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

RP-HPLC Method Development and Validation for Estimation of Metformin, Sitagliptin and Saxagliptin in Combined dosage Form

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Abstract
A new method was established for simultaneous estimation of Metformin, Sitagliptin and Saxagliptin RP-HPLC method. Metformin Sitagliptin was freely soluble in water and alcohol. Saxagliptin was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH3) was chosen as the mobile phase. The run time of the HPLC procedure was 5 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100µg / ml. The % recovery of Metformin Sitagliptin and Saxagliptin were found to be in the range of 99.25%-98.22%. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts.
Keywords: Metformin, Sitagliptin and Saxagliptin, RP-HPLC, Methanol.

A R T I C L E I N F O

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1. Introduction

Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake.

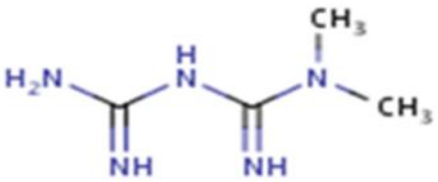

Fig 1: Chemical Structure of Metformin

Sitagliptin is a highly selective DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, thereby increasing the concentration and prolonging the action of these hormones.

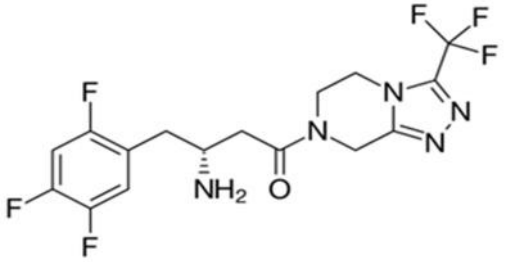

Fig 2: Chemical Structure of Sitagliptin

Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing production of sugar by the liver.

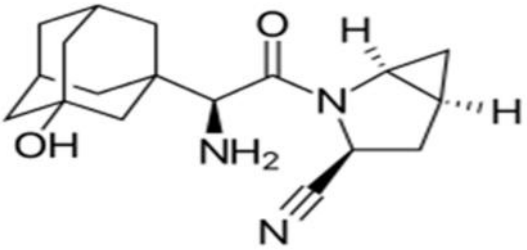

Fig 3: Chemical Structure of Saxagliptin

Literature review reveals that there is less analytical methods were reported for the analysis of Metformin, Sitagliptin and Saxagliptin by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was feltthat, there is a need of new stability indicating analytical method development for the simultaneous estimation of Metformin, Sitagliptin and Saxagliptin in pharmaceutical dosage form.Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Metformin, Sitagliptin and Saxagliptin. The developed method will be validated according to ICH guidelines.

2. Materials and Methods

Table 1: List of Equipment's

S. No.	Instrument Name	Model
1.	HPLC system	WATERS 2695 series (Empower software)
2.	Semi micro balance	Sartorius ME235P
3.	P^H Meter	Thermo electron corporation orion 2 star
4.	Sonicator	Ultrasonic cleaner power sonic 420
5.	Vacuum oven	Wadegati
6.	Constant temperature water bath	Thermolab GMP

Table 2: List of materials and chemicals

S.No.	Name	Manufacturer	Grade
1.	Potassium dihydrogen phosphate	Merck	GR
2.	Sodium perchlorate	Merck	GR
3.	Perchloric acid	Merck	GR
4.	Ortho phosphoric acid	Merck	GR
5.	Methanol	Merck	HPLC
6.	Acetontrile	Merck	HPLC
7.	Water	Milli-pore	Milli-Q
8.	0.45 µm Nylon filter	Axivia	S0761009

Selection of wavelength:

A solution of 10µg/ml of Metformin, Sitagliptin and Saxagliptin were prepared in milliQ water. The resulting solutions were scanned individually on HPLC PDA detector from 190 to 400 nm and also in UV-Visible spectrophotometer. The optimal response for three of them was obtained at 245 nm. Hence the complete method was processed at the wavelength of 240 nm.

Selection of chromatographic condition:

Proper selection of the method depends up on the nature of the sample (ionic/ ionisable/neutral molecule), its molecular weight and solubility. The drugs selected in the present study, were polar in nature. Thus reverse phase HPLC was selected for the initial separation because of its simplicity, suitability, ruggedness and its wider usage.

Initial separation condition:

The mobile phase selected to elute the drug from the stationary phase was milliQ water and HPLC methanol, because of its favourable UV transmittance, low viscosity and low back pressure. Metformin, Sitagliptin and Saxagliptin.

Preparation of standard solution: 10 mg of Metformin,5 mg of Sitagliptin and 25 mg of Saxagliptin were accurately weighed and transferred into a 100 ml clean dry volumetric flask, about 70 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 100 µg/ml,50µg/ml,250 µg/ml.(Stock solution) Further 1 ml was pipetted out fromthe above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent to

give a concentration of 10µg/ml, 5µg/ml and 25 µg/ml respectively.

