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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND SAXAGLIPTIN IN COMBINED DOSAGE FORM

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Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Metformin and Saxagliptin in combined-dosage form. A thermo hypersil BDS C8 (250*4.6*5µ) column with mobile phase containing water pH 3.0 adjusted with ortho phosphoric acid: methanol in the ratio of (70: 30, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 241 nm. The retention times of Metformin and Saxagliptin were 2.956min and 4.573 min, respectively. The correlation co-efficient for Metformin and Saxagliptin was found to be 1 and 0.999, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Metformin and Saxagliptin in formulations was found to be in the range of 100% and 99-100% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Metformin and Saxagliptin in combined dosage form.
Key**words** RP-HPLC, Metformin, Saxagliptin.

INTRODUCTION

Metformin is anti diabetic drug in biguanide class. Metformin (I, N, N-dimethyldiguanide) and used in the treatment of type 2 diabetes. Molecular formula for Metformin is found to be $C_4H_{11}N_5$ and molecular weight is 129.16 g mol^{-1}. Metformin decreases hepatic gluconeogenesis by interfering with respiratory oxidation in mitochondria. It suppresses gluconeogenesis from several substrates, including lactate, pyruvate, glycerol, and amino acids. In addition, Metformin increases intra-mitochondrial levels of calcium (Ca++), a modulator of mitochondrial respiration. It is a biguanide developed from galegine, a guanidine derivative found in Galega officinalis (French lilac). Chemically, it is a hydrophilic base which exists at physiological pH as the cationic species (>99.9%). Consequently, its passive diffusion through cell membranes should be very limited. Metformin is excreted unchanged in urine. The elimination half-life ($t_{½}$) of Metformin during multiple dosages in patients with good renal function is approximately 5 hours. Lactic acidosis is the feared adverse effect of the biguanide drugs but its incidence is very low in patients treated with Metformin. We suggest that the mean plasma concentrations of Metformin over a dosage interval be maintained below 2.5 mg/L in order to minimize the development of this adverse effect.

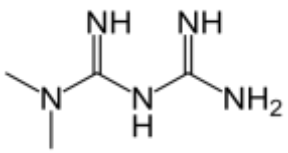
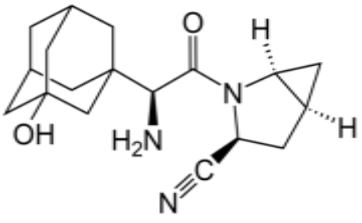


Fig- Metformin

Saxagliptin is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. IUPAC name for Saxagliptin is (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile. Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretion hormones. As a DPP-4 inhibitor, Saxagliptin slows down the breakdown of incretion hormones, increasing the level of these hormones in the body. Type 2 diabetes mellitus is a common chronic disease that causes significant morbidity and mortality worldwide. The primary goal of treatment is to target glycemic control by maintaining the glycosylated hemoglobin (HbA1c) level near 6% to 7% without predisposing patients to hypoglycemia. Currently available anti-diabetic agents work by different mechanisms to lower blood glucose levels. The usual adult dose is 2.5 to 5 mg once daily regardless of meals. A daily dose of 2.5 mg is recommended for patients with moderate to severe renal impairment or those who are taking potent CYP 3A4 inhibitors. In randomized clinical trials, Saxagliptin alone lowered HbA1c levels by about 0.5%; with better efficacy seen when combined with other agents.



Saxagliptin

MATERIAL AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and

thermo hypersil BDS column (250mmx4.6mm, particle size 5µm).

Chemicals and Reagents: Saxagliptin, Metformin was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of water (pH 3 adjusted with orthophosphoric acid) 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 70:30v/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 241nm and the run time was 8min. The volume of injection loop was 10µl 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:

Metformin: Accurately weighed quantity, 62.5 mg of Metformin was transferred into 50ml of volumetric flask and adds 30ml of water and sonicate for 15 min. make up the volume with water. Transferred above solution 20ml into 50ml volumetric flask and diluted to the mark with water.

Saxagliptin: Accurately weighed quantity, 5mg of Saxagliptin was transferred into 100ml of volumetric flask and adds 30ml of water and sonicate for 15mins make up the volume with water. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with water.

