INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS DETERMINATION OF METFORMIN AND SAXAGLIPTIN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

Aim: An RP-HPLC method has been developed for the simultaneous determination of metformin and saxagliptin in marketed formulation. Methods: This method is based on RP-HPLC separation of the two drugs on the Inspire C18 column (250 mm × 4.6 mm, 5.0µ); and mobile phase containing Buffer: Methanol in a ratio of 55:45 v/v at a flow rate of 1 ml/min, using UV detection at 208 nm. This method has been applied to formulation without any interference of excipients of formulation. Results: The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration ranges of 60–100 µg/ml for metformin and 0.6–1.0 µg/ml for saxagliptin respectively. The method was validated as per the ICH guidelines. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.17 µg/ml and 0.064 µg/ml for metformin and 0.08 µg/ml and 0.02 µg/ml for saxagliptin, respectively. Result of assay and recovery study was statistically evaluated for its accuracy and precision. Conclusion: According to the validation results, the proposed method was found to be specific, accurate, precise and economic for the determination of metformin and saxagliptin in pharmaceutical dosage forms.

Keywords: Metformin and Saxagliptin, RP- HPLC, Validation.

INTRODUCTION

Metformin hydrochloride- a biguanide improves glucose tolerance in patients with type 2 diabetes and it is mostly recommended drug compared to other antihyperglycemic drugs. Its chemical name is N, N –Dimethylimidodicarbonimidicdiamide. recommended dosage is 500mg orally twice a day or 500 to 2000mg once a day. Lactic acidosis is a rare, but serious, complication that can occur due to metformin accumulation. Hemodialysis is recommended to correct the acidosis and remove the accumulated Metformin.

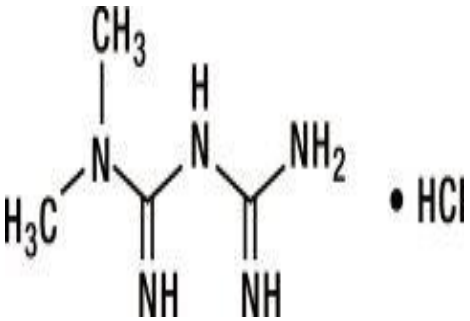

Fig. 1: Chemical structure of Metformin

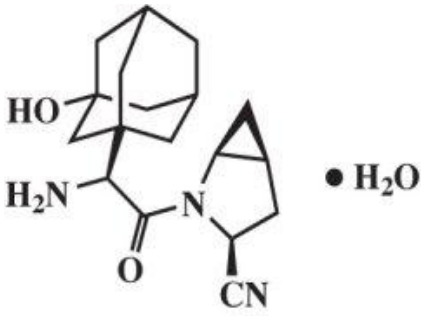

Fig. 2: structure of Saxagliptin

Saxagliptin is a competitive DPP4 inhibitor that slows the inactivation of the incretin hormones, thereby increasing their bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose-dependent manner in patients with type 2 diabetes mellitus. Recommended dosage is 2.5mg or 5mg orally once daily.[1, 2]

Literature survey revealed that number of methods has been reported for the individual analysis of Metformin and Saxagliptin by UV Spectrophotometric, RP-HPLC and in combination with other drugs. The suggested method was validated as per ICH guidelines. The validation parameters studied was accuracy, precision, linearity, LOD and LOQ, Robustness .However, none of the previous method reports was sensitive, selective and economic enough for the estimation of Metformin and Saxagliptin.The aim of present work was to develop the comparatively simple, economical, accurate RP-HPLC method for the estimation of Metformin and Saxagliptin in pharmaceutical dosage forms.[3-6]

MATERIALS AND METHODS

Materials & Reagents

Working standards of pharmaceutical grade was received as a gift sample from RV Chem Pharmaceuticals Limited, Hyderabad. Combinations tablet KOMBIGLYZE XR containing 500 mg Metrformin and 5 mg Saxagliptin was used for study. All the chemicals used were of HPLC grade.

