INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND METFORMIN HCl BY USING RP-HPLC METHOD

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Article Received on: 29/05/18 Approved for publication: 29/06/18

DOI: 10.7897/2230-8407.096106

ABSTRACT

A simple, rapid and selective HPLC method has been developed for quantitation of Saxagliptin and Metformin HCl from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of methanol and phosphate buffer pH-5.0 (70:30 v/v) at the flow rate of 1 ml/min. Waters C-8 (25 cm x 4.6 mm, 5 µm.) column was used as stationary phase. The retention time of Metformin HCl and Saxagliptin were 2.8 min. and 4.9 min. respectively. Linearity was observed in the concentration range of 2.5-12.5 µg/mL for Saxagliptin and 250-1250 µg/ml for Metformin HCl. Percent recoveries obtained for Saxagliptin and Metformin HCl were 102 and 101 respectively. The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Saxagliptin and Metformin HCl.

Keywords: Saxagliptin, Metformin HCl, RP-HPLC Method, Validation

INTRODUCTION

Saxagliptin is chemically designated as (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxyadamantan-1-yl)acetyl]-2- azabicyclo [3.1.0] hexane-3-carbonitrile. It is an anti-diabetic drug. Metformin HCl is 1-carbamimidamido-N,N-dimethylmethanimidamide and used as anti-diabetic drug. Detailed survey of literature for Saxagliptin and Metformin HCl revealed several methods based on techniques viz. HPLC spectrophotometric for its determination in pharmaceutical dosage form. However, very few methods has been developed for estimation of these drugs in combined dosage form. This paper presents simple, rapid, reproducible and economical method for RP-HPLC simultaneous estimation of Saxagliptin and Metformin HCl in bulk and pharmaceutical dosage form. [1-3]

MATERIALS & METHOD

Reagents and Chemicals

The solvents used were of HPLC grade. Double distilled water was used in preparation of mobile phase. Pure drug sample of Saxagliptin was produce from Swapnroop Drugs and Pharmaceuticals and Metformin HCl from Lupin Ltd.

Apparatus and Chromatographic Conditions

Chromatographic separation was performed on a Thermo U.S.A HPLC system consisting of P1000 pump, UV 200 detector, Hamilton syringe with 20 µl loop volume and windows based ChromQuest 4.1 software. An C18 RP-Column (Waters 4.6 mm x 25 cm, 5 µm) was used for separation. The elution was carried out isocratically at flow rate of 1 ml/min using methanol:phosphate buffer pH-5.0 (70:30 v/v) mobile phase.

Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 5.0 mg of Saxagliptin and 500.0 mg of Metformin HCl in 10 ml Methanol that give concentration 500 and 50000 µg/ml for Saxagliptin and Metformin HCl respectively. From the standard stock solutions, mixed standard solutions of Saxagliptin and Metformin HCl was prepared.

Preparation of sample solution

Four tablets were weighed, and the average weight was determined. Accurately weighed tablet powder equivalent to 5 mg Saxagliptin and 500 mg of Metformin HCl (i.e. 1197 mg) was transferred in a 10 ml volumetric flask and methanol was added. It was sonicated for 10 to 15 minutes. Later the volume was made up to mark with methanol. The solution was filtered through 0-0.45 µm filter paper.

VALIDATION [4-12]

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Precision

The repeatability and intermediate precision studies were carried out by estimating the corresponding response three times on the same day and on three different days for two same concentrations and result was reported in terms of the relative standard deviation.

Accuracy

The accuracy of an analytical method was determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy was calculated from the test results as the percentage of analyte recovered by the assay. The results of recovery studies and statistical data are recorded.

Limit of Detection and Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of the analyte that was reproducibly quantified. These two parameters were calculated using formula based on standard deviation of the response and slope. LOD and LOQ were calculated by the equation, LOD=3.3 x σ/s and LOQ= 10 x σ/s, where s = standard deviation, S = slope of calibration curve.

Robustness

It is measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provide an indication of its reliability under normal usage.