PREPARATION OF SAMPLE SOLUTION:

Accurately weighed 8 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 750mg of sample into 100ml of volumetric flask added 25ml of water and sonicated for 30mins and make up the volume with water and filtered through the 0.45µm filter paper

Table1: System Suitability Parameters

Parameters	Metformin	Saxagliptin
Correlation Coefficient	0.999	0.999
Regression Equation	y = 16616x – 26439	y = 19288x – 32595
LOD	3.127	0.2494
LOQ	10.423	0.8313
Theoretical plates	5631	7466
Tailing	1.260	1.197

Specificity: The specificity was established by preparing a Metformin and Saxagliptin standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

ACCURACY AND PRECISION:

The accuracy of the method was determined by recovery experiments. 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, added recoveries of standard drugs were found to be accurate (Table-3&4).

Transfer above solution 5ml into 50 ml volumetric flask and make up the volume with water.

Fig. 1: Standard chromatogram for Metformin and Saxagliptin

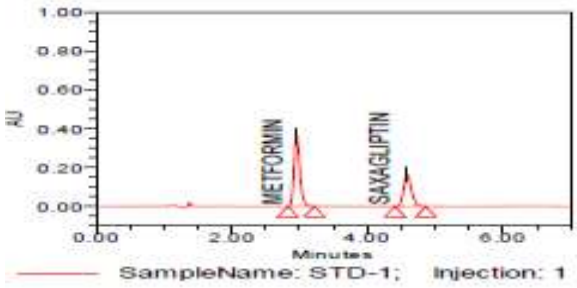
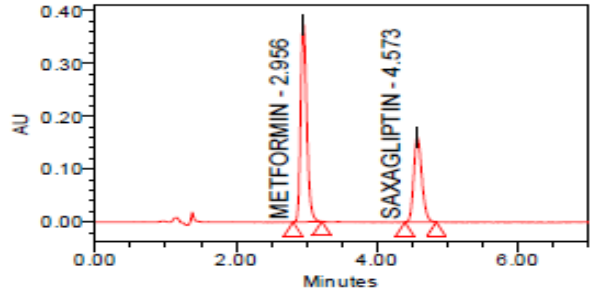


Fig. 2: Formulation chromatogram for Metformin and Saxagliptin



METHOD VALIDATION:

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method**.**

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

Table 2 : Precision Studies

S. No.	Sample weight	Area (Met)	Area (Saxa)	%Assay (Met)	Assay (Saxa)
1	708.0	2166490	1305724	99	100
2	708.0	2161828	1304116	99	100
3	708.0	2166514	1304919	99	100
4	708.0	2162047	1305421	99	100
5	708.0	2166035	1308586	99	99
6	708.0	2161459	1301781	99	98

