2007).

Structurally, saxagliptin (SAXA) and Metformin hydrochloride is shown in Figure 1 (Prameela *et al*., 2018).

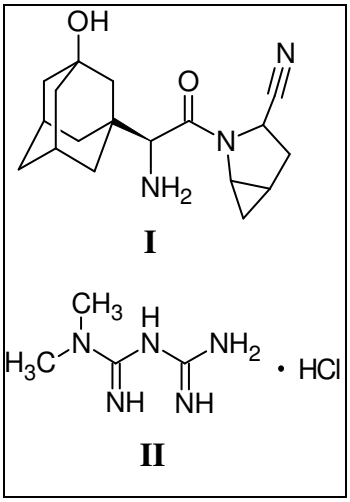

Fig. 1 : Structure of saxagliptin (I) and metformin hydrochloride (II).

Published literature revealed that different analytical methods such as UV-Vis spectrophotometric (Moneeb, 2013, Nyola and Govindasamy, 2012; Prajapati *et al.*, 2020), RP-HPLC (Merey *et al.*, 2017; Scheeren *et al.*, 2015; Yunoos and Sankar, 2015), HPTLC (El-Kimary *et al.*, 2015, Ahmed *et al.*, 2020) and electrochemical (Abdallah and Ibrahim, 2019) for quantitative analysis of saxagliptin single or with other drugs in raw, tablet and plasma samples (Kabra *et al.*, 2014a). Several analytical techniques were published for quantitative estimation of metformin single or in combination such as UV spectroscopic method (Jani *et al.*, 2015), electrophoresis (Hamdan *et al.*, 2010, Song *et al.*, 1998), potentiometry (Hassan *et al.*, 1999), spectrofluorimetry (Hassan *et al*., 1999), HPTLC (Ghassempour *et al.*, 2006, Modi and Patel, 2012), RP-HPLC(Al-Rimawi, 2009) and UPLC (Ayoub, 2015, Mowaka and Ayoub, 2017). The RP-HPLC for quantitative analysis of saxagliptin with metformin hydrochloride that have been reported is not cost-effective in concerns of run time and consumption of solvent; thus, the current study was performed. The goal of current investigation was to validate RP-HPLC for concurrent quantitative analysis of saxagliptin and metformin hydrochloride.

Materials and Methods

Instruments

Shimadzu Corporation, LC-2010CHT model (Kyoto, Japan) equipped with a vacuum degasser, isocratic quaternary pump, column oven, auto sampler injector and 100 µL volume injection loops was used in this study. Separation was achieved by HyperClone (Phenomenex) C18 column (250 mm × 4.6 mm I.D., 5 µm, BDS 130 A°). Detection was accomplished employing SPD-M20A photodiode array (PDA, Shimadzu Corporation, Kyoto, Japan) and UV detector connected to a HP computer installed with LC solution 5.57. Himedia nylon syringe filters (0.22µ) and Himedia cellulose nitrate membrane filters (0.22µ) employed for sample filtration and mobile phase respectively. The Ultrasonic Cleaner-15L (Equitron-Medica Instrument Mfg. Co., Mumbai, India) used to sonicate mobile phase. HANNA instruments (Italy) pH ep® used to check pH.

Chemical and solvents

Metformin hydrochloride was got as a gift from Cipla Ltd. (Baddi, India). Saxagliptin was obtained from Wockhardt Ltd. (Aurangabad, India). The other analytical grade chemicals were bought from S.D. Fine Chemicals Ltd.

Selection of wavelength

Saxagliptin and Metformin hydrochloride solution were scanned over wide range of wavelength 200-400 nm. The

isobestic points for saxagliptin and metformin hydrochloride were noticed at 220 nm and 250 nm (Figure 2). In this study wavelength 220 nm was selected for simultaneous quantitative analysis of both drugs (Kumar *et al.*, 2016, Sahani and Jain, 2019).

