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RESEARCH ARTICLE

Application of Validated High-performance Liquid Chromatography Method for Degradation Study of Saxagliptin and Metformin HCl

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ABSTRACT

A novel and simple reversed-phase liquid chromatographic method has been established for the determination of saxagliptin and metformin HCl Saxagliptin and metformin HCl is used to control Type 2 diabetes. The proposed work was performed on Young Lin (S.K) isocratic System UV Detector. Saxagliptin and metformin HCl is used to control Type 2 diabetes. The proposed work was performed on Young Lin (S.K) isocratic System UV Detector C18 column (150 mm × 4.6 mm). A mixture of potassium phosphate, mobile phase in this method with flow rate of 0.7 mL/min (UV detection at 203 nm) and the method was validated as per the ICH guidelines. Forced degradation studies were performed by exposing the drug saxagliptin and metformin HCl to acidic, alkaline, oxidation, and thermal stress degradations. The proposed reversed-phase-high-performance liquid chromatography method was found to be robust and specific, and this method is suitable for the assay of pharmaceutical dosage forms as well as kinetic studies.

Keywords: Metformin HCl, reversed-phase-high-performance liquid chromatography, saxagliptin, stability indicating, validation.

INTRODUCTION

Saxagliptin[1,2] is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affect the action of natural hormones in the body called incretins. Incretins cut blood sugar by cumulative consumption of sugar by the body, mainly through increase insulin production in the pancreas, and by dropping production of sugar by the liver (Bristol-Myers Squibb press release). DPP-4 is a membrane related peptidase which is found in many tissues, lymphocytes, and plasma. DPP-4 has two main mechanisms of action, an enzymatic function and additional mechanism where DPP-4 fixes adenosine deaminase, which carries intracellular signals through dimerization when activate. Saxagliptin forms a revocable, histidine-assisted covalent bond among its nitrile group and the S630 hydroxyl oxygen on DPP-4. The inhibition of DPP-4 increase levels active of glucagon-like peptide 1, which inhibit glucagon manufacture from pancreatic alpha cells and increase production of insulin from pancreatic beta cells.

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EXPERIMENTAL

Chemicals and reagents

Saxagliptin and metformin HCl was provided from Merck Laboratories Ltd. high-performance liquid chromatography (HPLC) grade potassium phosphate buffer pH - 3.2 with orthophosphoric acid, acetonitrile, and sodium hydroxide were procured from Merck Ltd. High pure water was prepared by using Millipore Milli Q plus purification system. Saxagliptin 50 mg and metformin 500 mg JALRA M were selected as a formulation for study.

HPLC instrumentation and conditions[3-7]

A HPLC framework, with LC arrangements information and the isocratic framework was utilized to develop method and further

subsequent analysis. The data were recorded using Autochro-3000 solutions software. The sample separation was performed on a Shimadzu Primesil C18 (4.6 mm × 150 mm) with the mobile phase consisting of acetonitrile and potassium phosphate buffer pH - 3.2 with a ratio of 40:60 (v/v) at ambient temperature. The flow rate was kept at 0.7 mL/min, and the determination wavelength was 218 nm. Retention time for metformin HCl was found out to be 2.66 min, and saxagliptin was found out to be 5.44 min.

Mobile phase

Acetonitrile: Phosphate buffer (acetic acid 0.05% OPA), (25+75% v/v) was set as mobile phase.

Standard solution

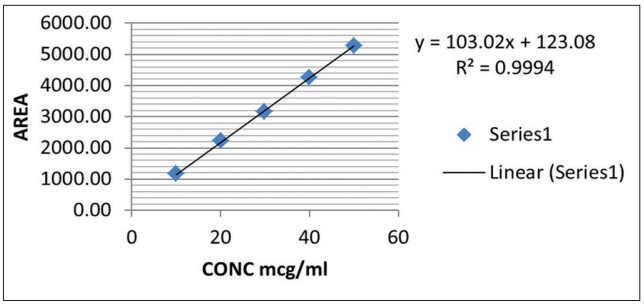
The standard was dissolved with mobile phase to 5 mcg/mL. The test samples were dissolved with the mobile phase. With the optimized chromatographic conditions, a steady baseline was recorded, the standard solution was injected, and the chromatogram was recorded. The procedure was repeated for the sample solution [Figure 3].

