METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF METFORMIN AND SAXAGLIPTIN BY RP-HPLC

[1]Bangaruthalli.J, [2]Swathi.T, [3]Kusumapriya.N, [4]N. Hemalatha.U, [5]Sailaja Kumari.R

[1]Department of Pharmaceutical Analysis, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.
[2]Department of Pharmaceutical Analysis, Yalamarty Pharmacy College, Visakhapatnam.
[3]Department of Pharmaceutical Analysis, Raghu College of Pharmacy, Visakhapatnam.
[4,5]Department of Pharmaceutical Technology, Bhaskara institute of pharmacy,Vizianagaram.

***Abstract*: A simple, sensitive, stability indicating and precise HPLC method for estimation of Saxagliptin and Metformin tablets has been developed and validated for determination in commercial dosage forms. The compound were well separated isocratically on a Agila C-18 (ODS) RP-Column, (25cm x 4.6mm, 5µ) using a mobile phase consisting of Acetate buffer : Acetonitrile : Methanol in the ratio of 70: 20 :10, at a flow rate of 1ml/min with UV detection at 225 nm. The retention time of Metformin at 3.539 min and Saxagliptin peak was at 6.005 mins and . The method was linear over the concentration range for Saxagliptin 10-50µg/ml and for Metformin 5-25 µg/ml. The recoveries of Metformin and Saxagliptin were found to be 100.32% and 99.9 respectively. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.**

***Keywords*: Metformine, Saxagliptine, RP-HPLC, ICH-guidelines, validation**

Introduction:

Metformin:

Metformin is an oral antidiabetic drug and is chemically, 11-dimethyl biguanide hydrochloride. It has high efficacy, safety profile, beneficial cardiovascular and metabolic effects and it also has therapeutic benefit in assocation with other antidiabetic drugs. Hence this drug is included in first line therapy to treat patients with type 2 diabetes mellitus. The main action of Metformin is to decrease fasting plasma glucose levels and is achieved by suppressing excessive hepatic glucose production and improving glucose clearance. It is the principal component in combination therapies intended for diabetes and is frequently used in high doses of about 500 to 850 mg.

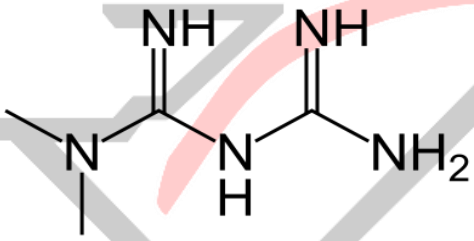

Fig. 1: Chemical structure of Metformin

Saxagliptin:

Saxagliptin is oral antidiabetic drug and is belongs to new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Chemical name is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile. It is used for the treatment of type II diabetes in combination with metformin, a sulphonylurea.

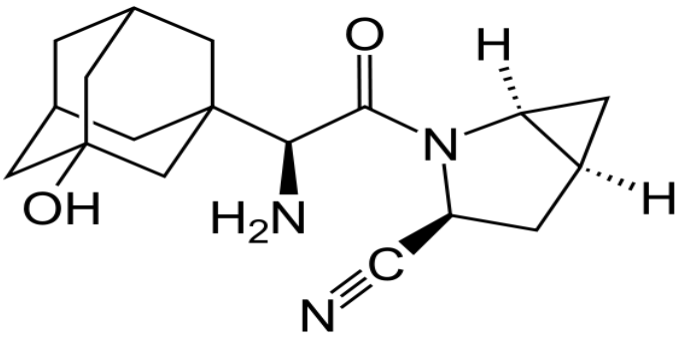

Fig -2:Chemical Structure of Saxagliptin

Materials and Methods

Chemicals and Drugs

Metformin & Saxagliptin were obtained as a gift sample from Mylan laboratories Ltd. (Hyderabad, India). Potassium dihydrogen orthophosphate (KH2PO4), Sodium Dihydrogen ortho Phosphate and HPLC grade solvents like Methanol and Acetonitrile were purchased from SD fine chemicals Ltd., India.

Equipment:

The chromatographic system consisted of Agilent 1100 series, Chromatography system consisted of WATERS HPLC fitted with Prominence LC 20 AD Series pump and PDA detector using Empower, Agilent chem station software as data handling system. Agila C-18 (ODS) RP-Column, (25cm x 4.6mm, 5µ) was used for this method. Ultrasonic bath (Toshcon by Toshniwal), digital Ph meter (Systronics model 802) were used in the study.