Instrumentation

The instrument used was Waters HPLC system equipped with a rheodyne injecting facility programmed at 20µl capacity per injection was used. The detector consisted of UV–Visible detector operated at wavelength 208 nm. Method was performed by using empower 2.0 software. The column used was Inspire C-18 (250mm x 4.6mm, 5µm). Analytical balance used for weighing was Afcoset digital balance.

Preparation of Mobile Phase

Mobile phase was prepared by mixing buffer: methanol in a ratio of 55:45 v/v. The mobile phase was ultrasonicated for 10 minutes and then it was filtered through 0.45 µ membrane filter.

Preparation of Standard Stock Solution

An accurately weighed quantity of metformin (10.0mg) and saxagliptin (10.0mg) was transferred to 10 & 100 ml volumetric flask dissolved and diluted up to mark with mobile phase and sonicated and volume made to 10ml with solvent. From this solution, 1.0 ml of saxagliptin was transferred to 10.0 ml volumetric flask and diluted to the mark with mobile phase. Further pipette 0.8ml of metformin and saxagliptin of above stock solution into 10 ml volumetric flask and diluted upto mark. The solution was found to be stable during the period of study.

Analysis of Formulation

Accurately weighed 10 tablets, each containing 500 mg metformin and 5 mg saxagliptin was crushed to fine powder and accurately weighed quantity equivalent to about 10.0 mg metformin and 5 mg saxagliptin was transferred to 10ml and 100ml volumetric flasks, dissolved and diluted up to mark with mobile phase. From this solution, 1.0 ml saxagliptin was transferred to 10.0 ml volumetric flask and diluted to the mark with mobile phase. Further, pipette 0.8ml of metformin and saxagliptin into a 10ml volumetric flask and made upto mark. The solution was filtered through 0.45 µ membrane filter. Equal volumes of standard and sample solution (20µL) were injected into the column and chromatographed using optimized chromatographic conditions.

METHOD VALIDATION PARAMETERS

The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity

The standard solution was prepared by dilution of stock solution containing 1000µg/ml Metformin and 100µg/ml Saxagliptin.Linearity test solutions were prepared at five different concentration levels ranging from 60 to 100 µg/ml Metformin and 0.6 to 1.0 µg/ml Saxagliptin concentration.

Standard solution was injected for six times. The peak area was plotted against the corresponding concentration to obtain the calibration graphs and calculate the correlation coefficient.

Precision

The precision study of metformin and saxagliptin was carried out by estimating the correspondence responses six times on the same day and intermediate precision study was carried out on different day by estimating the correspondence responses six times. The standard solutions were injected for six times and their peak areas were recorded. The repeatability of sample application and measurement of peak area for active compound were expressed in terms of relative standard deviation (%R.S.D.)

Limit of Detection & Limit of Quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated for the proposed method which was based on the signal to noise ratios.

LOD is calculated from the formula:

S/N=3

LOQ is calculated from the formula:

S/N=10

Robustness

To evaluate HPLC method robustness, a few parameters were deliberately varied. The parameters included variation of flow rate (±0.2ml), mobile phase composition (±10%).

RESULTS AND DISCUSSION

The proposed method describes a new RP-HPLC method for the determination of metformin and saxagliptin in pharmaceutical dosage form employing Waters HPLC system, UV/VIS detector, Inspire C-18 (4.6 × 250mm, 5µm) column and mobile phase

comprising of Buffer: Methanol (55:45 v/v). This method was found to be sensitive, accurate and economical. To optimize the RP-HPLC parameters, several columns & mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Buffer: Methanol (55:45 v/v) at 1.0 ml/min flow rate. The optimum wavelength for detection was set at 208 nm at which better detector responses of drugs were obtained. The retention time was found to be 2.37 for metformin and 3.94 for saxagliptin. The obtained chromatogram is shown in **Figure 3**.