Forced degradation studies

Saxagliptin and metformin HCl was allowed to hydrolyze in different strengths of base and acid (0.1 N) and hydrogen peroxide (0.1 N). The combination was studied for its neutral degradation. Further, it is important to note that from the chromatograms (Figure), it is evident that although the degraded peaks are observed. The combination saxagliptin and metformin HCl is stable under the applied stress conditions such as acid, base, oxidative, and neutral degradation states [Table 1][Figure 4].

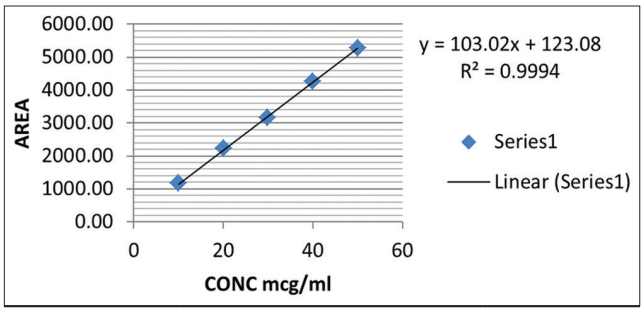
Linearity

The calibration curve showed good linearity in the range of 10–50 mcg/mL for metformin HCl and 1–5 mg/mL for saxagliptin HCl. The combination with RSD - 0.95 (Figure). A typical calibration curve has the regression equation of Y=103.0X+123 R^2 = 0.999 for metformin HCl



Concentration	Average area
100	2059.24
200	7961.57
300	14051.25
400	19836.51
500	25966.30

Figure 1: Linearity graph and data of metformin



Concentration	Average area
1	216.59
2	399.66
3	595.23
4	783.9
5	978

Figure 2: Linearity graph and data of Saxagliptin for Saxagliptin

Table 1: Results of forced degradation studies of saxagliptin and metformin HCl

Stress condition/duration/solution	Degradation (%)
Acid degradation (0.1 N) after 1 h	1.49
Acid degradation (0.1 N) after 2 h	1.15
Alkaline degradation (0.1 N) after 1 h	1.14
Oxidative degradation (0.1 N) after 1 h	1.13
Neutral degradation (0.1 N) after 1 h	0.97
Photolysis degradation (0.1 N) after 2 h	0.86

and Y=85.97X-3.638 R^2 = 0.999 for saxagliptin [Figure 1 and 2].

Precision

Intra-day and inter-day precision studies on reversed-phase - HPLC (RP-HPLC) method for saxagliptin and metformin which shows the high