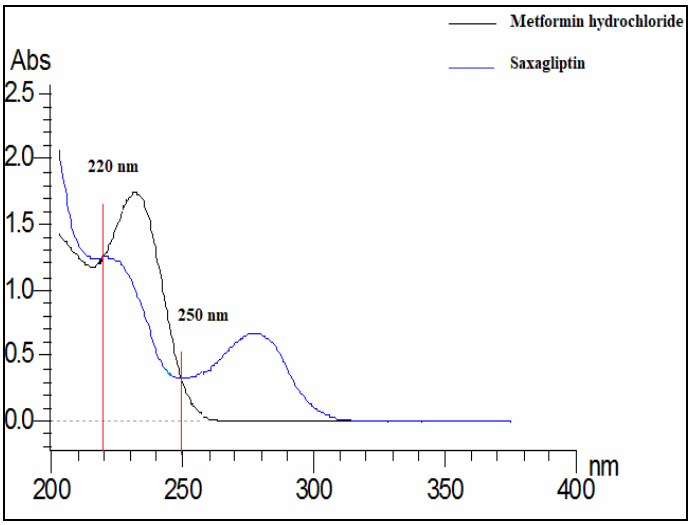

Fig. 2 : Overlaid spectra of metformin hydrochloride and saxagliptin: two isobestic point at 220 nm and 250 nm.

Standard solution preparation

Accurately weighed metformin hydrochloride (50 mg) and saxagliptin (5 mg) were transferred separately in 50 ml of volumetric flask holding methanol and shaken vigorously for five minutes followed by making up with methanol up to 50 ml. The standard stock solution diluted with methanol to made working standard solution (Argekar and Sawant, 1999).

Mobile phase preparation

The optimized mobile phase consisting of acetonitrile: buffer: water (20:30:50) was used. Phosphate buffer was made by transferring 1.3609g of potassium dihydrogen phosphate (10 mM) to volumetric flask (1000 ml) followed by making up volume with water. Buffer pH was corrected to 3 with ortho phosphoric acid (10%) and permeated through 0.22µ membrane filter.

Chromatographic condition

Chromatographic separation and analysis were executed on Hyper Clone (Phenomenex) C18 column (250 mm × 4.6 mm I.D., 5 µm particle size column, BDS 130 A˚) column with a flow rate 0.8 ml/min for 15 minute. Elutes were quantified at 220 nm at ambient temperature (35°C).

Method validation

The different validation parameters such as specificity, linearity, accuracy and precision were estimated as per ICH Q2 (R1) guideline (Bhadra *et al.*, 2011). Specificity of method is ascertained by evaluating retention time, resolution and tailing factor (Prajapati *et al.*, 2020). Linearity was confirmed by plotting a calibration curve for metformin hydrochloride and saxagliptin over concentration range 20-120 and 2-12 µg/ml for metformin hydrochloride and saxagliptin, respectively. Accuracy was determined by standard addition method (Patel *et al.*, 2014). Three concentrations level (80%, 100% & 120%) of standard solution of both drug was added to sample solution and analyzed sample in triplicate. Repeatability and intermediate precision determined to ascertained precision of method (Betz *et al.*, 2011). To determine the robustness, the chromatographic parameters were deliberately changed and the assay of sample solution was performed in each condition (Prajapati *et al.*, 2020; Shabir *et al.*, 2007). LOD and LOQ were determined as per below formulas:

$$LOD = 3.3\frac{\sigma}{S} \qquad ...(1)$$

$$LOQ = 10\frac{\sigma}{S} \qquad ...(2)$$

Where, 'σ'= standard deviation of 'y' intercept of linearity curve; 'S' = slope of the linearity curve.

Force degradation study

To determine the analytical procedure was stability-indicating, forced degradation was carried out under different stress conditions such as acidic, basic, hydrolytic, and oxidative stress conditions (Kabra *et al.*, 2014b). The objective of forced degradation study was not to characterize degradation products but to access the interference due to degradation products (Bansal *et al.*, 2007). Forced degradation study was carried by preparing 50 µg/ml metformin hydrochloride and 5µg/ml saxagliptin in 0.1 N HCl, 0.1 N NaOH, water and 3% H2O2. All the solutions were kept at elevated temperature of 80˚C for six hours. The resulting solutions were filtered through 0.22µ syringe driven membrane filters. 20µl of these solutions were injected in HPLC column under the conditions of the developed method. Chromatograms of these solutions were compared with chomatograms of fresh solutions of saxagliptin & metformin hydrochloride having same concentration and identical conditions.