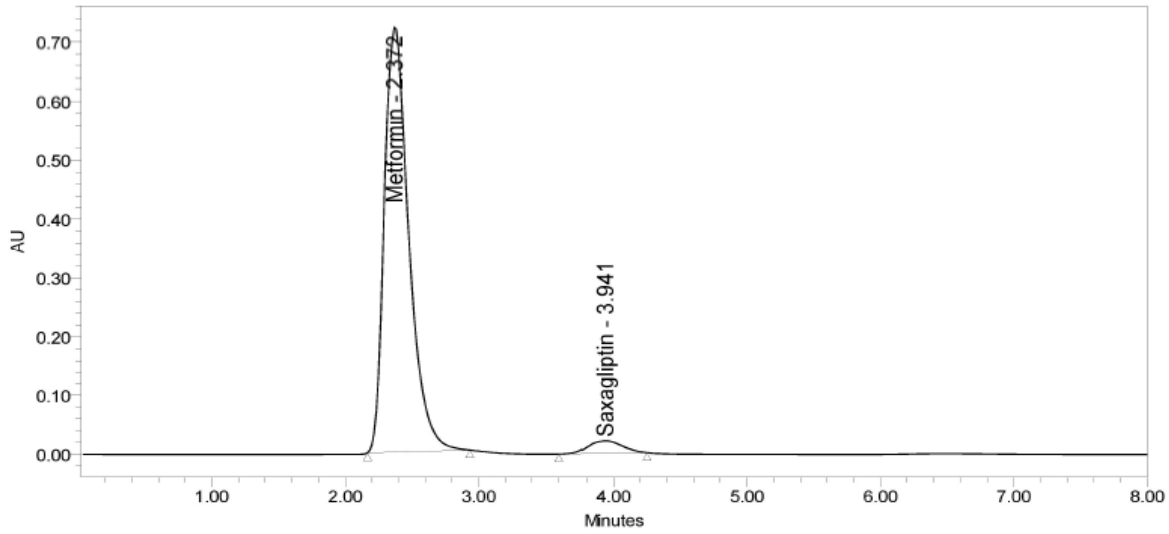

Fig. 3: Typical Chromatogram of Metformin & Saxagliptin (Rt 2.372&3.941)

System Suitability Testing

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in **Table No. 1**.

Linearity

Calibration curve was obtained in a concentration range from 60-100µg/ml for Metformin. The response of the drug was found to be linear in the investigation. The linear regression equation was Y= 200545x - 06 with correlation coefficient 0.999. The standard calibration curve of Mean Peak Area vs. Concentration is depicted in **Figure 4**.

Calibration curve was obtained in a concentration range from 0.6-1.0µg/ml for Saxagliptin. The response of the drug was found to be linear in the investigation. The linear regression equation was Y= 99238x – 91298 with correlation coefficient 0.999.The standard calibration curve of Mean Peak Area vs. Concentration is depicted in **Figure 5**.

Precision

Different concentrations of working standard solution of Metformin and Saxagliptin were prepared. All the solutions were analyzed thrice, in order to record any variation in the result. The results obtained for precision are shown in the **Table No. 2.** For intermediate precision study, six times solutions were injected. The results obtained for intermediate precision are shown in the **Table No. 2**.

Limit of Detection & Limit of Quantitation

The sensitivity of method is described in terms of Limit of Detection and Limit of Quantitation. LOD and LOQ values for Metformin and Saxagliptin were found to be 3.02, 2.95 and 9.95, 9.98 respectively. The results of LOD and LOQ studies are shown in **Table No. 3**.

Accuracy

The RP- HPLC area responses for accuracy determination are depicted in **Table No. 04.** The results show that best recoveries (101.07%, 101.25%) of the spiked drug were obtained at each added concentration; it indicates accuracy of the method.

Table No: 1 System Suitability Parameters

S.NO	PARAMETER	METFORMIN	SAXAGLIPTIN
1.	RESOLUTION	3.95	
2.	TAILING	1.41	1.04
3.	NO.OF THEORICAL PLATES	2850	2108
4.	RETENTION TIME	2.372	3.941

