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Research Article

Validated RP-HPLC Method for the Estimation of Simvastatin and Sitagliptin

Sujani PV[1*], Y Padamanabha Reddy[2], N Devanna[3], SS Phanindra[4]
[1]Department of Pharmaceutical Analysis, Procadence Institute of Pharmaceutical Sciences, Rimmanaguda, Medak (dt), Andra Pradesh, India
[2]Principal, RIPER, Anantapur, Andra Pradesh, India
[3]Associate professor of Chemistry, JNTU-College of Engineering, Anantapur, Andra Pradesh, India
[4]Department of pharmaceutical analysis, Procadence Institute of Pharmaceutical Sciences, Rimmanaguda, Medak (dt), Andra Pradesh, India

***Corresponding author**
Sujani PV
Email: sujani.ssp@gmail.com

Abstract: A simple, sensitive, rapid, economic, robust RP-HPLC method was developed for the estimation of Sitagliptin-Simvastatin in Pure and Tablet dosage forms. An Agilent C8 Column (250 x 4.6 mm, 5µ) was used with a mobile phase containing a mixture of Methanol and water in the ratio of 25:75. The procedure was carried out at pH-2.9 and TEA was used as the Ion pairing agent. The flow rate was maintained at 1.0 ml/min. results were determined at 266 nm with fixed wavelength PDA detector. The linearity for Sitagliptin was found between 20-120 µg/ml and between 10-50 µg/ml for Simvastatin. The retention times were found as 3.227 and 15.760 for Sitagliptin and Simvastatin respectively. Validation parameters like Precision, Accuracy, Robustness and System suitability parameters were determined and examined by applying validated parameters.
Keywords: Sitagliptin, Simvastatin, Method development, Validation, RP-HPLC

INTRODUCTION

Sitagliptin (Fig-1) is (3R) -3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5h- [1, 2, 4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one, an oral hypoglycaemic agent that blocks the dipeptidyl peptidase 4 (DPP-4) enzyme activity. This enzyme inhibition will leads to increased amount of active incretins, glucagon like peptide- 1 (GLP-1) and gastric inhibitory polypeptide (GIP), which significantly increases insulin secretion. And in turn decreases blood glucose level.

Simvastatin (Fig-2), a methylated analogue of lovastatin, is -(+)-{1S,3R,7S,8S,8aR)-1, 2, 3, 7, 8, 8a-hexahydro-3,7-dimethyl-8-[2-(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-naphthyl-2,2-dimethyl butanoate. It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug is converted into ß hydroxy acid of Simvastatin, which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver.

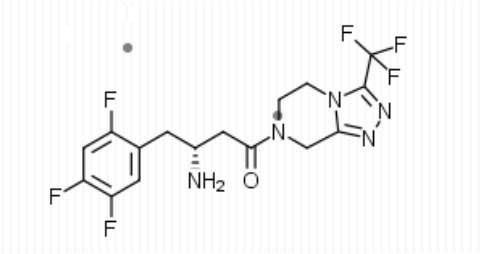

Fig. 1: Sitagliptin

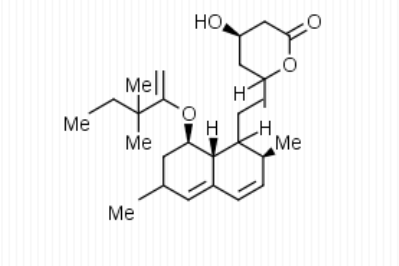

Fig. 2: Simvastatin

The literature review suggested that there were methods available for the estimation of Sitagliptin [1-7] and Simvastatin [18, 19] individually with the help of instruments like UV-Spectrophotometer, HPLC and for simultaneous determination there were methods found on UV-Spectrophotometer [10-12] and a very few on

HPLC[15-17]. An attempt was made to develop a method which is precise, simple, robust and most economic method so far for their determination.

MATERIALS AND METHODS

All the reagents used in the experiment were of HPLC grade solvents. After many trails it was observed that a a mixture of Methanol and Water (25:75) at pH-3 suits best for the elution. Orthophosphoric acid was used to adjust the pH and Triethanolamine was used as an ion pairing agent. Pure drugs of Sitagliptin and Simvastatin were obtained from Mylan Laboratories and the tablets were purchased from local pharmacy. The specifications of the instruments and all the conditions maintained were shown in Table 1.

Table1: Optimized Chromatographic Conditions

Parameters	Method
Column	Agilent C_8 Column (25 x 4.6 mm, 5µ)
Mobile phase	methanol : water (pH: 3) 25:75%v/v
Flow rate (ml/min)	1.0
Run time (minutes)	16
Injection volume	20 µl
Detection wavelength	253nm
Detector	PDA Detector
Elution	Isocratic
t_R (min)	Sitagliptin-3.2, Simvastatin- 15.7

Preparation of Mobile phase

For the mobile phase, 250 ml of Methanol and 750 ml of Distilled Water were mixed together and its pH was adjusted to 3 with ortho phosphoric acid. The mobile phase was then sonicated in an ultrasonicator to remove undissolved gasses and impurities to remove unwanted peaks in the chromatogram**.**

Preparation of standard stock solutions

Accurately weighed 10mg of sitagliptin and simvastatin, transferred to separate 10ml volumetric flasks and dissolved in methanol with the help of sonicator. The flasks were shaken and volumes were made up with methanol to give solutions containing 1,000µg/ml concentration of each.

