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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SAXAGLIPTIN AND METFORMIN IN TABLET DOSAGE FORM

Asiya Begum*****, Shilpa K, A. Ajitha, V. Uma Maheshwar Rao

Department of pharmaceutical analysis and quality assurance, CMR college of Pharmacy, Kandlakoya(v), Medchal, Hyderabad.

***Corresponding author e-mail:** asiyabegum889@gmail.com

ABSTRACT

A sensitive selective and precise stability indicating-high performance liquid chromatographic (HPLC) method was developed for Saxagliptin and Metformin in Tablet dosage form. An isocratic separation was carried out using Zorbax C18 (250 x 4.6 mm, 5 µm) column and Potassium dihydrogen Phosphate: Methanol (60:40 v/v) as mobile phase.With quantification carried out at a wavelength of 248nm. The stability studies under stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation were also carried out for Saxagliptin and Metformin.The Retention time of Saxagliptin and Metformin were observed to be 3.241and 2.191 minutes, respectively with theoretical plate count and asymmetry as per the ICH limits. The % assay of Saxagliptin and Metformin were 99.640% and 99.021%. The flow rate was found to be 1ml/min .The linear regression analysis data for the calibration plots showed a good linear relationship for Saxagliptin and Metformin over a concentration range of 50-1500 µg/ml with correlation co-efficient of 0.999 for Saxagliptin and 0.999 for Metformin. The limit of detection and Quantitation were found to be 2.857,2.918& 9.52,9.72µg/ml, respectively.the method was validated as per ICH guidelines and it was found to be acccurate, precise and selective stability-indicating high performance liquid chromatographic (HPLC) for the determination of Saxagliptin and Metformin in tablet dosage form.

Keywords: Saxagliptin, Metformin, HPLC, Stability

INTRODUCTION

Metformin is *N*,*N*-Dimethylimidodicarbonimidic diamide, reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Metformin may act via 3 mechanisms: Reduction of hepatic glucose production by inhibiting gluconeogenesis and glycogenolysis in muscle; modestly increasing insulin sensitivity, improving peripheral glucose uptake and utilisation; delaying intestinal glucose absorption.

Saxagliptin is (1*S*,3*S*,5*S*)-2-[(2*S*)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile, a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. DPP-4 is an enzyme that breaks down incretion hormones glucagon-like peptide 1 (GLP-1). As a DPP-4 inhibitor, Saxagliptin slows down the breakdown of incretion hormones, increasing the level of these hormones in the body.

A Saxagliptin/Metformin extended-release tablet is a dipeptidyl peptidase-4 (DPP-4) inhibitor and biguanide combination. It works by increasing the amount of insulin released by your body. It decreases the amount of sugar that the liver produces and the intestines absorb. It also helps to make your body more sensitive to the insulin that you naturally produce.

The US Food and Drug Administration (FDA) November 8, 2010 , has approved the first and only once-daily combination tablet featuring Saxagliptin and extended-release (XR) Metformin HCl to improve glycemic control in adults with type 2 diabetes mellitus.

The literature survey reveals that several, HPLC, fluorescence,uv-vissible methods [11-17] , have been reported for the analysis of Saxagliptin and

Metformin a single drug or in combination in pharmaceutical dosage form. This paper describes simple, precise, accurate and sensitive- HPLC method development and validation as well as stability studies (hydrolysis, oxidation, photo-degradation and thermal degradation) as per international conference on harmonization guidelines.
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MATERIALS AND METHODS

Instrumentation: The separation was carried out on HPLCsystem with Waters 2695 alliance with binary HPLC pump,Waters 2998 PDA detector, Waters Empower2 software and c18 Zorbax column (250mmx4.6mm, particle size5µm).

Reagents and Chemicals: Saxagliptin and Metformin pure drug samples were provided by Rainbow Pharma Training Lab Hyderabad. Potassium dihydrogen phosphate and Methanol were of HPLC grade . Fixed dose combination Tablet (Brand name: Kombiglyze XR) containing 5mg of Saxagliptin and 500mg of Metformin were procured from local pharmacy, Hyderabad, India.