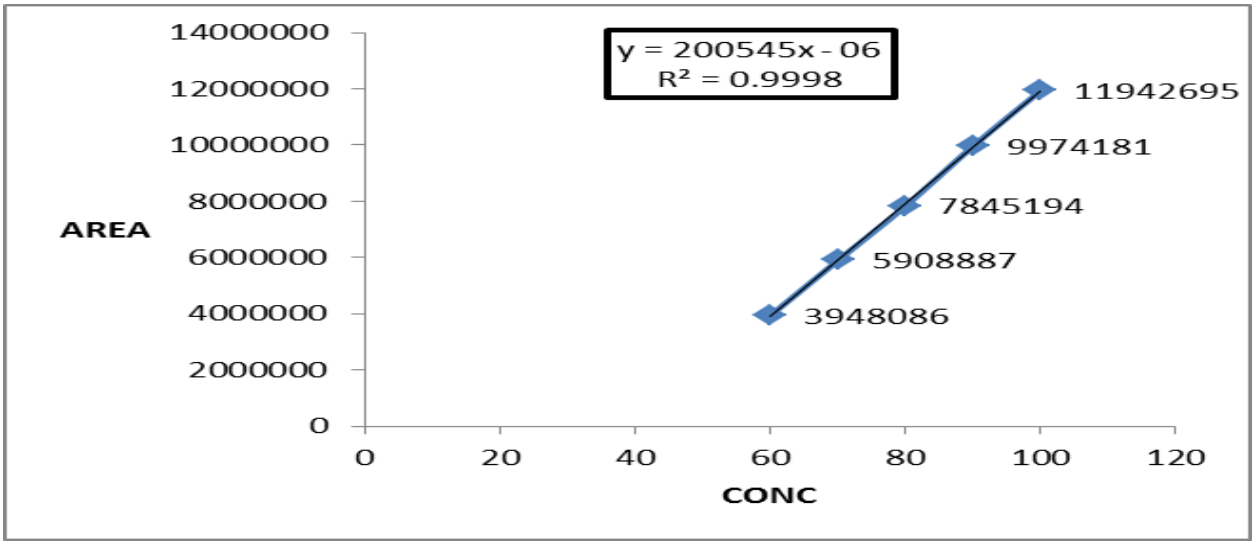

Fig. 4: Linearity plot of metformin

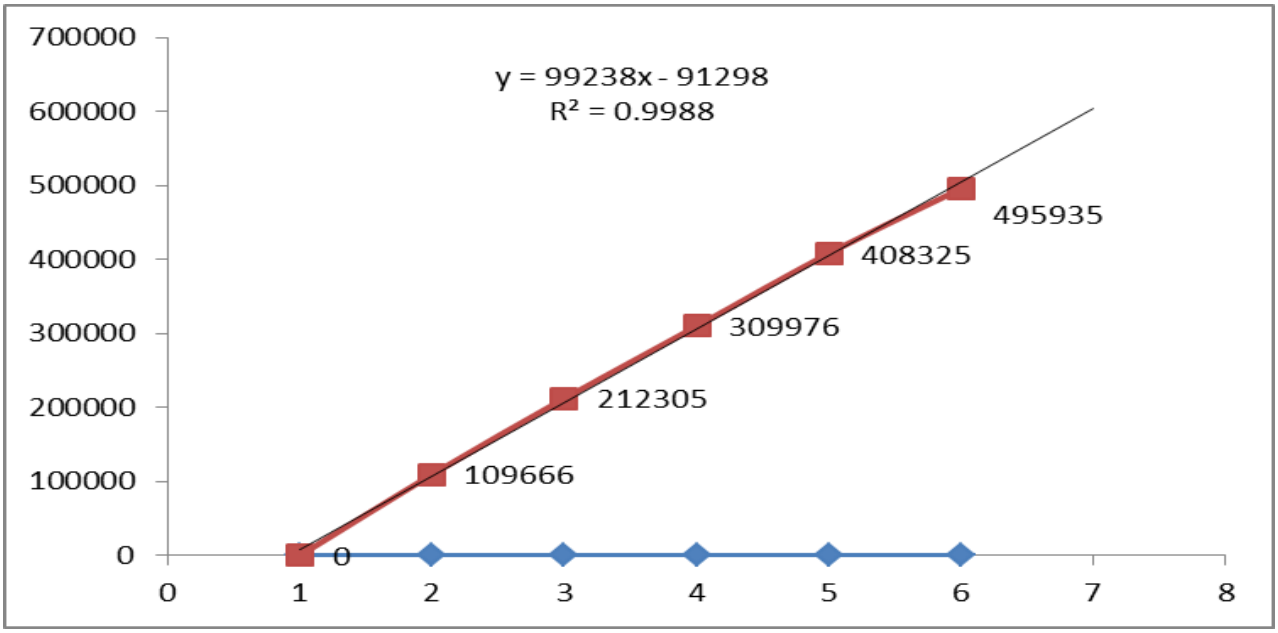

Fig. 5: Linearity plot of saxagliptin

Table 2: Results for Precision and Intermediate Precision

Injection	Precision		Intermediate precision	
	Area of metformin	Area of saxagliptin	Area of metformin	Area of saxagliptin
Injection-1	9313715	359525	7729031	288674
Injection-2	9303880	360111	7769889	282344
Injection-3	9269496	361664	7697780	293593
Injection-4	9286249	359130	7763422	289450
Injection-5	9293910	359935	7725057	293682
Injection-6	9303884	350265	7766843	293493
Average	9295189	358438.3	7742004	290206
S.D	15722.84	4096.471	29204.59	4451.703
%RSD	0.17	1.14	0.38	1.53

Table 3: Results for LOD & LOQ

	LOD			LOQ		
Drug name	**Base line(µV)**	**Signal obtained(µV)**	**S/N ratio**	**Base line(µV**	**Signal obtained(µV)**	**S/N ratio**
Metformin	42	127	3.02	42	419	9.95
Saxagliptin	42	124	2.95	42	417	9.98

Table 4: Results for Accuracy

% concentration	Amount added(mg)	Amount found (mg)	%Recovery	Mean recovery	Amount added (mg)	Amount found(mg	%Recovery	Mean recovery
50%	5.0	5.05	101.06%		5.0	5.08	101.61%	
100%	10.0	10.15	101.51%		10.0	10.30	100.98%	
150%	15.0	15.10	100.64%	101.07%	14.9	15.17	101.16%	101.25%

Robustness

The result of robustness study of the developed assay method was established in **Table No. 05**. The result indicates during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Table 5: Result of Robustness Studies

Flow rate (ml/min)	Plate count of Metformin	Plate count of Saxagliptin	Tailing for Metformin	Tailing for Saxagliptin
