Original Article

Reverse phase high-performance liquid chromatography method for the simultaneous estimation of sitagliptin with simvasatin in bulk and tablet formulation

Abstract

Aim: A simple, sensitive, precise, and rapid reverse phase high-performance liquid chromatography method was developed and validated for simultaneous estimation of sitagliptin (SIT) and simvastatin (SIM) in bulk drug and tablet dosage forms. **Materials and Methods:** The separation was achieved by using Prontosil 120-5-CN (250 mm × 4.6 mm, 5 μm) column with a mobile phase consisting of acetonitrile:methanol:phosphate buffer (45:10:45 v/v). The mobile phase was delivered at a flow rate of 1.0 ml/min. Analysis was performed at ambient temperature with detection at 254 nm. **Results:** The retention times of SIT and SIM were found to be 4.8 and 6.0 min and the calibration curves were linear (R^2 = 0.999) over a concentration range from 1 to 30 μg/ml for SIT and SIM, respectively. Limit of detection and limit of quantitation were 0.6 μg/ml and 0.7 μg/ml for SIT and 0.1 μg/ml and 0.2 μg/ml for SIM, respectively. **Conclusion:** The developed method was validated for parameters such as system suitability, specificity, linearity, accuracy, precision, ruggedness, and robustness as per International Conference on Harmonization guidelines and the results were found to be within the limits. Hence, it can be used for the routine quality control of SIT and SIM in the bulk sample and tablet dosage forms.

Key words:
Reverse phase high-performance liquid chromatography, simvastatin, sitagliptin

Introduction

Sitagliptin (SIT) chemically is (3R)-3-amino-1-[3-(trifluoro methyl)-6,8-dihydro-5h-[1,2,4]triazolo[3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one, Figure 1 shows structure of SIT, an oral anti-diabetic agent that blocks dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin increased incretin levels (glucagon-like peptide-1 and gastric inhibitory polypeptide), which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion.[1-3] Simvastatin (SIM) chemically is 2,2-dimethyl butanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8ahexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6 oxo2H pyran-2yl]ethyl]1-napthalenyl ester; Figure 2 shows a structure of SIM, used as 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor in the treatment of primary hypercholesterolemia and is effective in reducing total and low-density lipoprotein cholesterol as well as plasma triglycerides and apolipoprotein B.[3-6] The literature reveals that few methods have been reported for simultaneous estimation of SIT and SIM by ultraviolet (UV),[7-9] high-performance liquid chromatography (HPLC),[10-12] and high-performance thin layer chromatography.[13] Hence, attempt was made to develop new versatile, accurate, precise, and economical reverse phase (RP)-HPLC method for the simultaneous estimation of SIT and SIM in bulk and tablet dosage form and method was validated as per International Conference on Harmonization (ICH) guidelines.[14,15]

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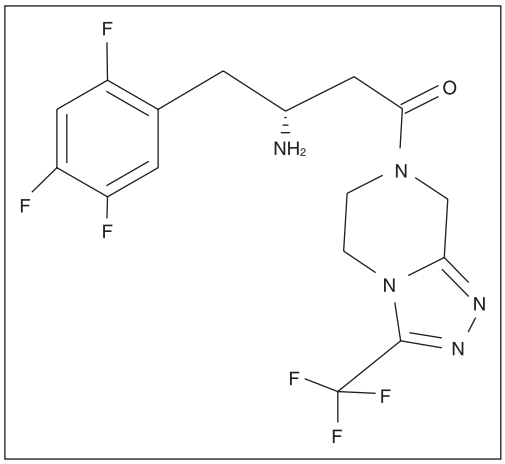

Figure 1: Structure of sitagliptin

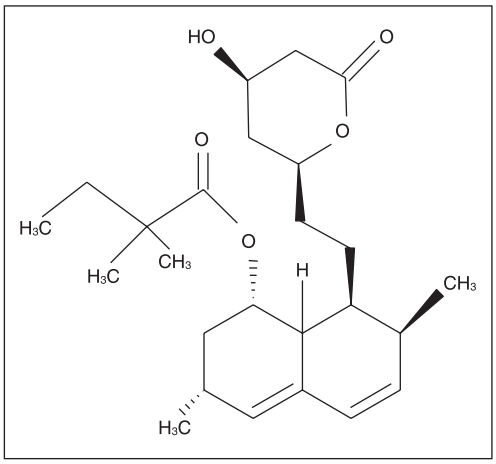

Figure 2: Structure of simvastatin

Materials and Methods

Chemicals and reagents

High-performance liquid chromatography grade methanol, acetonitrile and all other analytical grade reagents were purchased from Merck, India. HPLC grade water was prepared using Milli-Q water purification system. JUVISYNC tablets were purchased from local markets of Bangalore. Class A glassware is used throughout the experiment. Reference standard SIT and SIM gift samples were obtained from Watson Laboratories, Mumbai.