Robustness of method was studied by deliberately changing the chromatographic parameters such as flow rate, mobile phase composition & wavelengths.

Assay

20 µl of standard and sample solutions were injected into an injector of liquid chromatograph, from the peak area of Saxagliptin and Metformin HCl amount of drug in samples were computed.

Table 1: Chromatographic Condition

Chromatographic mode	Chromatographic condition
HPLC system	Thermo (USA)
Pump	P1000
Detector	UV200
Data processor	ChromQuest 4.1
Stationary phase	RP C_8 (Waters)
Mobile phase	Methanol:Phosphate buffer pH-5.0 (70:30 v/v)
Wavelength	228 nm
Flow rate	1 ml/min
Sample size	20 µl
Column temperature	Ambient

Table 2: System suitability parameter

	Sr. No.	Peak Area		Tailing Factor		Theoretical Plate	
		Saxa	Met	Saxa	Met	Saxa	Met
	1	585.23	8583.68	1.6865	1.1091	4634	2967
	2	586.23	8580.60	1.6476	1.1466	4600	2956
	3	583.98	8581.68	1.7199	1.1400	4675	2919
Mean	-	585.1467	8581.987	1.6846	1.1319	4636.333	2947.333
±SD	-	1.12	1.56	0.04	0.02	37.55	25.14
%RSD	-	0.19	0.01	2.37	1.76	0.80	0.85

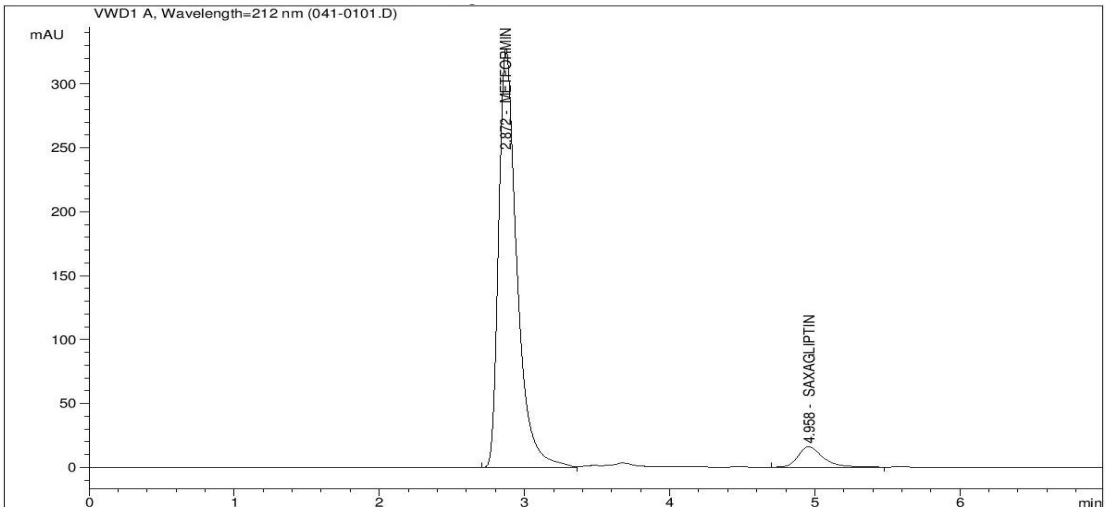

Figure 1. Chromatogram of Metformin HCl and Saxagliptin

Table 3. Linearity

Saxagliptin		Metformin HCl	
Conc. (µg/ml)	**Peak area**	**Conc. (µg/ml)**	**Peak area**
2.5	191.45	250	2843.40
5.0	384.60565	500	5696.725
7.5	585.77195	750	8673.685
10.0	755.0683	1000	11115.25
12.5	954.69	1250	13751.20