Preparation of sample solution:

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of Sitagliptin present in 10 tablets (1854.4mg) was transferred into a 100 ml clean dry volumetric flask, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 100 µg/ml,50 µg/ml,250 µg/ml ,allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as par with standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system.

Preparation of mobile phase:

(For Optimized Conditions)

Take 2.5 gm of potassium dihydrogen ortho phosphate into 1000ml volumetric flask dissolved in hplc grated water and adjust phupto 3 with ortho phosphoric acid. From the above prepared buffer take 350 ml (35%) and 650ml of Methanol HPLC (65%) were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, typical analytical performance characteristics that should be considered in the validation of the type of methods are system suitability, specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness.

Optimized chromatographic conditions

Mobile phase: Methanol: phosphate buffer P^H 3.5 (65:35)
Flow rate: 1.0 ml per min
Column : Agilent C_{18} (4.6 x 150mm, 1.7µm)
Detector wavelength : 245 nm
Column oven : Ambient
Injection volume : 10 µl
Run time : 10 min

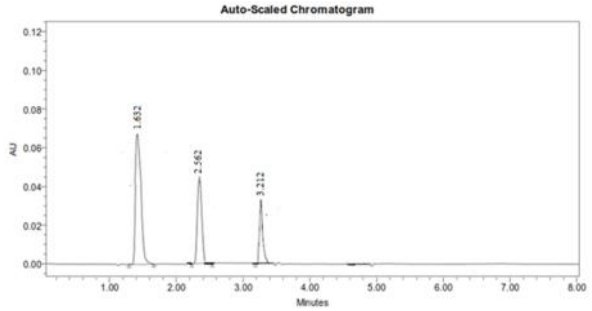

Fig 4: Optimized Chromatogram

3. Results and Discussion

Selection of wavelength:

A solution of 10µg/ml of Metformin Sitagliptin and Saxagliptin were prepared in milliQ water. The resulting solutions were scanned individually on HPLC PDA detector from 190 to 400 nm and also in UV-Visible spectrophotometer. The optimal response for three of them was obtained at 245 nm. Hence the complete method was processed at the wavelength of 245 nm.

System Suitability:

Sample solution of Metformin Sitagliptin and Saxagliptin were injected three times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections.

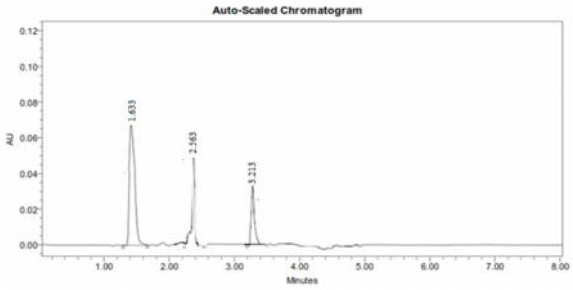

Fig 4: Chromatogram for System suitability

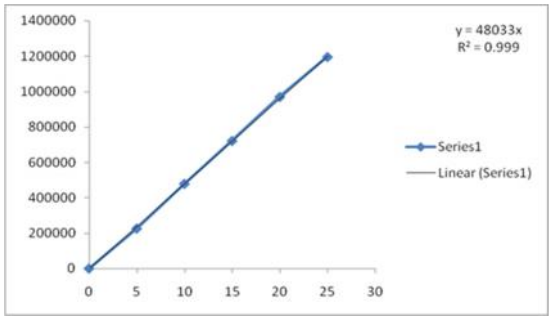

Fig 5: Calibration curve of Metformin

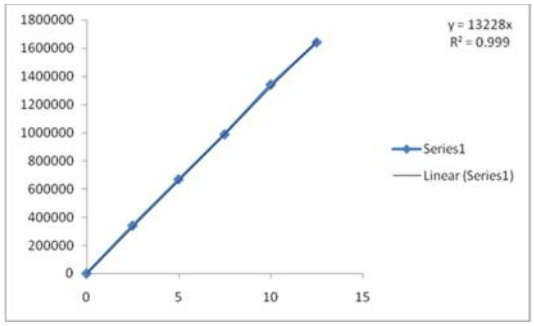

Fig 6: Calibration curve of Sitagliptin

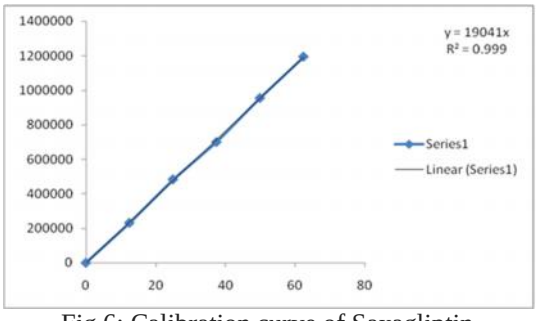

Fig 6: Calibration curve of Saxagliptin

Linearity:

10 mg of Metformin, 5mg of Sitagliptin and 25 mg of Saxagliptin were accurately weighed and transferred into a 100 ml clean dry volumetric flask, about 70 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 100µg/ml, 50 µg/ml, 250 µg/ml. (Stock solution).