Result and Discussion

A number of trials were evaluated to optimize the mobile phase, initially methanol was tried as organic phase but good symmetric peaks were obtained with acetonitrile. Based upon all observations C18 HPLC column with low concentration of organic phase (acetonitrile) along with buffer pH 3 was selected for the analysis. The chromatograms of optimized method are shown in figure 3 along with the peak of individual components.

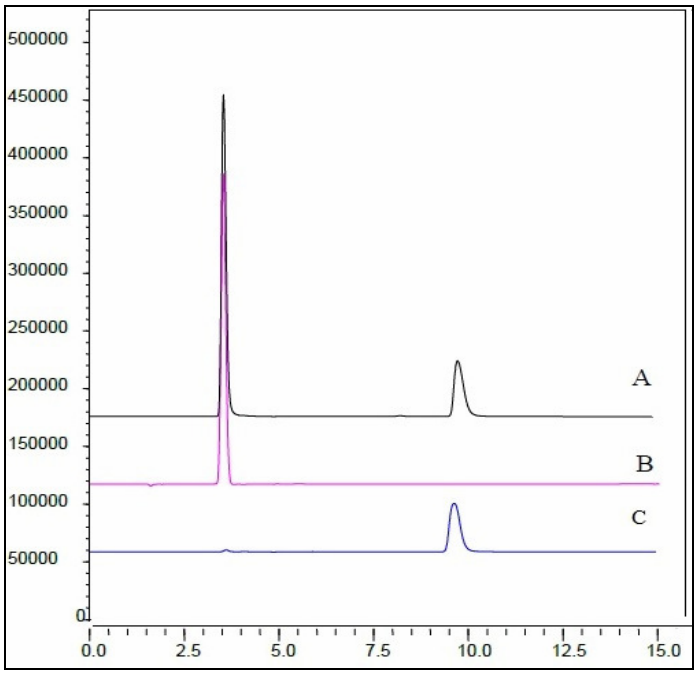

Fig. 3 : Overlay of chromatograms; A: mixture of metformin hydrochloride and saxagliptin B: metformin hydrochloride, and C: saxagliptin

Method validation

System suitability test

System suitability was carried by testing characteristics such as tailing factor, number of theoretical plates, resolution and repeatability. System suitability tests check for adequate system performance before and during analysis. All the parameter (Table 1) complies with USP limit for system suitability. Repeatability is checked in term of percent % RSD of response and % RSD was less than 2. Theoretical plates for both the peaks were found to be greater than 2000 and tailing factor was less than 2.

Table 1 : System suitability parameter for method (n=6)

Parameters	Metformin Hydrochloride			Saxagliptin		
	Mean	SD	% RSD	Mean	SD	% RSD
Area (mV)	11850108.17	69808.09	0.59	637241.50	5952.53	0.93
Theoretical plates	3661.83	30.36	0.83	6959.33	102.83	1.48
Tailing factor	1.22	0.01	0.66	1.64	0.02	1.48
Retention time (min)	3.54	0.04	1.16	9.71	0.08	0.86
Resolution	17.92	0.05	0.30	16.16	0.29	1.79

Specificity

The optimized chromatogram for mixture of saxagliptin and metformin hydrochloride shows good symmetrical peaks at 3.54 and 9.71 min for metformin hydrochloride and saxagliptin respectively (Figure 3). All peaks conforms the system suitability parameters and shows good resolution. Peak purity index for both peaks have been calculated using PDA detector. Peak purity index of 0.9997 and 1 was obtained for metformin hydrochloride and saxagliptin (Figure 4).