Calibration of standards

Based on the label claim of dosage form to be analysed, different volumes of stock solutions of each drug were transferred accurately to 10ml volumetric flask and diluted to mark to give a series of concentrations of solution equal to 20, 50, 80, 110, and 120 µg/ml of sitagliptin and 10, 20, 30, 44, and 50 µg/ml of simvastatin. These solutions were injected into the optimized condition. The calibration line was plotted with mean peak area (n =3) on Y-axis and concentration of drugs on X-axis.

Assay

Sample preparation

20 tablets of Juvisync (50mg/20mg) containing 50mg and 20mg of sitagliptin and simvastatin respectively were powered finely. weight of tablet powder equivalent to 100 mg of sitagliptin (which also contain a tablet powder equivalent to 40 mg of simvastatin) was taken in a 100ml volumetric flask.60ml of methanol was transferred to the flask and sonicated for 15 minutes and the volume was made up to the mark with methanol. The above solution was filtered using Whatman No.1 filter paper. From the above solution 0.5 ml was withdrawn and made up to 10 ml with methanol to get 50 µg/ml of sitagliptin (Also contains 20 µg/ml simvastatin) . The resulting solution was analyzed under optimized condition. The Results were shown in the Table 7. Chromatogram was shown in Fig 3.

Estimation Method

The amount of sitagliptin and Simvastatin present in each dosage form was calculated by using Linear line Equation of calibration curve.

$$Y = m X \pm C,$$

Where Y is Peak Area,
X is Concentration of the sample
m is slope
C is Y intercept

Amount Found= Concentration x Dilution Factor

Method Validation

The developed method was validated as per ICH guide lines [20]. All the solutions were prepared according to the procedures given under preparation of standard and sample solutions.

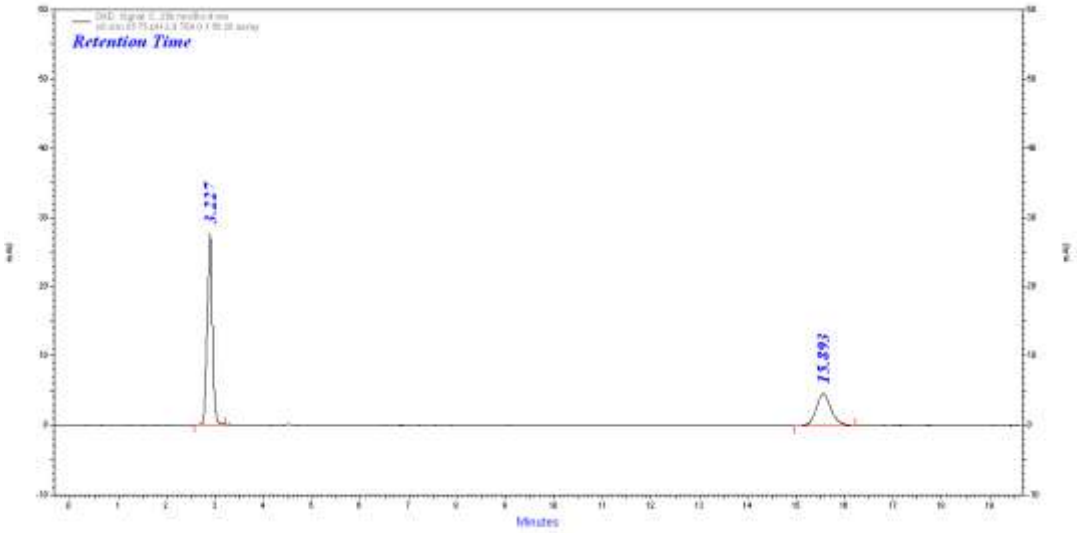

Fig. 3: Assay chromatogram for Sitagliptin and Simvastatin

RESULTS AND DISCUSSION

An attempt was made to develop a RP-HPLC method which is economic, accurate, precise and robust for the determination of sitagliptin and Simvastatin in combined dosage form. The chromatographic conditions were optimized by changing the composition of mobile phase, pH, buffers and their concentration during many trails run on the instrument. Finally a mixture of 25 parts of methanol with 75 parts of water at pH-3 was found suitable for best separation of two components. Different concentrations of standard solutions were injected to predict the linearity range for both drugs. Sitagliptin was found linear between the concentrations 20 to 120µg/ml and 2 to 44µg/ml for Simvastatin. Their correlation coefficients were found from the linear graph as 0.9997 and 0.9991 for sitagliptin and Simvastatin respectively. The retention times were found as 3.227 and 15.760 for sitagliptin and Simvastatin respectively.