Preparation of Mobile Phase:

700 ml of Acetate buffer(pH was adjusted to 4.8 using glacial acetic acid), 200 ml of HPLC grade Acetonitrile and 100 ml of HPLC grade Methanol were transferred in to a mobile phase bottle and mixed thoroughly for few minutes and then degassed with the help of sonicator for 5-10 minutes.

PREPARATION OF SAMPLE SOLUTIONS:

Preparation of Stock Solution:

10 mg of drug substance in the form of API was taken and dissolved it in 10ml of the diluent (mobile phase) to prepare 10ml of 1000 ppm concentration sample solution which is used as stock solution.

Preparation of Standard Solution:

1 ml of 1000 ppm concentration sample solution was transferred into 10 ml volumetric flask and the volume was made up to 10ml to prepare 10ml standard solution (100ppm).

Results and Discussion:

Table No 1: Optimized chromatographic conditions:

Mobile phase	Acetate buffer : Acetonitrile : Methanol in the ratio of 70: 20 :10
Column	Agila C18,250×4.6mm ID, 5µm Particle size
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	225 nm
Injection volume	20 µl
Run time	10 min

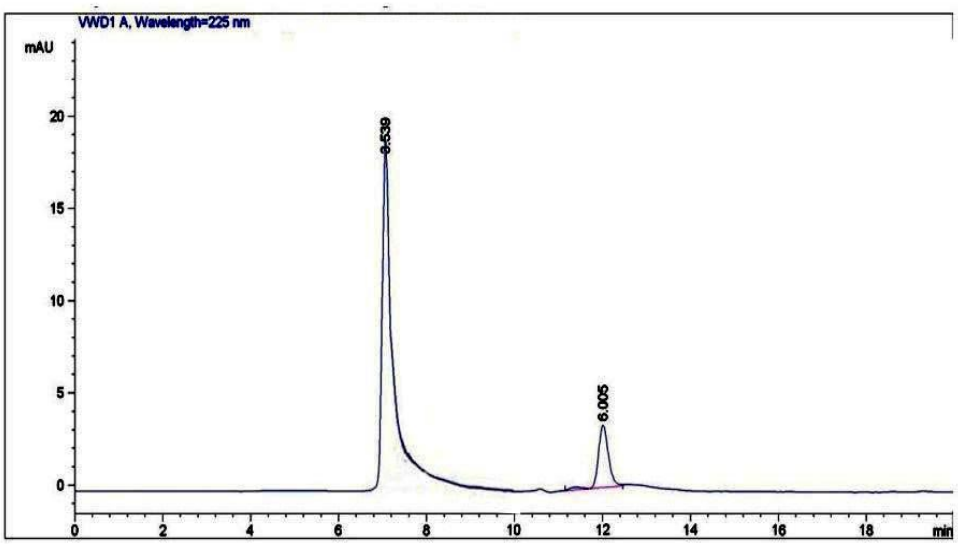

Fig 3 HPLC chromatogram of Metformin and Saxagliptin

Validation:

System suitability:
The System suitability solution and Standard Solution were prepared and analyzed as per test method to evaluate the system suitability parameters and the results were found to be within the limits. The standard solution was injected six times to evaluate system precision and the result is found to be within the limit.

Table No:2 Results of System Suitability

S.No	Name	Retention Time	USP Resolution		USP Tailing		USP Plate Count	
			Observed	ICH Standard	Observed	ICH Standard	Observed	ICH Standard
1	Saxagliptin	6.005	-	NLT 2.0	1.20	NMT 2.0	3133	NLT 1000
2	Metformin	3.539	4.36	NLT 2.0	1.19	NMT 2.0	5822	NLT 1000

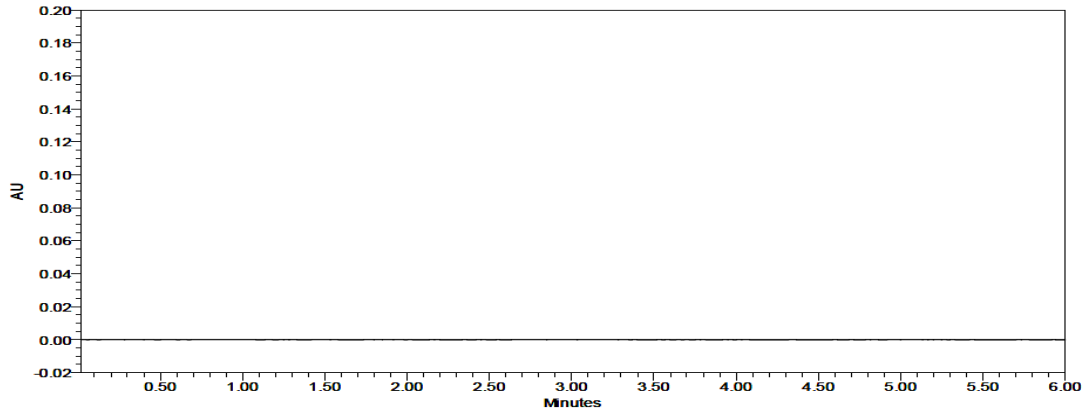

Fig.4: Chromatogram of Blank

Linearity:

