Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 2| Issue 4 |OCT-DEC |2012|161-167

Research Article
Pharmaceutical Sciences

A NEW RP – HPLC METHOD FOR DETERMINATION OF METFORMIN HCL AND SAXAGLIPTIN IN TABLET DOSAGE FORM

Patil Prafulla Prakash[*1,] Kalkotwar Ramesh.S[1],*
Patil Vikas V[2], Jadhav Vijay.B[2], Patil Nilesh .P[2]

*[*1]Department of Quality Assurance, SND College of Pharmacy, Pune University, Yeola Dist, Nasik, India*
[2] Department of Quality Assurance & Pharmaceutical Chemistry, SND College of Pharmacy, Pune University, Yeola Dist, Nasik, India

***Corresponding Author Email:** patilprafulla2 @gmail.com

ABSTRACT

A new simple economical reverse phase high performance liquid chromatographic method was developed for the determination of Metformin Hcl [MFH] and Saxagliptin [SGP] in bulk and dosage form. The separation was eluted on a Zodiac C_{18} column (150 mm x 4.6 mm; 5µ) using a mobile phase mixture of Phosphate buffer pH 6.8 and acetonitrile in a ratio of 94:6 v/v at a flow rate of 1.0ml/min. The detection was made at 248 nm. The retention times were 1.6min for [MFH] and 4.1min for [SGP]. Calibration curve was linear over the concentration range of 12.5-75 µg/ml for (MFH) and 0.125 to 0.75 µg/ml for [SGP]. The propose method was validated as per the ICH guidelines parameters like Linearity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

KEY WORDS

Method development and validation, Saxagliptin, Tablets, C_{18} column, RP-HPLC.

1. INTORDUCTION

Metformin Hcl is 1, 1-dimethylbiguanide hydrochloride[1]; saxagliptin (1s, 3s, 5s) -2- [(2s) -2-amino-2- (3-hydroxy-1-adamantyl) acetyl] -2-azabicyclohexane-3-carbonitrile is a new dipeptidylpeptidase-4-inhibitor[2]. MFH is official in Indian pharmacopoeia [1].but there is no official method for the combination. Both drugs in combination of tablet dosage form in the ratio of 500:5 MFH: SGP. As per literature survey many methods have been reported the estimation of MFH and SGP individually or in combination with some other drugs[2-7]. With this present proposed method both MFH and SGP estimates simple and economical in tablet formulation.

2. MATERIAL AND METHODS

2.1 Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with a Zodiac C_{18} column (150 mm x 4.6 mm ; 5µ) and 2489 UV-Visible detector, autoinjector, autosampler with Empower 2 software from Waters corporation, Milford USA was employed in the study. HPLC grade acetonitrile , water were purchased from E.Merck Co; Mumbai, India, and Potassium dihydrogen phosphate, ortho phosphoric acid AR grade were purchased from SD Fine Chem Mumbai, India were used in the study.

2.2 Drug Samples

The reference samples were obtained from M/s. Bio-Leo Analytical Labs India Pvt Ltd, Hyderabad, India, and the formulation samples were purchased from local market.

2.3 Mobile phase

A mixture of water and acetonitrile in the ratio 94:6 v/v was filtered through 0.22µ membrane filter and was degassed. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered and sonicated by using Bio-Technics india, Mumbai before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 30ºC and the detection of the drug was carried out at 248nm.

2.4 Preparation of stock and working standard solution of Montelukast sodium and Fexofenadine HCl

About 500mg of Metformin HCl and 5 mg of Saxagliptin was weighed accurately on Sartorius semi micro balance model-CPA225D and transfers in to 100ml volumetric flask the solution was sonicated and the resulting solution was diluted with the mobile phase to get a working standard solution of 50 µg/ml MFX AND 0.5 µg/ml SGP.

2.5 Sample Preparation

Weighed accurately 872 mg of previously weighed and crushed 20 tablets powder transferred to 100ml volumetric flask make upto the mark with mobile phase sonicated and filtered through 0.22µ membrane filter paper.Further dilute 1ml to100 ml with mobile phase.