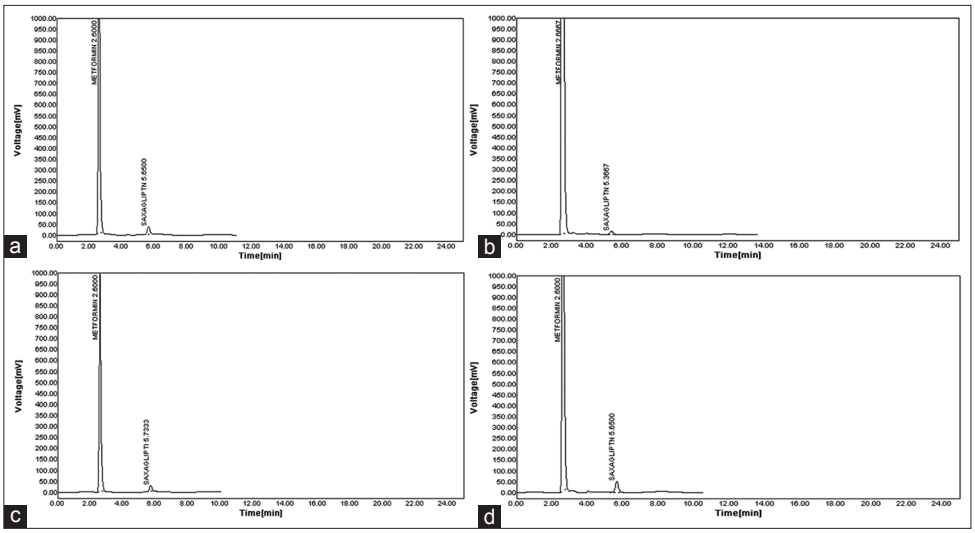


Figure 3: (a-d) Chromatogram of saxagliptin and metformin HCl

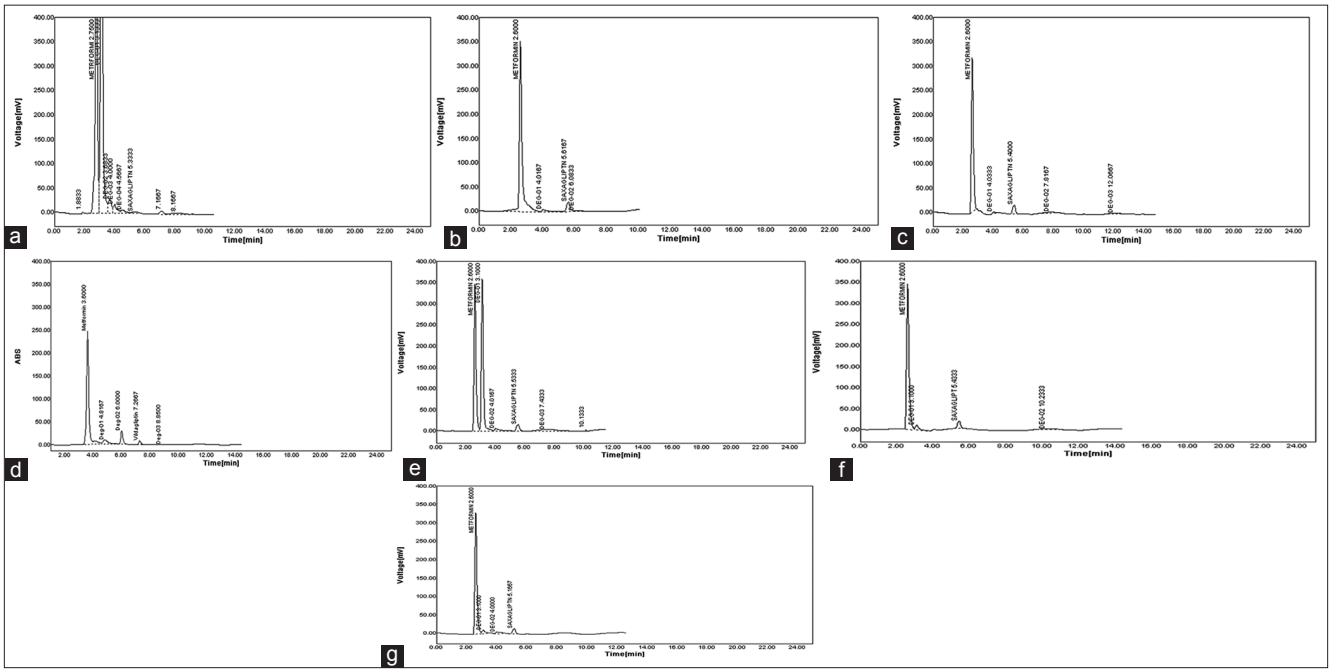


Figure 4: Chromatograms of (a) oxidative degraded sample, (b) acid hydrolyzed degraded, (c,d,) alkali degradation, (e,f) neutral degradation, and (g) photolysis degradation

precision percentage amount in between 98% and 102% indicates to an analytical method that concluded [Tables 2 and 3].

Intra-assay and inter-assay

The intra- and inter-day variation of the method was carried out and high values of the mean assay and low values of standard deviation and percentage RSD within a day and day-to-day variations for saxagliptin and metformin HCl revealed that the proposed method isaccurate precise and repeatability of method was good [Tables 2, 3,4,5].