Chromatographic Conditions: The mobile phase consisting of Potassium dihydrogen phosphate and Methanol(HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 248nm and the run time was 8min. The volume of injection loop was 3µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of standard solution: Accurately weigh 500mg of Metformin and 5mg of Saxagliptin into a 50ml of volumetric flask and dissolve the sample using water and sonicate it for 15min then finally make up the volume to 50ml.Now pipette out 1ml of this solution into 25ml of volumetric flask and make up the volume upto mark using mobile phase as shown in figure 3.

Preparation of sample solution: Accurately weighed 2 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 1175.35mg of sample into 50ml of volumetric flask added with methanol and water and sonicated for 30mins and make up the volume with water and filtered through the0.45µm millipore filter paper Transfer above solution 5ml into 25ml volumetric flask and make up the volume with mobile phase chromatogram is shown in figure 4.

Development and Validation of HPLC method: Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Saxagliptin and Metformin in tablet dosage forms. The experiment was carried out according to the official specifications of ICH- 1996, Global Quality Guidelines- 2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness.

System suitability: A standard solution was prepared using Saxagliptin and Metformin working standard as per the test method and was injected six times into the HPLC system. The parameters namely USP plate count, peak asymmetry factor and resolution for the standard solutions were calculated.

Selectivity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Saxagliptin and Metformin from impurities.

Linearity: Linearity of the method was determined by constructing calibration curves. Standard solutions of Saxagliptin and Metformin at different concentrations level level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of Saxagliptin and Metformin to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy: Accuracy is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed.

Precision: Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are

recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2%.

Robustness: The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 ml/min, amount of diluents (10% to 15%) the temperature of the column (28°C to 32°C) and pH of the mobile phase.

Limit of detection and limit of quantitation: Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Saxagliptin and Metformin was made from standard solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

Stress Degradation Studies of Bulk Drug: Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared. The blank subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation were carried out in solid state.

Alkaline hydrolysis: 1 ml working standard solution o (1000 µg/ml) was mixed with 1 ml of 0.1 N methanolic NaOH and 8 ml of Methanol. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted with mobile phase to 10 ml (10 µg/ml) and then was injected into the system.

Acidic hydrolysis: 1 ml working standard solution (1000 µg/ml) was mixed with 1 ml of 0.1 N methanolic HCl and 8 ml of methanol. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted with mobile phase to 10 ml (10 µg/ml) and then was injected.

Oxidation: 1 ml working standard solution (1000 µg/ml) was mixed with 1 ml of 30 % solution of H2O2 and 8 ml of methanol. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted to 10 ml with mobile phase (10 µg/ml) and then was injected.

Degradation under dry heat: Dry heat studies were performed by keeping drug sample in oven (1000 C) for a period of 1 hour. Sample was withdrawn after 1 hour and processed as per standard solution preparation procedure mentioned under Preparation of Standard stock solution to get 10µg/ml as final concentration and was injected.

Photo-degradation studies: Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr.Sample was withdrawn after exposure and processed as per standard solution preparation procedure mentioned under Preparation of Standard stock solution to get 10 µg/ml as final concentration and was injected.

RESULTS AND DISCUSSION

Method Development System suitability: The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. Table1 summarized the test results of system suitability study.

Linearity: The linearity of the developed method was determined in triplicate at different concentrations ranging from50%, 75%, 100%, 125%, and 150% were used for Saxagliptin and Metformin. The Regression coefficient (R2) was 0.99 for both drugs, showing good linearity. The results confirmed the linearity of the standard curves over the range studied and the excellent reproducibility

Accuracy Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained in added recoveries of standard drugs were found to be accurate as shown in table 2(a & b).

Precision: Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2% shown in table 4.

Robustness As part of the robustness, deliberate changes in the flow rate and detection wavelength were made to evaluate the impact on the method and retention times were significantly changed.

