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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Metformin and Sitagliptin by Using RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Metformin and Sitagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Sitagliptin by using Symmetry C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol:phosphate buffer($KH_2PO_4$and K_2HPO_4) pH 3 (pH was adjusted with orthophosphoric acid),detection wave length was 258nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The % purity of Metformin and Sitagliptin was found to be 100.27% and 99.87% respectively.The system suitability parameters for Metformin and Sitagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Metformin and Sitagliptin was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.27 and 0.40, % RSD for intermediate precision was 0.27 and 0.94 respectively. The precision study was precise, robust, and repeatable.LOD value was 2.17 and 6.60, and LOQ value was 0.032 and 0.1125 respectively.

Keywords: Symmetry C18, Metformin and Sitagliptin, RP-HPLC

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. It is not associated with weight gain. It is taken by mouth.

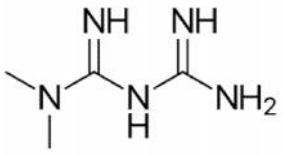

Fig 1: Structure of Metformin

Sitagliptin, sold under the brand name Januvia among others, is a medication used to treat diabetes mellitus type 2. It is generally less preferred than metformin or a sulfonylurea. It is taken by mouth. It is also available within a single pill as metformin/sitagliptin.

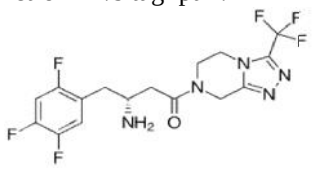

Fig 2: Structure of Sitagliptin

2. Materials and Methods

Instrumentation:

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine.

Chemicals:

Metformin and Sitagliptin, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized chromatographic conditions

Column **:** Symmetry C18 column (4.6×150mm)5µ
Mobile phase ratio **:** 70:30 methanol : phosphate buffer
Detection wavelength **:** 258nm
Flow rate **:** 1ml/min
Injection volume **:** 20µl
Column temperature**:** Ambient
Auto sampler temperature **:** Ambient
Run time **:** 10min

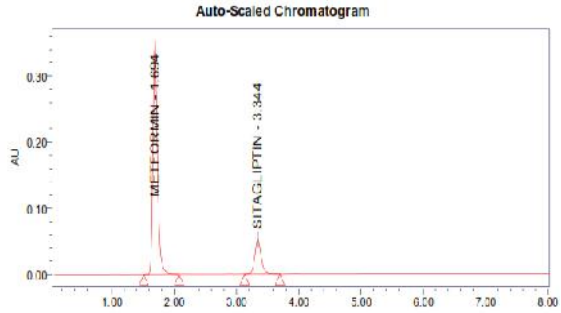

Fig 3: Chromatogram from optimized conditions

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks.

Sample solution preparation:

10 mg of Metformin and 1mg Sitagliptin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig. and results are tabulated in Table.

Standard solution preparation:

10mg Metformin and 1mg Sitagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion

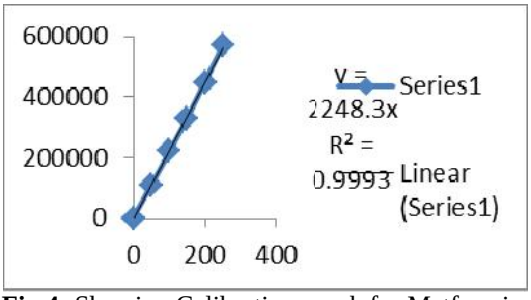

Fig 4: Showing Calibration graph for Metformin

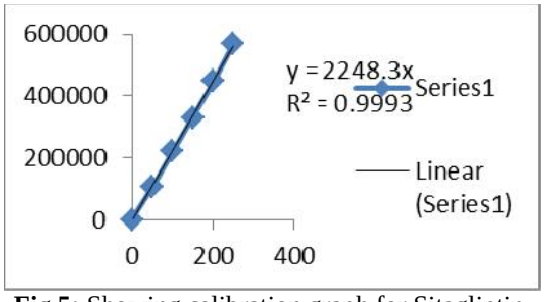

Fig 5: Showing calibration graph for Sitagliptin

4. Conclusion

The developed UV and RP-HPLC methods are precise, specific, accurate. Statistical analysis proves that these methods are suitable for the analysis of Saxagliptin and Metformin by using RP-HPLC.

Table 1: Linearity Results for Metformin

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	221543
2	II	100 ppm	456277
3	III	150 ppm	654999
4	IV	200 ppm	856124
5	V	250 ppm	1102139
Correlation Coefficient			0.999

Table 2: Linearity Results for Sitagliptin

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	231543
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Table 3: Showing accuracy results for Metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7371253	5	4.96	99.91%	99.56%
100%	14634226.7	10	9.98	99.18%	
150%	2243270.7	15	15.02	99.60%	

Table 4: Showing accuracy results for Sitagliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	99.47%
100%	967998	1.0	1.05	99.38%	
150%	145437	1.5	1.495	99.52%	

Table 5: Showing% RSD results for Metformin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Metformin	1.688	1817589	368060	3001.1	1.2
2	Metformin	1.690	1834970	371075	2862.2	1.3
3	Metformin	1.689	1840643	373296	2945.5	1.3
Mean			1831067.5		2936.2	1.3
Std.Dev.			12012.5			
%RSD			0.7			

Table 6: Showing %RSD results for Sitagliptin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Sitagliptin	3.282	376633	54987	54987	1.1
2	Sitagliptin	3.277	380765	55523	5345.1	1.1
3	Sitagliptin	3.277	382506	56202	5453.2	1.1
Mean			379967.9		5445.4	1.1
Std.Dev.			3016.1			
%RSD			0.8			

Table 7: Showing results for intermediate precision of Metformin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Metformin	1.690	1837154	369568	3022.6	1.2
2	Metformin	1.689	1844969	376764	2910.0	1.3
3	Metformin	1.693	1856220	370802	2797.0	1.3

Mean			1846114.3		2909.9	1.3
Std.Dev.			9584.3			
%RSD			0.5			

Table 8: Showing results for intermediate precision of Sitagliptin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Sitagliptin	3.273	380522	56875	5623.5	1.1
2	Sitagliptin	3.275	380632	56469	5600.2	1.1
3	Sitagliptin	3.278	383855	54278	5084.0	1.1
Mean			381669.4		5435.9	1.1
Std.Dev.			1893.4			
%RSD			0.5			

Table 9: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Sitagliptin	5401.60	479884400	0.0372

Table 10: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	371827.90	563365963	6.60
Sitagliptin	5401.60	479884400	0.112

Table 11: Showing system suitability results for Metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	**1**	**2294**	**1.27**
3	1.2	2146	1.26

Table 12: Showing system suitability results for Sitagliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

Table 13: Showing system suitability results for Metformin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2347	1.44
2	*Actual	2294	1.27
3	5 % more	2239	1.13

Table 14: Showing system suitability results for Sitagliptin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	0.99
2	*Actual	4891	1.03
3	5 % more	4817	1.05

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