Fig. 3: Accuracy Chromatograms-50% of Metformin and Saxagliptin

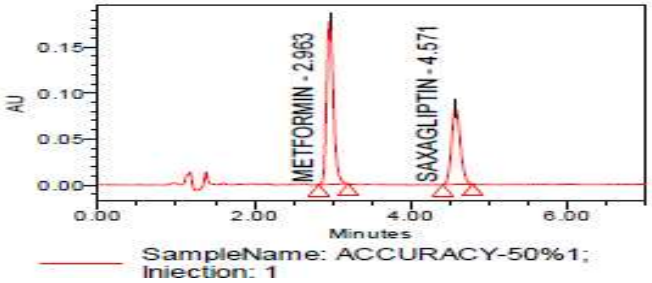


Fig. 4: Accuracy Chromatograms-100% of Metformin and Saxagliptin

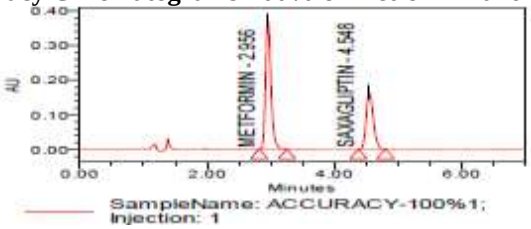


Fig. 5: Accuracy Chromatograms-150% of Metformin and Saxagliptin

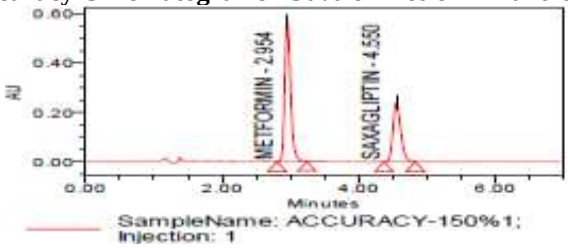


Table 3: Accuracy for Metformin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	354.00	1081355	495.000	494.78	100	100
50%	354.00	1085570	495.000	496.71	100	
50%	354.00	1083742	495.000	495.88	100	
0%	354.00	1082063	495.000	495.11	100	
50%	354.00	1086939	495.000	497.34	100	
50%	354.00	1086554	495.000	497.16	100	
100%	708.00	2165630	990.000	990.90	100	100
100%	708.00	2163398	990.000	989.88	100	
100%	708.00	2163941	990.000	990.13	100	
150%	1062.00	3253813	1485.000	1488.81	100	100
150%	1062.00	3255842	1485.000	1489.74	100	
150%	1062.00	3253180	1485.000	1488.52	100	
150%	1062.00	3257393	1485.000	1490.45	100	
150%	1062.00	3258137	1485.000	1490.79	100	
150%	1062.00	3259291	1485.000	1491.32	100	

Table 4: Accuracy for Saxagliptin

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	354.00	653284	5.000	4.99	100	100
50%	354.00	653451	5.000	4.99	100	
50%	354.00	653716	5.000	4.99	100	
50%	354.00	653784	5.000	4.99	100	
50%	354.00	653685	5.000	4.99	100	
50%	354.00	653064	5.000	4.98	100	
100%	708.00	1303566.00	10.000	9.95	99	100
100%	708.00	1307053.00	10.000	9.98	100	
100%	708.00	1307988.00	10.000	9.98	100	
150%	1062.00	1956188	15.000	14.93	100	100
150%	1062.00	1954348	15.000	14.92	99	
150%	1062.00	1950134	15.000	14.88	99	
150%	1062.00	1959903	15.000	14.96	100	
150%	1062.00	1955815	15.000	14.93	100	
150%	1062.00	1956874	15.000	14.94	100	

LINEARITY AND RANGE:

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x - 26439 (R^2=0.999) for Metformin and y = 19288x - 32595 (R^2=0.999) for Saxagliptin. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Metformin and Saxagliptin shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

Fig 6: Overlay chromatograms of Linearity for Metformin and Saxagliptin

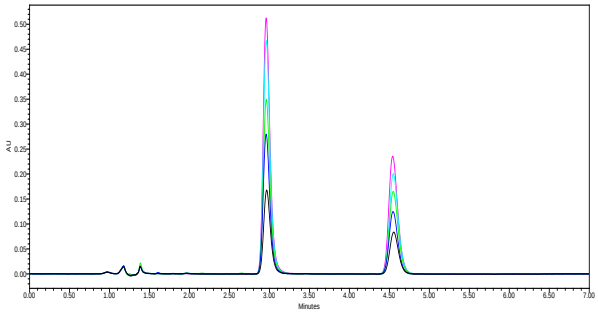


Fig. 7: Linearity Curve for Metformin

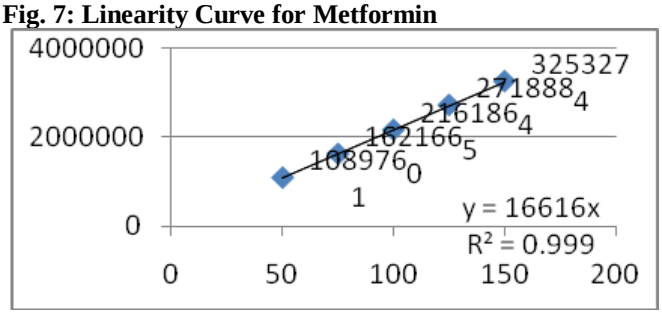
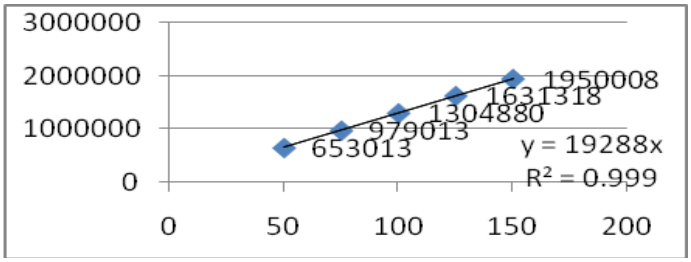


Fig. 8: Linearity Curve for Saxagliptin



ROBUSTNESS:

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust (Table-5&6).