2.6 Linearity and Construction of Calibration Curve

Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working standard dilution of MFX and SGP in the range of 12.5-75µg/ml and 0.125 to 0.75 µg/ml respectively. 20µl quantity of the dilution was injected each time in to the column. The drug in the elutes was monitored at 248 nm and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. This regression equation was later used to estimate the amount of MFX and SGP in pharmaceutical dosage form. A representative chromatogram for the separation of MFX and SGP presented in **Fig.1**

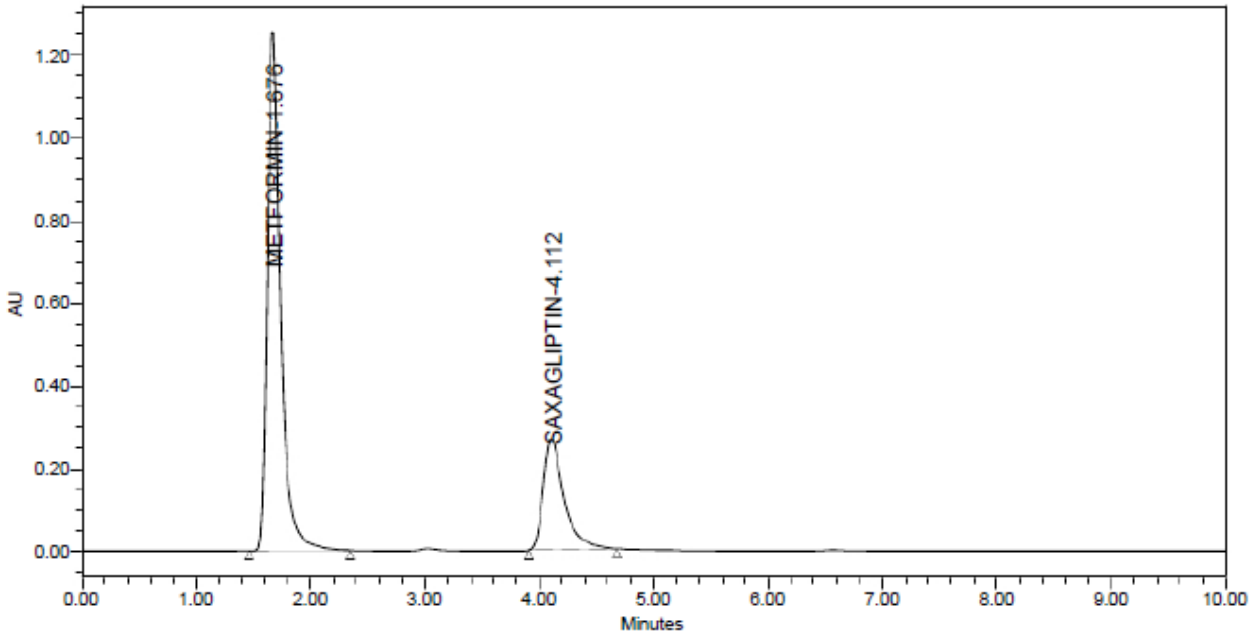
2.7 System Suitability Testing

The system suitability parameters such as Theoretical plates, tailing Factor and resolution were performed to verify the system is adequate for the analysis to be performed. The results are performed in **Table 1**.

Table 1: System suitability parameters

Parameters	Metformin	Saxagliptin
Tailing Factor	1.41	1.50
Theoretical plates	3329	3741
Resolution	--	9.67
LOD(µg/ml)	5.8802	0.0107
LOQ(µg/ml)	17.8188	0.0326

Figure 1: Chromatogram of MFH (50mcg/ml)& SGP(0.5mcg/ml).



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	METFORMIN	1.676	10212765	1254521	75.03		1.41	3256
2	SAXAGLIPTIN	4.112	3358453	268864	24.97	9.69	1.48	3854

RESULTS AND DISCUSSION

The present study was aimed at developing a simple economical precise and accurate HPLC method for the analysis of MFX and SGP in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a C_{18} stationary phase. A mixture of phosphate buffer pH 6.8 : acetonitrile in a proportion of 94:6 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for MFX was 1.67±0.1 min and for MKS was 4.1±0.1min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of MFX and SGP were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed for MFX and (r^2=0.999) was observed for SGP. The regression concentration and areas are given in **Table 2**. And the regression characters are given in **Figure 2&3**. When test solutions were analysed by the proposed method for finding out intra and inter-day variation, low co-efficient of variation was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method are given in **Table 3**.