Method robustness

Influence of small changes in chromatographic conditions such as change in flow rate (10%), organic content in mobile phase (2%), wavelength of detection (5%), and pH of buffer in mobile phase (0.2%) studied to determine the robustness of the method are also in favor [Table 6] of the developed RP-HPLC.

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

The LOD and LOQ of saxagliptin were found to

Table 2: Linearity and precision data for metformin

Linearity					
Concentration mcg/mL	**Area-I**	**Area-II**	**Mean**	**SD**	**% RSD**
100	2083.11	2035.36	2059.24	33.76	1.64
200	7981.01	7942.32	7961.67	27.36	0.34
300	14012.84	14089.65	14051.25	54.31	0.39
400	19662.68	20010.33	19836.51	245.83	1.24
500	25797.28	26135.32	25966.30	239.03	0.92

Precision						
Concentration mcg/mL	**Area-I**	**Area-II**	**Mean**	**Amount found (%)**	**SD**	**RSD**
200	7954.06	7965.32	7959.69	199.24 (99.62)	7.96	0.10
300	14160.7	14090.25	14125.47	302.55 (100.85)	49.82	0.35
400	25586.6	25932.23	25894.41	499.75 (99.95)	244.40	0.94

Table 3: Linearity and precision data for saxagliptin

Linearity					
Concentration	**Area-I**	**Area-II**	**Mean**	**SD**	**RSD**
1	217.723	215.45	216.59	1.61	0.74
2	396.95	402.36	399.66	3.83	0.96
3	590.14	600.32	595.23	7.20	1.21
4	787.5	780.3	783.90	5.09	0.65
5	975.12	980.87	978.00	4.07	0.42

Precision						
Concentration	**Area**	**II**	**Mean**	**Amount found (%)**	**SD**	**RSD**
2	405.76	415.25	410.51	2.03 (101.78)	6.71	1.63
3	598.38	595.64	597.01	3.01 (100.40)	1.94	0.32
4	785.89	773.97	779.93	3.08 (102.97)	8.43	1.08

Table 4: Repeatability for metformin and saxagliptin

Repeatability					
Concentration mcg/mL	**Peak area**	**Amount found (%)**	**Mean**	**SD**	**RSD**
200	7945.94	199.01 (99.50)	199.13 (99.56)	0.16 (0.08)	0.08 (0.09)
200	7960.23	199.24 (99.62)			
2	401.23	1.98 (99.28)	1.99 (99.72)	0.01 (0.62)	0.71 (0.62)
2	404.54	2.00 (100.15)			

Table 5: Accuracy data for (a) metformin and (b) saxagliptin

	Percentage received (%)		
	80	**100**	**120**
a			
Mean	99.90	99.53	100.83
SD	0.28	0.35	1.96
%RSD	0.28	0.36	1.94
b			
Mean	99.01	100.67	100.30
SD	0.94	1.04	1.53
%RSD	0.95	1.03	1.53

be 0.08 (mcg/mL) and 8.24 (mcg/mL), analytical method that concluded. The LOD and LOQ of metformin were found to be 5.96 (mcg/mL) and 18.06 (mcg/mL), respectively.

RESULTS AND DISCUSSION

The present examination is the give an account of steadiness demonstrating measure of blend saxagliptin and metformin HCl in the nearness of debasement items by HPLC. In this technique, the isocratic elution strategy was chosen for examination