Linearity of detector response was established by plotting a graph between concentrations versus area. A series of dilutions of Saxagliptin and Metformin standard were prepared in the concentration range as given below analyzed as per test method.

Table No: 3 Results of Linearity for Saxagliptin and Metformin

S.No	Saxagliptin		Metformin	
	Concentration (µg/mL)	Peak Area	Concentration (µg/mL)	Peak Area
1	50	2639906	50	1861092
2	75	3955939	75	2793872
3	100	5275523	100	3730805
4	125	6596118	125	4662793
5	150	7914048	150	5592989

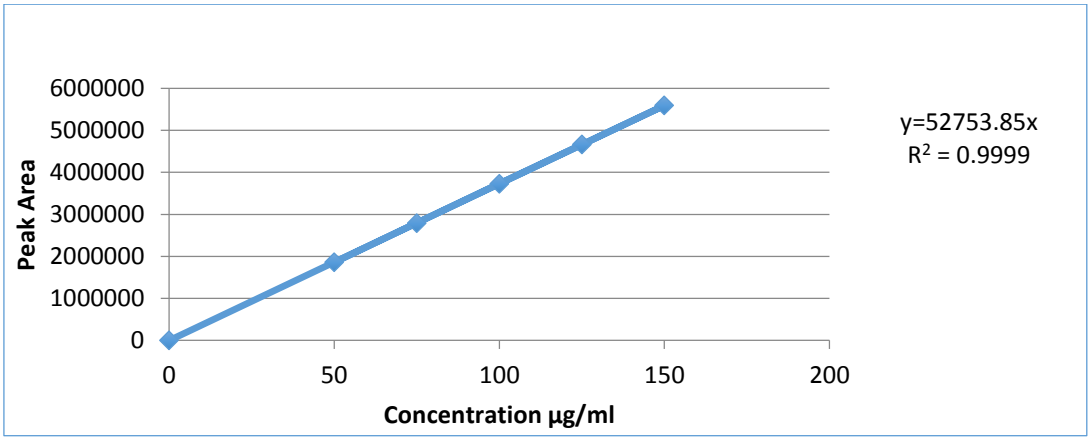

Figure -5: Calibration curve of Saxagliptin

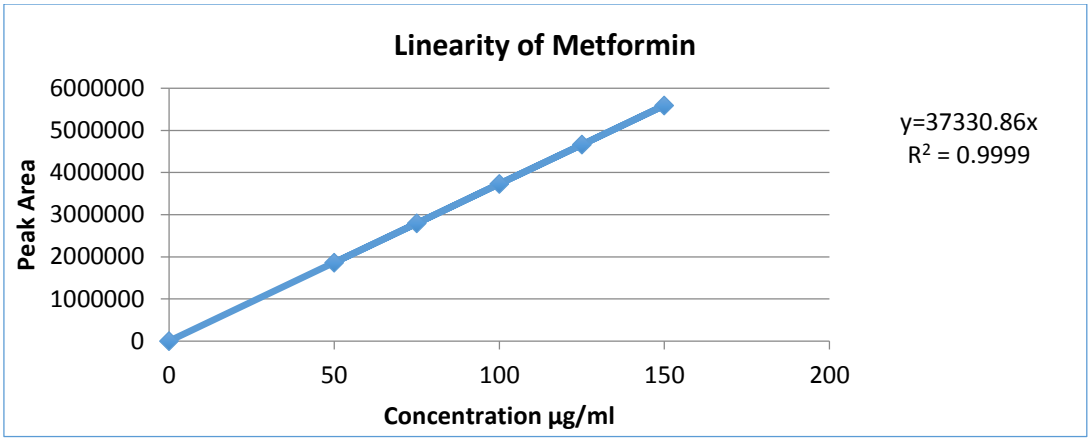

Figure-5 : Calibration curve of Metformin

Accuracy:

A study of recovery was conducted for Saxagliptin and Metformin from about 50% to 150% of the initial assay concentration.