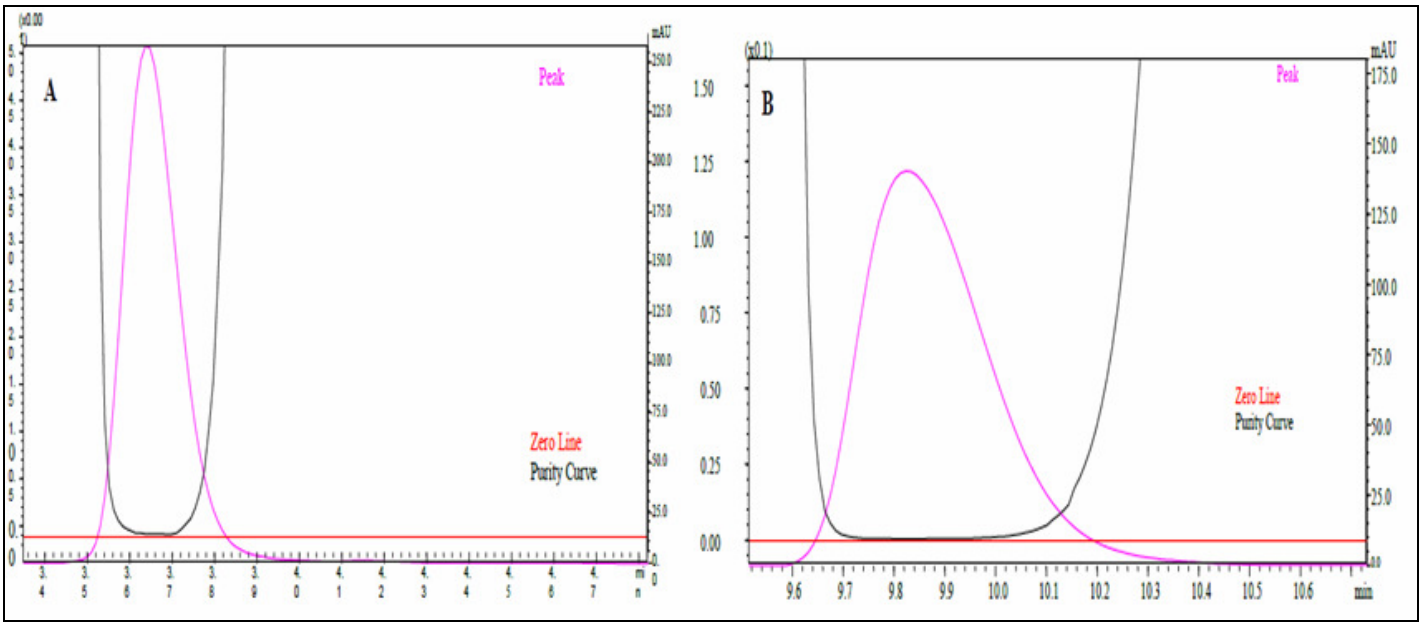

Fig. 4 : Peak purity curves for metformin hydrochloride (A) and saxagliptin (B).

Linearity

The calibration curve (n=3) for both drugs was linear over wide concentration range (20-120µg/ml for metformin hydrochloride and 2-12µg/ml for saxagliptin) with correlation coefficient (r^2) closer to one (Table 2). The value of correlation coefficient nearer to one revealed good linearity of responses in the given concentration range (Figure 5).

Table 2 : Linearity data of method (n=3)

Parameter	Metformin Hydrochloride	Saxagliptin
Concentration (µg/ml)	20-120	2-12
Coefficient (r^2)	0.9998	0.9995
Slope	116361	60215
Intercept	794225	31816