Table 6: Robustness study of metformin and saxagliptin

	Flow change		**0.9 mL**		**Flow change 0.8 mL**	**1.1 mL**	
S. No.	**Concentration mcg/ml**		**Area**	**Sr No.**	**Concentration mcg/ml**		**Area**
1	200		7811.36	1	200		6335.65
2	200		7840.23	2	200		6358.65
		Mean	7825.80			Mean	6347.15
		SD	20.41			SD	16.26
		%RSD	0.26			%RSD	0.26
Mobil phase volume			24 + 76			26 + 74	
S. No.	**Concentration mcg/mL**		**Area**	**S. No.**	**Concentration mcg/mL**		**Area**
1	200		6518.66	1	200		7131
2	200		6570.54	2	200		7257.32
		Mean	6544.60			Mean	7194.16
		SD	36.68			SD	89.32
		%RSD	0.56			%RSD	1.24
Wavelength change = 223 nm		211 nm			213 nm		
S. No.	**Concentration mcg/ml**		**Area**	**S. No.**	**Concentration mcg. mL**		**Area**
1	200		7459.7	1	200		6905.39
2	200		7558.3	2	200		7023.6
		Mean	7509.00			Mean	6964.50
		SD	69.72			SD	83.59
		%RSD	0.93			%RSD	1.20
	Flow change	**0.9ml**			**Flow change 0.8 ml**	**1.1**	
S. No.	**Concentration mcg/mL**		**Area**	**S. No.**	**Concentration mcg/mL**		**Area**
1	2		399.02	1	2		389.61
2	2		405.65	2	2		390.85
		Mean	402.34			Mean	390.23
		SD	4.69			SD	0.88
		%RSD	1.17			%RSD	0.22
Mobile phase volume			24 + 76			26 + 74	
S. No.	**Concentration mcg/mL**		**Area**	**S. No.**	**Concentration mcg/mL**		**Area**
1	2		322.67	1	2		923.12
2	2		325.41	2	2		920.32
		Mean	324.04			Mean	921.72
		SD	1.94			SD	1.98
		%RSD	0.60			%RSD	0.21
Wavelength change = 223 nm		211 nm			213 nm		
S. No.	**Concentration mcg/mL**		**Area**	**S. No.**	**Concentration mcg/mL**		**Area**
1	2		339.64	1	2		300.22
2	2		342.25	2	2		302.25
		Mean	340.95			Mean	301.24
		SD	1.85			SD	1.44
		%RSD	0.54			%RSD	0.48

of the mixture. Since, it gave a better pattern partition and pinnacle width, which is appropriate for routine examination of mixtures the created technique was approved according to ICH rules.

Stability testing frames an imperative piece of procedure of drug product advancement. The

motivation behind stability testing is to give prove on how the medication quality substance shifts with time under the impact of different natural factors, for example, temperature, moistness, and light, and empowers proposals of capacity conditions, retest periods, and time span of usability to be built up.

CONCLUSION

The procured pure drug of Saxagliptin and Metformin were found to melt in the range of 149-169°C respectively. The drug was found to be freely soluble in Acetonitrile, potassium buffer, Sparingly soluble in water, alcohol. UV absorption of 10 µg/mL solution of Saxagliptin and Metformin methanol was generated and absorbance was taken in the range of 200-400 nm. λmax of Saxagliptin and Metformin in Acetonitrile was found to be 214 nm and 234 nm respectively. it has been observed that, using mobile phase of Acetonitrile+Ph, buffer (25+75%v/v) pH - 3.2 gave adequate retention time at 240.72 min and 3083.11 min. with good peak shape. Tablet Assay for %Label claim for %RSD Calculated. Analysis process was repeated five times with tablet formulation. Tablet Assay for % Label claim for %RSD Calculated. Recovery studies were done to validate the accuracy of established method. To pre analyzed tablet solution, a definite concentration of standard drug (80%,100%, and 120%) To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Saxagliptin and Metformin system suitability parameters were studied. The method was established by analyzing various replicates standards of Saxagliptin and Metformin. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded respectively. The Robustness of a method is its capability to remain unpretentious by small thoughtful changes in parameters. To assess the robustness of the proposed method, small but careful variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in(±1 ml/ min^{-1}) proportion and the flow rate was varied by(±1ml/min^{-1}), and wavelength change(±1 ml/ min^{-1})of optimized chromatographic condition. %RSD for peak area for Metformin was calculated which should be less than 2%.the result revealed in analytical method that determined. %RSD for peak area for Saxagliptin was calculated which should be less than 2%.the the result revealed in analytical method that determined. Forced degradation studies of both the drugs namely Saxagliptin and Metformin were carried out individually and in combination under different stress conditions like acid hydrolysis, alkaline hydrolysis, hydrogen peroxide oxidation and photolysis. Liquid chromatography is a technique of physical separation in which the components of a liquid mixture are distributed between two immiscible phases; this has provided us the information regarding the degradation part of the drug under study.

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