Limit of detection and limit of quantification: Limit of detection and limit of quantification represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Saxagliptin and Metformin was made from standard solution. The samples were injected in the system and measured signal from the samples was compared with those of blank samples. LOD and LOQ was calculated from linear curve using formulae LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope (Where σ = the standard deviation of the response and S = Slope of calibration curve) shown in table 6.

FORCED DEGREDATION STUDIES: A study was conducted to demonstrate the effective separation of degradents. Seprate portions of drug products and placebo was exposed to induced degradation under stressed conditions like acid(2N HCL),base(2N NaOH),Peroxide(5%H_2O_2),UV Light(200wts.hr/cm^2 for 55hrs,heat(105^0 for 48 hrs).Stressed samples into HPLC system using photo diode array(PDA) detector for above test method conditions. The chromatograms of stressed samples were evaluated.

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Saxagliptin and Metformin using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Hence, this method can easily and conveniently adopt for routine quality control analysis of Saxagliptin and Metformin in its pharmaceutical dosage forms.

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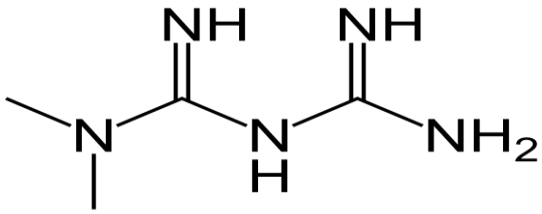