Instrument and chromatographic conditions

The chromatographic system consists of LC-20AT SHIMADZU-SPD-M20A equipped with photodiode array (PDA) detector and rheodyne injector with 20 µl loop volume. Chromatographic separations were carried out using Prontosil 120-5-CN (E) column (250 mm × 4.6 mm i.d.) packed with 5µm diameter particles. The mobile phase consisting of acetonitrile methanol and 10 mM phosphate buffer (1.36 g of potassium dihydrogen orthophosphate in 1000 ml of HPLC water) in the ratio of 45:10:45 v/v. The flow rate was 1 ml/min and detection was carried out at 254 nm using PDA detector.

Preparation of standard stock solutions of sitagliptin and simvastatin

Accurately 10 mg of SIT and SIM were weighed separately into a clean and dry 10 ml volumetric flasks, dissolved with sufficient volume of diluent. The volumes were made up to 10 ml with diluent to get a concentration of 1000 µg/ml each drug (stock I). About 1 ml of each resulting stock solution was further diluted separately into 10 ml volumetric flasks with diluent to get a concentration of 100 µg/ml for both SIT and SIM, respectively (stock II). From stock II, 2 ml of SIT and 1 ml of SIM were transferred together into a 10 ml volumetric flask and final volume was then made up to 10 ml with diluent to get a concentration of 20 µg/ml and 10 µg/ml of SIT and SIM, respectively.

Preparation of sample stock solution

Twenty tablets of JUVISYNC each containing 100 mg of SIT and 40 mg of SIM were weighed and finely powered. Powder equivalent to 100 mg of SIT and 40 mg of SIM was taken together and transferred into a clean, dry 100 ml volumetric flask. The powder was first dissolved in methanol and sonicated for 10 min. The resulting mixture was then filtered through Whatmann Filter No. 0.45 µ. The final volume of filtrate was made up to 100 ml with diluent. About 1 ml of the resulting solution was diluted to 10 ml with diluent to get a concentration of 100 µg/ml and 40 µg/ml of SIT and SIM, respectively.

Method Validation

The developed method has been validated with respect to various parameters, in accordance with the ICH guidelines.[14,15]

Linearity

The various concentration of working standard solutions of SIT and SIM were made by pipetting 0.1–3.0 ml from stock II separately into a series of 10 ml volumetric flask and diluted to 10 ml to get the final concentration of 1-30 µg/ml solutions. About 20 µl of each of these working standard solutions of SIT and SIM ranging from 1 to 30 µg/ml were injected into a chromatograph at flow rate of 1 ml/min. Retention time and peak area obtained were recorded and standard calibration curve was plotted for SIT and SIM and linearity equations were derived. The calibration curves were shown in Figures 3 and 4.

Specificity

The specificity of the proposed method was demonstrated that the excipients from sample and diluents do not interfere in the drug peak. The chromatogram was presented in Figure 5.

System suitability parameter

This test ensures that the analytical system is working properly and can give accurate and precise results. Figure 6 and Table 1 shows the results.