The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in **Table 4**. The lowest value of LOD and LOQ as obtained by the proposed method by calculated using

3.3xstdev/slope for LOD and 10xstdev/slpoe for LOQ. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit system suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor and the number theoretical plate are in the acceptable limits.

The system precision was established by six replicate injections of the standard solution containing analytes of interest. The values of relative standard deviation were found within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found within the limit, indicating the injection repeatability of the method. The results were presented in **Table 5&6**.

The diluted preparations of marketed tablets were injected in duplicate and the results were calculated and presented in **Table 7**.

The specificity of the HPLC method was determined by the complete separation of MFX and SGP. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH and Heat degradation at 105°C. The method does not permit detection of degradation product for MFX and SGP.

Hence it can be concluded that the proposed HPLC method is simple economical sensitive and reproducible for the analysis of Metformin Hcl and Saxagliptin in pharmaceutical dosage form.

Table 2: Calibration data of the proposed method

Fexofenadine HCl		Montelukast Sodium	
Conc (mcg/ml)	Mean Area	Conc (mcg/ml)	Mean Area
12.5	2511450	0.125	821977
25.0	5117952	0.25	1704973
37.5	7677954	0.375	2560352
50.0	10161139	0.5	3342924
62.5	12667974	0.625	4183569
75.0	15055516	0.75	5008839

Table 3: Accuracy data (Triplicate values at 50,100 &150 percent levels)

	Amount taken (µg)	Amount found (µg)	Percent Recovery	Percentage of mean recovery
Metformin	50	50.30	100.61	100.61
	100	99.75	99.75	99.75
	150	149.22	99.48	99.48
Saxagliptin	50	50.19	100.37	100.37
	100	99.94	99.94	99.94
	150	149.69	99.79	99.79

*Each value is a mean of three readings

Table 4: Robustness Study

Drug name	Variations	Chromatographic parameters				
		Retention time	Area	Height	Theoretical plates	Asymmetry
Metformin	Buffer change ± 2.5%					
	91.65% v/v	2.00	10695948	1329058	3392	1.23
	94.0%v/v	1.677	10198692	1256731	3329	1.41
	96.35% v/v	1.413	10165789	1341202	3256	1.41
	Change in flow rate at ±0.1ml/min					
	1.flow rate at 0.90ml/min	1.851	11354681	1296739	3146	1.42
	2.flow rate at 1.0ml/min	1.677	10198692	1256731	3329	1.41
	3.flow rate at 1.10ml/min	1.529	9251842	1187628	3395	1.45
Saxagliptin	Buffer change ± 2.5%					
	96.35% v/v	4.789	3468327	299921	3413	1.48
	94.0%v/v	4.112	3398519	269096	3741	1.50
	91.65% v/v	3.330	3423289	278466	3760	1.46
	Change in flow rate at ±0.05ml/min					
	1.flow rate at 0.95ml/min	4.552	3888918	269163	3620	1.52
	2.flow rate at 1.0ml/min	4.112	3398519	269096	3741	1.50
	3.flow rate at 1.05ml/min	3.729	3195243	255894	3366	1.62

Table 5: Precision Study

	Metformin		Saxagliptin	
S.No.	RT	Area	RT	Area
1	2.471	2495186	7.172	1702069
2	2.462	2503650	7.14	1715593
3	2.462	2503654	7.14	1715591
4	2.46	2500933	7.095	1714933
5	2.491	2512543	7.1	1720894
6	2.445	2499990	7.002	1714041
avg	2.465167	2502659	7.108167	1713854
stdev	0.015198	5755.24	0.059351	6256.01
%RSD	**0.62**	**0.23**	**0.83**	**0.37**

Table 6: Method Precision study

	Metformin		Saxagliptin	
S.No.	**RT**	**Area**	**RT**	**Area**
1	1.678	10137662	4.116	3375005
2	1.677	10190195	4.114	3366450
3	1.677	10198692	4.112	3398519
4	1.675	10143276	4.113	3367459
5	1.676	10165345	4.112	3357621
6	1.677	10176932	4.112	3362165
avg	1.676667	10168684	4.113167	3371203
stdev	0.001033	24699.44	0.001602	14583.44
%RSD	**0.06**	**0.24**	**0.04**	**0.43**

Table 7: Assay Results

Drug	Amount present/tablet	% of Assay
Metformin	497.62 mg	99.52
Saxagliptin	4.983 mg	99.67