Preparation of Level – I (5ppm of Metformin, 2.5ppm of Sitagliptin & 12.5 ppm of Saxagliptin):

0.5 ml of stock solution was taken in 10 ml of volumetric flask diluted up to the mark with **Preparation of Level – II (10 ppm of Metformin, 5ppm of Sitagliptin & 25 ppm of Saxagliptin):**

1.0ml of stock solution was taken in 10 ml of volumetric flask diluted up to the mark with

Preparation of Level– III (15ppm of Metformin, 7.5ppm of Sitagliptin & 37.5ppm of Saxagliptin):

1.5 ml of stock solution was taken in 10 ml of volumetric flask diluted up to the mark with

Preparation of Level – IV (20ppm of Metformin, 10ppm of Sitagliptin & 50 ppm of Saxagliptin):

2.0 ml of stock solution was taken in 10 ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – V (25ppm of Metformin, 12.5 ppm of Sitagliptin & 62.5 ppm of Saxagliptin): 2.5 ml of stock solution was taken in 10 ml of volumetric flask diluted up to the mark with diluent to give the respective concentrations i.e.50 ppm, 50 ppm and 50ppm.

Procedure:

Each level solution was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated.

Precision:

The standard solutions were injected for five times and the areas for all five injections were measured in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Robustness:

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition, temperature variations which may differ but the responses were still within the specified limits of the assay.

a) Effect of variation of flow rate*:*

A study was conducted to determine the effect of variation in flow rate. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. Standard solution 10ppm of Metformin,5ppm of Sitagliptin& 25 ppm of Saxagliptinwere prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10 %.

b) Effect of variation of mobile phase composition:

A study was conducted to determine the effect of variation in mobile phase ratio by changing the ratio of mobile phase. The Organic composition in the Mobile phase was varied from 30 % to 70 %.Standard solution 10 µg/ml of Metformin,5µg/ml of Sitagliptin& 25 µg/ml ofSaxagliptin were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

Table 3: System suitability Results

S.No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Metformin	1.632	638986	835168	2487	1.62	1.2
2	Sitagliptin	2.562	655764	103296	2281	1.51	3.04
3	Saxagliptin	3.212	119002	73286	2594	1.25	13.23

Table 4: Calibration parameters for Metformin, Sitagliptin and Saxagliptin

Parameter	Results for Metformin	Results for Sitagliptin	Results for Saxagliptin
Slope	19813	14314	78352
Intercept	65496	49165	48075
Correlation coefficient	0 .9993	0.99917	0.99903

Table 8: Precision Results for Metformin, Sitagaliptin and Saxagliptin

Injection No	Peak Area		
	Metformin	Sitagaliptin	Saxagliptin
1	1248257	935136	954857
2	1247578	929455	937616
3	1245272	930458	950692
4	1245264	934387	940253
5	1248573	924058	927055
Avg	1246487	927858.7	935424.3
SD	2865.61	5875.15	6301.561
% RSD	0.23783	0.5231	0.562

Table 9: Robustness results for Metformin (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min	1.0ml/min	1.2ml /min
1	Metformin	1.636	1.635	1.635
USP Plate count		2512	2495	2488
USP Tailing		1.65	1.63	1.67

Table 10: Robustness results for Sitagliptin (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8 ml/min	1.0ml/min	1.2m l/min
1	Sitagliptin	2.562	2.561	2.561
USP Plate count		2178	2467	2287
USP Tailing		1.46	1.47	1.47

Table 11: Robustness results for Saxagliptin (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min	1.0ml/min	1.2m l/min
1	Saxagliptin	3.216	3.215	3.215
USP Plate count		2347	2546	2087
USP Tailing		1.25	1.26	1.26

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 12: Robustness results for Metformin

S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	Metformin	1.634	1.635	1.633
USP Plate count		2511	2397	2595
USP Tailing		1.44	1.63	1.65

Table 13: Robustness results for Sitagliptin

S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	Sitagliptin	2.562	2.561	2.561
USP Plate count		2434	2263	2522
USP Tailing		1.34	1.47	1.6

Table 14: Robustness results for Saxagliptin

S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	Saxagliptin	3.214	3.213	3.215
USP Plate count		2483	2545	2135
USP Tailing		1.23	1.25	1.32

*Results for actual Mobile phase composition (65:35Methanol: Buffer) have been considered from Accuracy standard.

4. Conclusion

For establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100µg/ml. The % recovery of Metformin Sitagliptin and Saxagliptin were found to be in the range of 99.25% - 98.22 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be

suitable for the routine analysis of Metformin Sitagliptin and Saxagliptin in Bulk drug and Pharmaceutical formulation.

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