0.8	2685	2134	1.78	1.52
1.0	2850	2108	1.41	1.04
1.2	2657	2664	1.81	1.60

Table 6: Result of Robustness Studies

Change in organic composition in mobile phase	Plate count of Metformin	Plate count of Saxagliptin	Tailing for Metformin	Tailing for Saxagliptin
10% less	2660	2947	1.70	1.01
*Actual	2850	2108	1.41	1.04
10% more	2750	2857	1.95	1.88

Table 7: Summary table

S.NO	PARAMETERS	LIMIT	OBSERVATION
1	System suitability (%RSD of tailing factor)	Tailing NMT 2.0 Theoretical plates NLT 2000	Met 1.41 2850 saxa 1.04 2108
2	Precision: A) System Precision B) Intermediate Precision	RSD NMT 2.0%	Met saxa 0.17 1.14 0.38 1.53
3	Linearity	Correlation coefficient NLT 0.999	Met 0.9999 Saxa 0.9999
4	Accuracy	%Recovery range98-102 %	Met 101.07 saxa 101.25
5	Robustness	RSD NMT 2%	Robust
6	LOD	S/N Ratio should be 3 for LOD solution	Met 3.02 saxa 2.95
7	LOQ	S:N ratio should be 10 for LOQ solution	Met 9.95 saxa 9.98

CONCLUSION

All these factors lead to the conclusion that the proposed method is simple, specific, accurate, precise and reproducible. Statistical analysis proves that the method is suitable for the analysis of Metformin and Saxagliptin.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Principal, KGRL college of Pharmacy, Bhimavaram, A.P for support during Research work.Thanks are also extended to Pharmatrain Laboratories ,Hyderabad for providing laboratory facilities and RV Chem Pharmaceuticals ltd, India for providing gift samples of the pure drugs for research work.

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