Sample solutions were prepared in triplicate for each level and analyzed as per test method. The individual % recovery, %average recovery and % RSD for recovery at each level were calculated and the results are found to be within limit.

Table No-4: Results of Accuracy for Saxagliptin and Metformin

% Level spiked	Sample No.	Saxagliptin				Metformin			
		Amount added (µg/ml)	Amount recovered (µg/ml)	%Recovery	Mean % Recovery	Amount added (µg/ml)	Amount recovered(µg/ml)	% Recovery	Mean % Recovery
50%	1	24.935	24.94	100	100	198.281	198.31	100.01	99.9
	2	24.935	24.93	100		198.342	198.03	99.94	
	3	24.935	24.87	99.97		198.513	198.65	100.26	
100%	1	49.854	49.84	100	99.97	396.432	396.72	100.91	100.3
	2	49.854	49.79	99.89		396.350	397.19	99.92	
	3	49.854	49.86	100.02		396.641	396.74	100.15	
150%	1	74.805	74.80	100	99.96	594.842	594.52	99.97	99.97
	2	74.805	74.76	99.96		594.574	594.19	99.76	
	3	74.805	74.73	99.94		594.753	594.86	100.19	

Table No-5: Results of Precision for Saxagliptin and Metformin

S.No	SampleName	Saxagliptin		Metformin	
		RT	Area	RT	Area
1	PRECISION1	6.005	5276971	3.539	3734715
2	PRECISION2	5.941	5271275	3.536	3737663

3	PRECISION3	5.991	5272732	3.526	3736030
4	PRECISION4	5.812	5278611	3.498	3730254
5	PRECISION5	5.855	5272896	3.491	3730888
6	PRECISION6	5.820	5275323	3.530	3731698
Mean		5274635		3733541	
Std. Dev		2816.876		3026.672	
% RSD		0.05%		0.0.8	

Limit of Quantitation (LOQ):

Different concentrations of impurities are injected to establish the LOQ by using Signal to Noise ratio(S/N) method. Noise is calculated by injecting six blank solutions.

Limit of Detection (LOD):

Different concentrations of impurities are injected to establish the LOD by using Signal to Noise ratio(S/N) method. Noise is calculated by injecting six blank solutions.

Table No-6: Result of LOD & LOQ

Drug	**s/n at LOD**	**s/n at LOQ**
Saxagliptin	4.02	9.4
Metformin	3.8	10.2

References;

[1] Sharma, B.K. "Instrumental methods of chemical Analysis", Krishna prakashan media (p) Ltd, Meerut, 18thEdn, 10-30,(1999).

[2] Beckett A.H., and Stenlake J.B., Practical Pharmaceutical Chemistry 4th edition, CBS publishers and distributors, 1997, pp 116-167.

[3] GurdeepChatwal, Sahm K. Anand, Instrumental methods of Chemical Analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, 1,1-1.8, 2.566-2.570.

[4] Huynh-Ba, K., Handbook of stability testing in pharmaceutical development regulations, methodologies, and best practices, Springer Verlag, 2008

[5] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, Wiley, New York, 1997, 295.

[6] ICH guideline, Q1A (R2)- Stability testing of new drug substances and products, International Conference on Harmonization, Food and Drug Administration, USA, February, 2003.

[7] Grimm, W., Storage conditions for stability testing in the EC, Japan and USA; the most important market for drug products. Drug Development and Industrial Pharmacy 1993, 19, (20), 2795-2830

[8] HimalPaudelChhetri, PannaThapa, Ann Van Schepdael. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharmaceutical Journal, Volume 22, Issue 5, November 2014, Pages 483–487,

[9] SrikanthInturi ,RavikanthInturi , Israel kumarTagaram. Validated novel lc determination of saxagliptin in pure bulk and pharmaceutical dosage forms. IJPRD, 2011; Vol 3(8): November 2011 (45 - 52).

[10] P. SrinivasaRao, D. Rama chandran, K. Murali and S. Srinivasu. Stability indicating Isocratic Reverse phase HPLC Method with PDA detector for the Estimation of Saxagliptin in bulk drugs and in its Formulation. International Journal of Pharma Sciences Vol. 3, No. 5 (2013): 333-342.

[11] Mohammad Abdul-Azim Mohammad, Ehab Farouk Elkady, Marwa Ahmed Fouad. Development and validation of a reversed-phase column liquid chromatographic method for simultaneous determination of two novel gliptins in their binary mixtures with Metformin. European Journal of Chemistry. Home > Vol 3, No 2 (2012) >Mohammad.