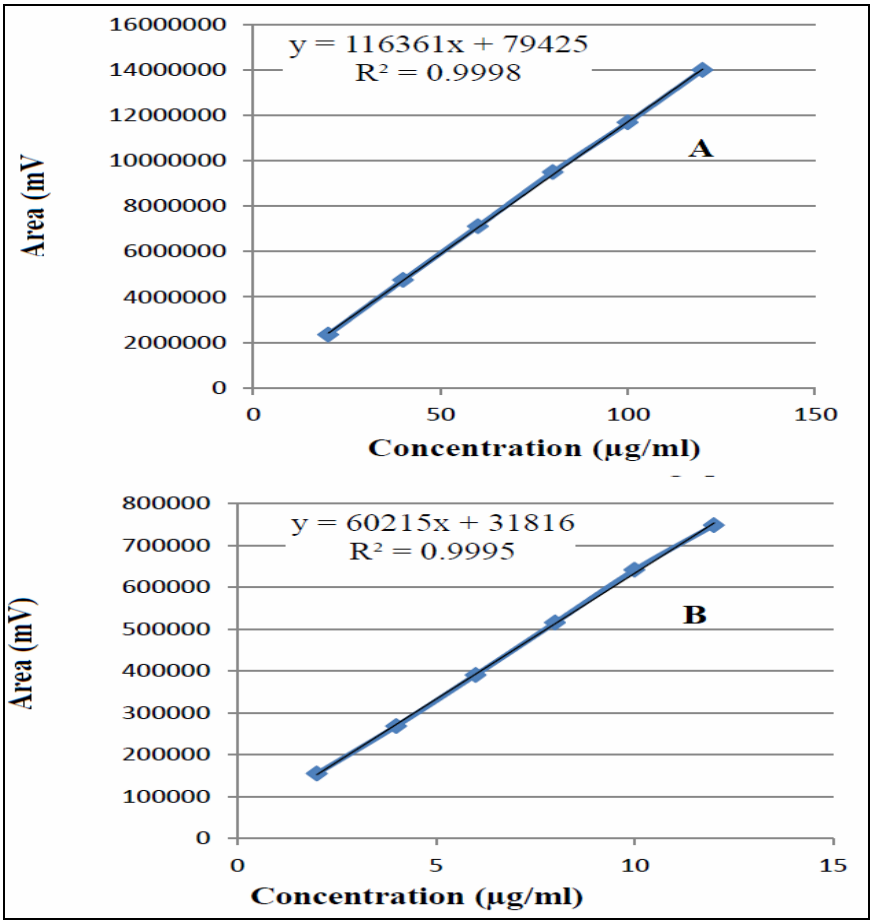

Fig. 5 : Linear calibration plot for metformin hydrochloride (A) and saxagliptin (B).

Limit of detection (LOD) and Limit of quantification (LOQ)

The LODs for metformin hydrochloride and saxagliptin were 1.98 and 0.33 µg/ml, respectively, and the LOQs for metformin hydrochloride and saxagliptin were 6 and 0.99 µg/ml, respectively.

Accuracy

Accuracy was studied by spiking the sample solution with standard drug at 80, 100 and 120 %. The mean percentage recovery for metformin hydrochloride and saxagliptin was found 101.02 to 101.63 and 98.75 to 101.17 respectively. The method accuracy showed excellent results for % RSD ranging from 0.33 to 0.71 and 0.15 to 0.39 for metformin hydrochloride and saxagliptin, respectively (Table 3).

Table 3. Accuracy data for the method (n=3).

Drug	Pre-analyzed Concentration (mcg/ml)	Amount added (mcg/ml)	% Recovery	% RSD
Metformin Hydrochloride	50	40	101.63	0.33
		50	101.88	0.35
		60	101.02	0.71
Saxagliptin	5	4	98.75	0.39
		5	101.80	0.15
		6	101.17	0.24

Table 4 : Precision data for method.

Drug	Amount added (mcg/ml)	Amount found			
		Intraday		Inter-day	
		% Found	% RSD	% Found	% RSD
Metformin Hydrochloride	40	100.27±0.18	0.18	99.19±0.39	0.39
	80	101.53±0.38	0.37	100.96±0.19	0.19
	120	99.72±0.08	0.08	99.13±0.61	0.62
Saxagliptin	4	99.22±1.28	1.29	98.61±0.29	0.29
	8	100.39±1.01	1.01	100.87±0.79	0.78
	12	99.25±0.14	0.15	98.23±0.25	0.26

Precision

Precision was ascertained by determining repeatability, intraday and inter-day precision. Repeatability of the method is finding out by analyzing six replicates of 100 µg/ml of metformin hydrochloride and 10 µg/ml of saxagliptin. The percentage RSD for studied responses was found to be less than 2 (Table 4). The outcomes for both intra-day and inter-day determinations ascertains the high precision and repeatability of the developed method where, % RSD was 0.5 and 0.78 % for metformin hydrochloride and saxagliptin respectively and never surpassed acceptance limit (% RSD < 2).