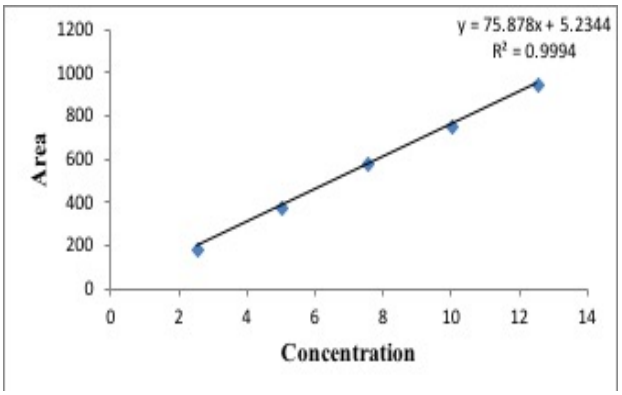

Figure 2. Calibration curve of Saxagliptin

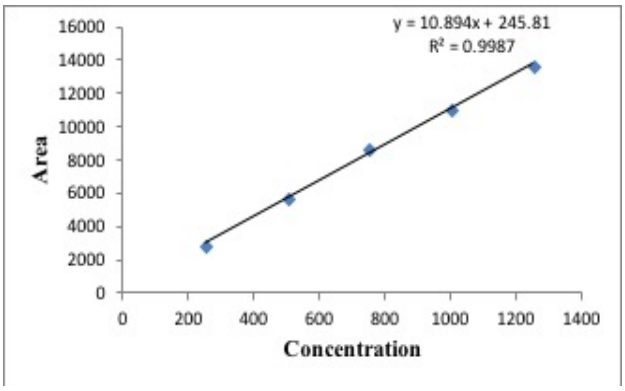

Figure 3. Calibration curve of Metformin HCl

Table 4.1. Precision studies for Saxagliptin

Sr. No.	Conc. µg/ml	Measured area ± S.D, RSD (%)	
		Repeatability (n=2)	**Intermediate Precision (n=2)**
1	2.5	187.33 ± 0.46, 0.25	189.67 ± 0.96, 0.51
4	7.5	585.80 ± 0.80, 0.14	582.33 ± 2.46, 0.42
3	12.5	954.98 ± 0.36, 0.04	952.61 ± 1.94, 0.20

Table 4.2. Precision studies for Metformin HCl

Sr. No.	Conc. µg/ml	Measured area ± S.D, RSD (%)	
		Repeatability (n=2)	**Intermediate Precision (n=2)**
1	250	2894.27 ± 2.14, 0.07	2894.78 ± 6.46, 0.22
4	750	8582.67 ± 1.42, 0.02	8574.22 ± 1.26, 0.01
3	1250	13762.5 ± 6.48, 0.05	13753.26± 3.82, 0.03

The % RSD in two replicates was not more than 2.0%, hence the method was found to be precise.

Table 5. Recovery data for Saxagliptin and Metformin HCl

Drugs	Spiked level %	% Recovery	% R.S.D.
Saxagliptin	80	101.50	0.91
	100	99.62	0.60
	120	97.38	1.02
Metformin HCl	80	101.55	0.13
	100	97.46	0.69
	120	96.23	0.18

Table 6. Results of LOD and LOQ

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Saxagliptin	0.14	9.33
Metformin HCl	0.44	28.30

Robustness results

Table 7.1. Effect of variation in Flow rate of mobile phase by ±1%

Sr. No.	Flow rate	Conc. (µg/ml)		Mean		S.D.		% R.S.D.	
		Saxa	**Met**	**Saxa**	**Met**	**Saxa**	**Met**	**Saxa**	**Met**
1	0.9	5	500	463.72	6884.14	0.33	28.47	0.07	0.41
2	1.1	5	500	331.34	4906.34	0.04	23.46	0.01	0.48

Table 7.2. Effect of variation in mobile phase composition by±1 % v/v

Sr. No.	Mobile Phase Composition	Conc. (µg/ml)		Mean		S.D.		% R.S.D.	
		Saxa	Met	Saxa	Met	Saxa	Met	Saxa	Met
1	M:W(69:31)	5	500	2306.66	1092.0	1.19	0.47	0.05	0.04
2	M:W(71:29)	5	500	2297.18	1111.28	0.95	1.37	0.04	0.12