Fig. 1: Structure of Metformin

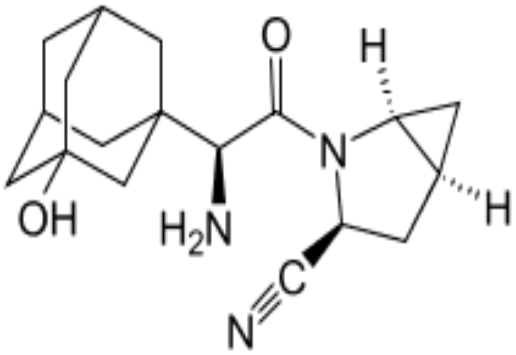

Fig. 2: Structure of Saxagliptin

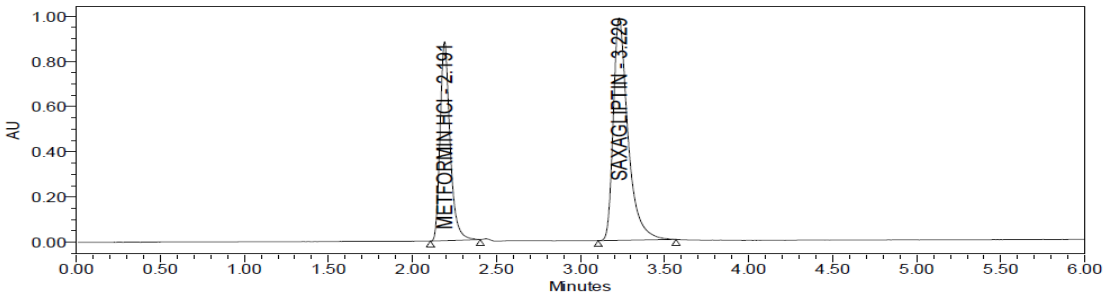

Fig. 3: Chromatogram of standard preparation

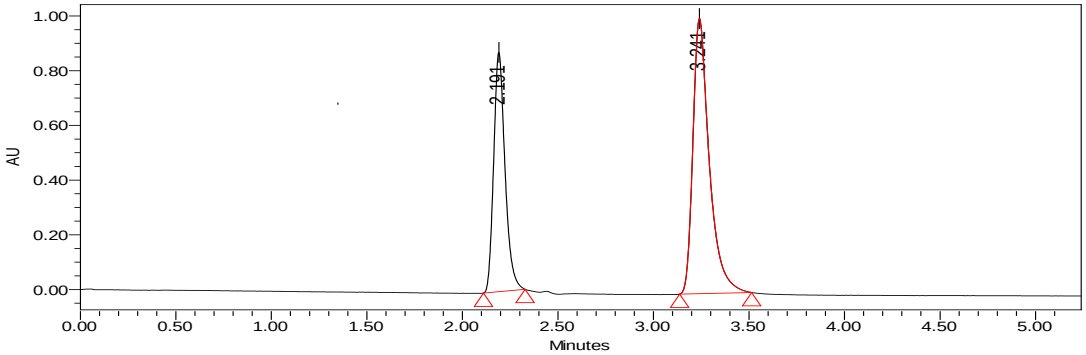

Fig. 4: Chromatogram of sample preparation

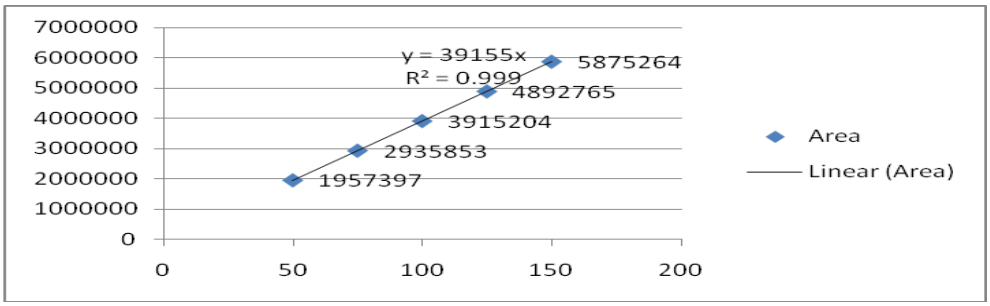

Fig 5: Linearity curve of Metformin

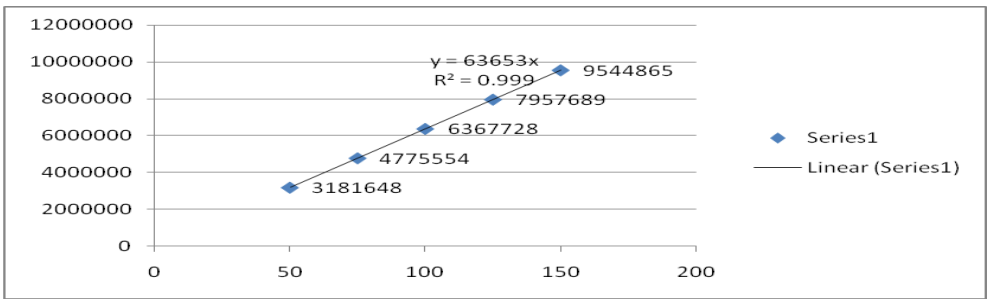

Fig 6: Linearity curve of saxagliptin

Table 1: Chromatogram data of standard drugs.

SAMPLE NAME	PEAK NAME	RT	AREA	MEAN	USP PLATE COUNT	USP RESSOLUTION	USP TAILING
STD1	SAXAGLPTIN	3.229	5969804	5969804	7184	7.74	1.56
STD1	METFORMIN	2.191	3636417	3636417	6751		1.31

Table 2: System suitability parameters

Parameter	Saxagliptin	Metformin
Correlation Coefficient	0.999	0.999
Regression Equation	Y=63653X+0	Y=39155X+0
LOD	2.85	2.91
LOQ	9.52	9.72
Theoretical plates	7301	6560
Tailing	1.53	1.24

Table 3(a): Recoveries of Metformin drugs

ACCURACY OF METFORMIN						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	588.00	1957634	991.548	990.84	99.929	99.756
50%	588.00	1954421	991.548	989.22	99.765	
50%	588.00	1950967	991.548	987.47	99.589	
50%	588.00	195386	991.548	988.69	99.712	
50%	588.00	1957086	991.548	990.57	99.901	
50%	588.00	1952011	991.548	988.00	99.642	
100%	1175.35	3914250	1982.000	1981.17	99.958	99.965
100%	1175.35	3916323	1982.000	1982.22	100.011	
100%	1175.35	3912988	1982.000	1980.53	99.926	
150%	1764.00	5871125	2974.644	2971.63	99.899	99.983
150%	1764.00	5874460	2974.644	2973.32	99.955	
150%	1764.00	5876283		2974.24	99.986	
150%	1764.00	5879749	2974.644	2975.99	100.045	
150%	1764.00	5877203	2974.644	2974.71	100.002	
150%	1764.00	5877686	2974.644	2974.95	100.010	