Robustness

Robustness was assessed by changing the method parameters. Sample solution was injected in triplicate and assayed for metformin hydrochloride and metformin. The mean, standard deviation, and RSD are presented in Table 5 and 6 for metformin hydrochloride and saxagliptin respectively.

Table 5 : Robustness for metformin hydrochloride.

S. No	Assay of metformin hydrochloride (µg/ml)								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	49.62	50.98	49.49	50.24	48.93	50.94	50.83	49.27	50.35
2	50.12	50.21	50.38	49.83	49.64	49.52	49.03	49.74	50.92
3	50.94	50.56	49.92	50.52	50.27	49.91	49.62	50.38	50.03
Mean	50.23	50.58	49.93	50.20	49.61	50.12	49.83	49.80	50.43
SD	0.67	0.39	0.45	0.35	0.67	0.73	0.92	0.56	0.45
RSD	1.33	0.76	0.89	0.69	1.35	1.46	1.84	1.12	0.89

Table 6 : Robustness for Saxagliptin

S. No	Assay of saxagliptin (µg/ml)								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	5.02	4.87	5.02	5.05	4.98	5.07	5.13	4.96	5.02
2	4.96	4.92	4.98	4.93	5.08	4.95	4.89	5.03	5.17
3	4.87	4.96	4.86	5.09	5.03	4.93	4.93	4.87	5.13
Mean	4.95	4.92	4.95	5.02	5.03	4.98	4.98	4.95	5.11
SD	0.08	0.05	0.08	0.08	0.05	0.08	0.13	0.08	0.08
RSD	1.53	0.92	1.68	1.66	0.99	1.52	2.58	1.62	1.52

Where, Run 1: control sample (flow rate 0.8 ml/min, acetonitrile: buffer: water in ratio of 20:30:50 %v/v/v, temperature 35°C and pH 3.0); Run 2: sample (pH 2.9); Run 3: sample (pH 3.1); Run 4: Sample (flow rate 0.7 ml/min); Run 5: Sample (flow rate 0.9 ml/min); Run 6: Sample (Column Temperature 30°C); Run 7: Sample (Column Temperature 40°C); Run 8: Sample (acetonitrile: buffer: water in ratio of 25:25:50); Run 9: Sample (acetonitrile: buffer: water in ratio of 15:35:50).

The statistical analysis of data performed using one way ANOVA. As evident from Table 7, the calculated value of F was greater than its tabulated value revealing no significant difference in any condition. The results revealed that the method was robust and response was not affected with minor change in method parameters.

Table 7 : ANOVA table for deviation

Drug	Source of Variation	SS	df	MS	F	P-value	F-crit
Metformin hydrochloride	Between Groups (BG)	2.399733	8	0.299967	0.829486	0.58827	2.510158
	Within Groups (WG)	6.509333	18	0.36163			
	Total (T)	8.909067	26				
Saxagliptin	Between Groups (BG)	0.0782	8	0.009775	1.495326	0.2269	2.510158
	Within Groups (WG)	0.117667	18	0.006537			
	Total (T)	0.195867	26				

Force degradation study

A force degradation study was performed on the sample solution. Using the peak purity test, the purity of the drug peaks were evaluated at each stage of force degradation studies. Chromatograms of sample are shown in Figure 6. .