Table 7.3. Effect of variation in wavelengths

Sr. No.	Wavelength Change (nm)	Conc. (µg/ml)		Mean		S.D.		% R.S.D.	
		Saxa	Met	Saxa	Met	Saxa	Met	Saxa	Met
1	230	5	500	385.17	5735.50	0.08	1.09	0.02	0.37
2	232	5	500	387.36	5578.29	1.02	2.20	0.33	0.06

Table 8. Results for estimation of Saxagliptin and Metformin HCl in marketed formulation

Drugs	Conc. (µg/ml)	Amount found	% label claim	S.D.	% R.S.D.
Saxa	5	4.83	96.60	0.31	0.28
Met	500	490.96	98.11	0.15	0.15

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop accurate and stable assay method. Waters C8 column with a mobile phase of mixture of methanol and phosphate buffer pH-5.0 (70:30 v/v), delivered at a flow rate of 1.0 ml/min with detection at 228 nm gave sharp and symmetrical peak with retention time 2.8 and 4.9 min for Metformin HCl and Saxagliptin respectively. The typical chromatogram of the sample is shown in Fig. 1.

Linearity response for both Saxagliptin and Metformin HCl were found to be linear in concentration range of 2.5-12.5 µg/ml and 250-1250 µg/ml respectively. The slope and intercept value for calibration curve was $y = 75.87x + 5.234$ ($R^2 = 0.999$) for Saxagliptin and $y = 10.89x + 245.8$ ($R^2 = 0.998$) for Metformin HCl. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above.

The % RSD in two replicates for precision studies was not more than 2.0 % hence the method was found to be precise. Percentage recovery for both drugs Saxagliptin and Metformin HCl was found in range of 97.38-101.50 % and 96.23-101.55 % indicating accuracy of the proposed work. The LOD value of Saxagliptin and Metformin HCl was found to be 0.14 µg/ml and 9.33 µg/ml respectively. The LOQ value of Saxagliptin and Metformin HCl was found to be 0.44 µg/ml and 28.30 µg/ml respectively. The results of the robustness study also indicated that the method is robust and is unaffected by deliberate variation in the chromatographic conditions. The % label claim of Saxagliptin and Metformin HCl was found to be 98.57 % and 98.05 % respectively with % RSD not more than 2.

Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, & selective and can be employed successfully for the estimation of Saxagliptin and Metformin HCl in bulk and pharmaceutical dosage formulation.

CONCLUSION

The developed UV and RP-HPLC methods are precise, specific, accurate. Statistical analysis proves that these methods are suitable for the analysis of Saxagliptin and Metformin HCl in bulk and pharmaceutical formulation without any interference from the excipients. These methods have been found to be better than previously reported methods, because of use of a less, economical and readily available solvent in mobile phase i.e. methanol as compared to acetronitrile and no use of tedious and time consuming procedures. All these factors using these methods make easy quantification of drugs in bulk and pharmaceutical dosage form. It can therefore be concluded that use of these methods can save much time and money and hence can be used in small laboratories with very high accuracy over a wide linear range.

ACKNOWLEDGEMENT

The authors are thankful to Pune District Education Association's, Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi, Pune, for providing all the necessary facilities, and to Swapnroop Drugs and Pharmaceuticals and Lupin Ltd., Pune for the gift samples of Saxagliptn and Metformin HCl are required for the research work. The authors are also thankful to all the teaching and non-teaching staff and the colleagues for their constant support. Hearty thanks to our parents.

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Cite this article as:

Vijaya U. Barge *et al*. Development and validation of analytical method for simultaneous estimation of Saxagliptin and Metformin HCl by using RP-HPLC method. Int. Res. J. Pharm. 2018;9(6):142-146 http://dx.doi.org/10.7897/2230-8407.096106

Source of support: Nil, Conflict of interest: None Declared