Table 3(b): Recoveries of Saxagliptin drugs

		ACCURACY OF METHYLCOBALAMIN				
Spiked level	**Sample weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	588.00	3181180	9.976	9.96	99.893	99.985
50%	588.00	3184646	9.976	9.98	100.002	
50%	588.00	3186776	9.976	9.98	100.069	
50%	588.00	3185510	9.976	9.98	100.029	
50%	588.00	3185742	9.976	9.98	100.036	
50%	588.00	3180897	9.976	9.96	99.884	
100%	1175.35	6363757.00	19.940	19.93	99.970	99.947
100%	1175.35	6361200.00	19.940	19.93	99.930	
100%	1175.35	6361955.00	19.940	19.93	99.942	
150%	1764.0	9540806	29.927	29.89	99.864	99.872
150%	1764.0	9540078	29.927	29.89	99.857	
150%	1764.0	9542123	29.927	29.89	99.878	
150%	1764.0	9540626	29.927	29.89	99.863	
150%	1764.0	9540930	29.927	29.89	99.866	
150%	1764.0	9544492	29.927	29.90	99.903	

Table-4: Precision studies of Saxagliptin and Metformin

S.No	**Sample Weight**	**Sample Area-1**	**Sample Area-2**	**Assay**	**% Assay**
1	1176.00	3912371	6368994	98.956	99.697
2	1176.00	3916122	6366580	99.051	99.659
3	1176.00	3912689	6364965	98.964	99.634
4	1176.00	3912237	6361053	98.953	99.573
5	1176.00	3916865	6362443	99.070	99.595
6	1279.36	3919368	6367828	99.133	99.679
Average				99.021	99.640
STD				0.075	0.048
% RSD				0.075	0.049

Table 5(a): Robustness of Saxagliptin

	Sample Name	**Peak Name**	**RT**	**AREA**	**USP TAILING**	**USP PLATE COUNT**
1	TEMP1	SAXAGLIPTIN	3.202	6174662	1.53	7018
2	TEMP2	SAXAGLIPTIN	3.200	6210297	1.54	7210
3	FLOW1	SAXAGLIPTIN	4.003	8616889	1.55	7696
4	FLOW2	SAXAGLIPTIN	2.666	5177656	1.54	6599

Table 5(b): Robustness of Metformin

	Sample Name	Peak Name	RT	AREA	USP TAILING	USP PLATE COUNT
1	TEMP1	METFORMIN	2.200	4101740	1.30	6869
2	TEMP2	METFORMIN	2.201	4125529	1.31	7031
3	FLOW1	METFORMIN	2.756	5793369	1.29	7224
4	FLOW2	METFORMIN	1.829	3427648	1.32	6439

Table 6: LOD and LOQ of Saxagliptin and Metformin

DRUG	LOD**(µg/ml)**	LOQ**(µg/ml)**
Saxagliptin	2.85	9.52
Metformin	2.91	9.72

Table-6: Forced Degradation of Saxagliptin

S.NO	Sample Weight	Sample Area 1	%Assay	%Degradation
Acid	1176	5021256	79	-21
Base	1176	5236073	82	-18
Peroxide	1176	5115763	80	-20
Light	1176	6032525	94	-6
Heat	1176	5936685	93	-7
Average Assay		4557050		
STD		2273141		

Table-7:Forced Degradation of Metformin

S.NO	Sample Weight	Sample Area 1	%Assay	%Degradation
Acid	1176	2531753	64	-35
Base	1176	2848071	72	-27
Peroxide	1176	2727720	69	-30
Light	1176	3616563	91	-8
Heat	1176	3524063	89	-10
Average Assay		2541361.7		
STD		1319785.7		

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