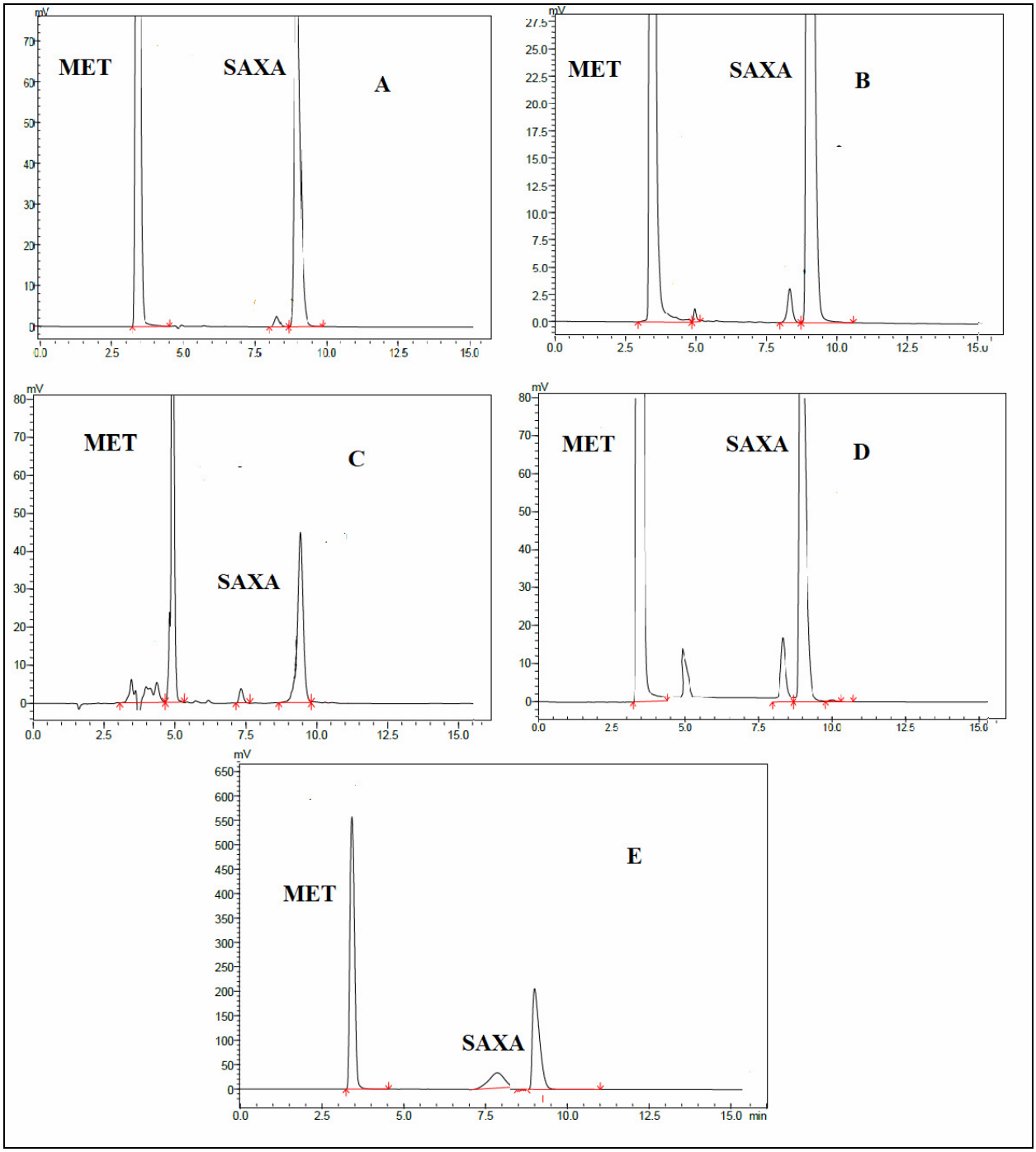

Fig. 6 : Force degradation study: acidic hydrolysis (A), basic hydrolysis (B), thermal (C), oxidative (D), and photolytic (E).

Table 8 : Force degradation study for metformin hydrochloride and saxagliptin.

Stress condition	Metformin hydrochloride			Saxagliptin		
	Area (µv*sec)	Assay (µg/ml)	Degradation (%)	Area (µv*sec)	Assay (µg/ml)	Degradation (%)
Acidic	5483230	46.44	7.12	322353.4	4.825	3.5
Basic	5284834	44.735	10.53	311093.2	4.638	7.24
Thermal	4572123	38.61	22.78	305162	4.5395	9.21
Oxidative	5592609	47.38	5.24	317355.5	4.742	5.16
Photolytic	4917715	41.58	16.84	318288.9	4.7575	4.85

The chromatograms in Figure 6 and data in Table 8 have shown that there was no interference of any degradation products with the peaks of both drugs.

Conclusion

The analytical procedure described for the concurrent quantitative analysis of metformin HCl/saxagliptin. The result of study concluded that RP-HPLC analytical method was validated and stability indicating method.

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Conflict of Interest

The authors declare no conflict of interest.

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