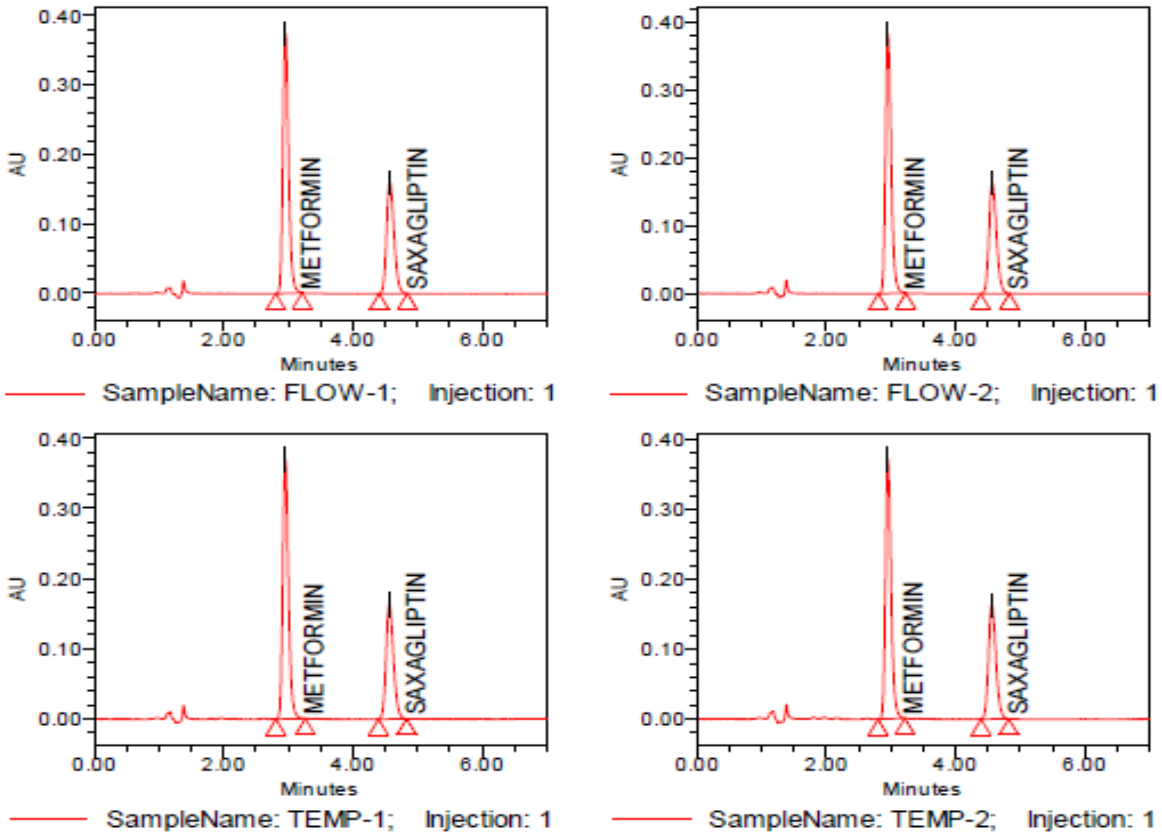


Table 5: Robustness for Metformin

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Metformin	3.156	2875625	1.258	5602
2	Flow2	1.2ml/min	Metformin	2.654	2330071	1.261	5571
3	Temp1	25°C	Metformin	3.254	2959438	1.269	5644
4	Temp2	35°C	Metformin	2.753	2251207	1.264	5633

Table 6: Robustness for Saxagliptin

S No	Sample name	change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Saxagliptin	4.673	1393929	1.211	7226
2	Flow2	1.2ml/min	Saxagliptin	4.370	1310696	1.191	7414
3	Temp1	25°C	Saxagliptin	4.765	1324786	1.208	7423
4	Temp2	35°C	Saxagliptin	4.360	1292387	1.210	7489

RESULTS AND DISCUSSION:

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Metformin and Saxagliptin standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Metformin and Saxagliptin studies. The method accuracy was evaluated by recovery studies. Metformin and Saxagliptin recovery was founded 99-100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be Y= 16646x – 26439 for Metformin and y = 19288x – 32595 for Saxagliptin. The intra day and inter day variations was calculated in terms of %RSD and results was found to be intra day and inter day respectively. Method robustness results were given by table 5&6. Stability studies are given in table 7&8.

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Metformin and Saxagliptin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Metformin and Saxagliptin in pure and its pharmaceutical dosage forms.

ACKNOWLEDGEMENT:
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