The assay (Table 7) was made for the combination tablets by preparing the solutions of concentrations from tablet powder which falls between the linear ranges of standard solution. The accuracy (Table 3) of the method was checked by performing recovery studies. The recovery was determined at three levels Vie- 80, 100 and 120% of the selected concentrations and performing three replicates at each recovery level. The precision (Table 4) of the method was determined from one lot of combined dosage forms. To determine the robustness (Table 5) of the developed method, experimental conditions were purposefully altered and the assay was performed.

Table-2: Specificity data for sitagliptin and simvastatin

Sl. No.	Injection	Results
1	Blank	No peaks were observed at t_R of analytes
2	Drug solution	Sitagliptin-3.2, Simvastatin- 15
3	Degradated sample	No peaks were co-eluted with analyte peaks

Table 3: Recovery studies for sitagliptin and simvastatin

Sl. No.	Preanalysed sample conc.(µg/ml)	Recovery level (%)	Amount added (µg/ml)	Amount of drug (µg/ml) Mean±SD	%RSD	% Recovery (n=3)
1	Sitagliptin-50	80%	40	90.18± 0.41	0.45	100.2
		100%	50	101.01± 0.78	0.78	101.0
		120%	60	109.92± 1.02	0.92	99.9
2	Simvastatin- 20	80%	16	36.28± 0.48	1.32	100.7
		100%	20	40.71± 0.59	1.44	101.7
		120%	24	43.92± 0.21	0.47	99.8

Table 4: Precision studies of sitagliptin and simvastatin

Sl. No.	Analyte	Conc. (µg/ml)	Intraday(n=3) Conc. ± SD	Interday(n=3) Conc. ± SD	%RSD	
					Intraday	Interday
1	Sitagliptin	20	20± 0.25	20± 0.29	1.23	1.40
		50	50± 0.58	50± 0.36	1.16	0.72
		120	120± 1.12	120± 1.29	0.93	1.07
2	Simvastatin	10	10± 0.12	10± 0.14	1.20	1.40
		20	20± 0.28	20± 0.21	1.40	1.05
		44	44± 0.56	44± 0.61	1.27	1.38

Table 5: Robustness Data for sitagliptin and Simvastatin

Sl. No.	Parameter	Variation* (ml/min)	Sitagliptin			Simvastatin		
			t_R(min)	T_f	% Assay	t_R (min)	T_f	%assay
1	Flow Rate	1.1	2.92	1.0	100.1	14.08	1.2	101.1
		0.9	3.58	1.0	101.1	17.30	1.2	100.3
2	Mobile Phase Ratio	23:77	3.21	1.2	99.8	12.57	1.1	100.8
		27:73	3.26	0.9	99.9	19.23	1.1	102.0
3	pH	2.8	3.14	1.1	101.8	15.71	1.2	101.8
		3.2	2.92	1.0	98.7	15.45	0.9	101.6
4	Wave Length (nm)	256	3.21	1.1	100.1	12.57	1.3	101.2
		250	3.23	1.0	100.4	15.81	1.3	101.9

*Mean of Five values

Table 6: Data for System Suitability Parameters of sitagliptin and Simvastatin

Sl. No.	Parameter	Values Observed		Acceptance criteria
		Sitagliptin	Simvastatin	
1	Number of Theoretical Plates	4622 ± 32	4759 ± 46	> 4,000
2	Capacity factor(K)	1.65	4.25	0.5<K<20
3	Tailing Factor	1.12 ± 0.01	1.23 ± 0.02	≤ 2
4	Retention Time	3.227	15.760	--

Table 7: Assay Data for sitagliptin and Simvastatin, Juvisync (50 mg/20mg)

Drug	% Assay	Mean Assay	SD	%RSD
Sitagliptin	100.6	100.2	0.3066	0.3059
	100.6			
	100.2			
	99.93			
	100			
	100			
Simvastatin	101.48	100.7	0.5652	0.5617
	101.2			
	100.8			
	100.6			
	100.1			
	100			

All the validated parameters were checked by applying statistical formulas such as standard and relative standard deviation. The results were found to fall within the prescribed limits.

CONCLUSION

The present combination Sitagliptin and Simvastatin is marketed as one formulation (Juvisync - 50mg/20mg)

Sitagliptin – 50 mg/tablet

Simvastatin – 20mg/tablet

The fixed dose combination tablet of Sitagliptin and Simvastatin was subjected to simultaneous estimation by reverse phase HPLC method. The proposed HPLC method was validated by evaluation of the validation parameters. The relative standard deviation of slope, correlation coefficient, within and between day repeatability, resolution and tailing factors for this techniques were obtained. Assay was performed within a short analysis time. Assay parameters used in this study reduced tailing for all peaks and improved the resolution.

Highly reliable and cost efficient HPLC method was developed for the quantitative estimation of Sitagliptin and simvastatin in combined tablet dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained.

From the forgoing it is concluded that the method developed is simple, rapid, specific and precise hence suitable for application in routine analysis of pharmaceutical preparations.

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