Figure 2: Linearity of Metformin

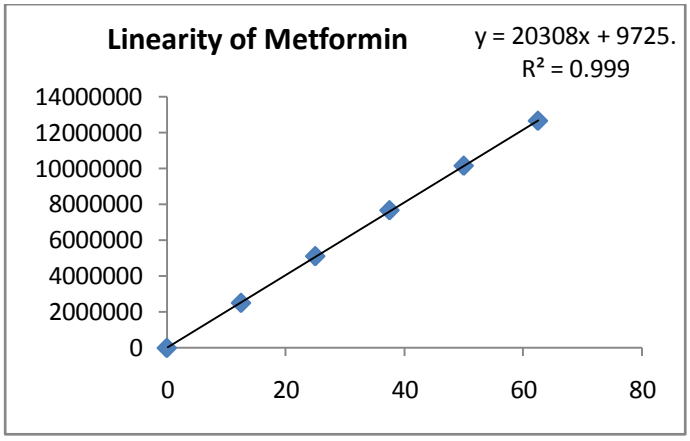
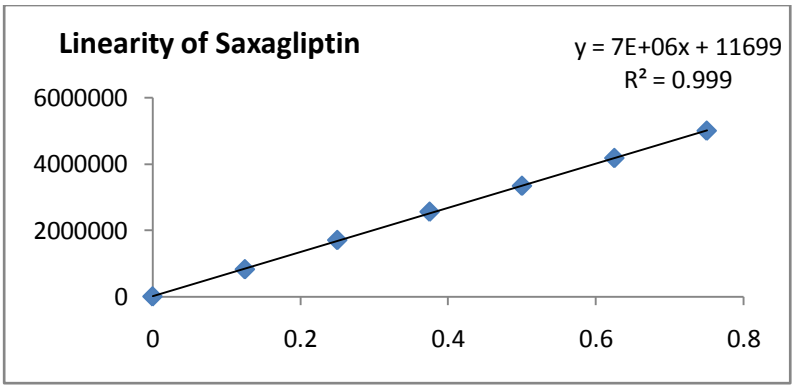


Figure 3: Linearity of Saxagliptin



ACKNOWLEDGEMENT

The authors are thankful to M/s BIO-LEO ANALYTICAL LABS INDIA PVT.LTD, HYDERABAD for providing a gift samples and laboratory facilities, the authors are also thankful to Department of Quality Assurance, SND College of Pharmacy, Pune University, Nasik, India for encouragement.

REFERENCES

1. Indian Pharmacopoeia. The Indian Pharmacopoeia Commision. Vol.II, Ghaziabad 2010; 1657-1658.
2. Augeri,D; Robl,J; Betebenner,D; Magnin,D; Khanna,A; Robertson,J. J.Med.Chem.2005,48, 5025-5037.
3. Mohammad Abdul-AzimMohammad, Ehab Farouk Elkadyb and Marwa Ahmed Fouadb, Development and validation of reverse phase column liquid chromatographic method for simultaneous determination of two novel gliptins in their binary mixtures with Metformin , EUROPEAN JOURNAL OF CHEMISTRY, 2012, 3(3) : 152-155.
4. Srikanth Inturi, Ravikanth Inturi, Israel kumar Tagaram, Sadasiva rao Galaba Novel HPTLC-denstometric method for the determination of Saxaglitin in bulk drug and tablet formulation, IJSPER, 2011, 1(1) : 27-35.
5. Srikanth Inturi,Ravikanth Intluri and Israel kumar tagaram, Validated novel LC Determination of Saxagliptin in pure Bulk and pharmaceutical dosage forms, IJPRD, 2011, 3(8): 45 : 52.
6. M.Sarat, P.Murali Krishna and C Rambabu, RP-HPLC method for estimation of Saxagliptin and pioglitazone in tablets, IRJP, 2012, 3(5): 399-402.
7. R.Kalaichelvi and E Jayendran, Spectroscopic method for estimation of Saxagliptin in pure and from tablet formulation, IJPPS, 2011, 3(3): 179-180.

***Corresponding Author:**
PATIL PRAFULLA PRAKASH
Vidya Vihar Colony, Plot No.43,
Near Kalyan Maruthi, Amalner Dist,
Jalgoan(MH).India.
Email: patilprafulla2 @gmail.com