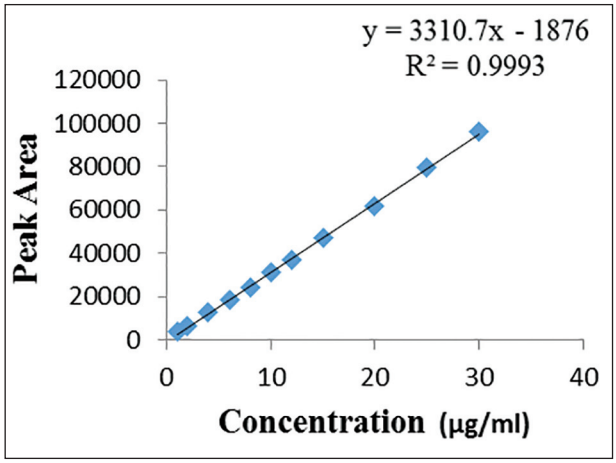

Figure 3: Linearity plot for sitagliptin

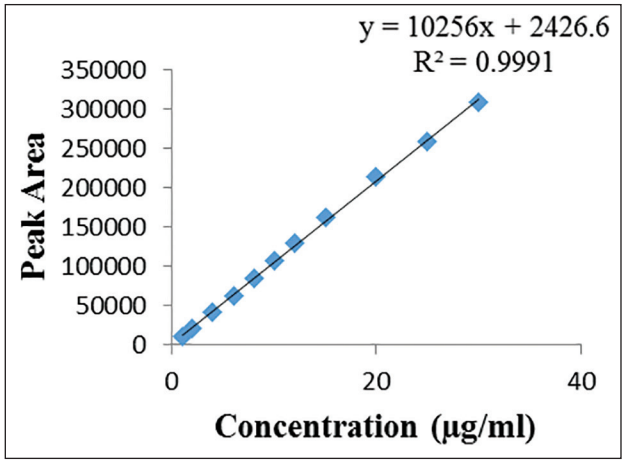

Figure 4: Linearity plot for simvastatin

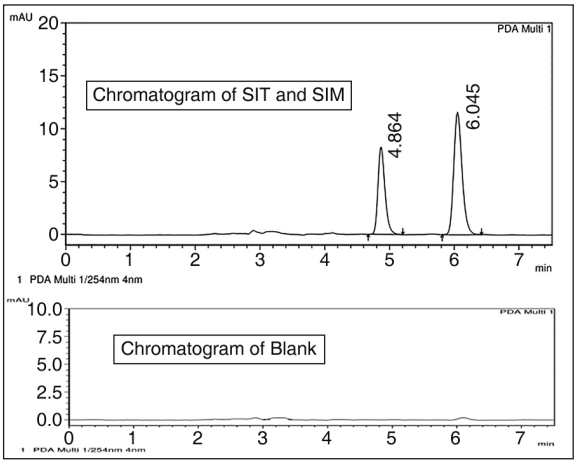

Figure 5: Chromatogram for specificity

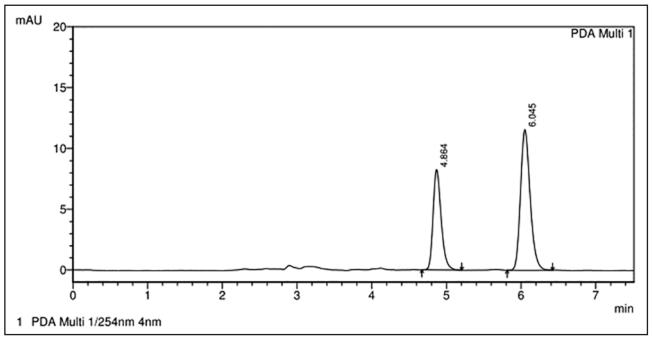

Figure 6: Chromatogram of sitagliptin and simvastatin

Precision

System precision

Successive six injections of standard solution (six replicates) were injected into a HPLC chromatograph, the peak area, and chromatograms obtained were recorded. The percentage relative standard deviation was calculated for peak areas of replicates. The results are shown in Table 2.

Method precision (repeatability)

Method precision was assessed by replicate injections and measurement of peak area for SIT and SIM in six replicates. The amounts of SIT and SIM in sample were calculated from their respective standard linearity equations. The results obtained are presented in Table 2.

Intermediate precision (ruggedness)

Intermediate precision was determined by the assay of sample sets by different analysts, the peak area and the chromatograms were recorded. The percentage assay of standard drug was calculated from the peak areas of replicates. The results obtained are mentioned in Table 3.

Table 1: System suitability parameters

System suitability factor	SIT	SIM	Acceptance criteria
Theoretical plates	54,771	64,787	> 2000
HETP (mm)	18.25	15.43	–
Tailing factor	1.58	1.32	< 2
Resolution	4.52		> 2

SIT – Sitagliptin; SIM – Simvastatin; HETP – Height equivalent to theoretical plate

Table 2: Results of precision parameters

Precision parameters	RSD*		Acceptance criteria, %
	SIT, %	SIM, %	
System precision	1.07	0.75	< 2.0
Method precision	1.16	1.07	< 2.0

*n = 6; SIT – Sitagliptin; SIM – Simvastatin; RSD – Relative standard deviation

Table 3: Results of intermediate precision (ruggedness)

Drug	Analyst 1*	Analyst 2*	Acceptance criteria
SIT, %	99.24	99.86	90-110
SIM, %	99.24	100.72	90-110

*n = 6; SIT – Sitagliptin; SIM – Simvastatin

Table 4: Results of accuracy of SIT and SIM

Level %	Amount of SIT stand drug added (µg/ml)	Amount of standard recovered (µg/ml)*	% recovery of SIT	Amount of SIM stand drug added (µg/ml)	Amount of standard recovered (µg/ml)*	% recovery of SIM
80	6	6.07	101.09	2.4	2.33	97.02
	6	5.98	99.64	2.4	2.34	97.51
	6	5.97	99.48	2.4	2.37	98.27
100	10	10.03	100.28	4	4.00	100.11
	10	9.87	98.73	4	3.98	99.42
	10	9.86	98.56	4	3.95	98.84
120	14	13.82	98.73	5.6	5.56	99.27
	14	13.82	98.75	5.6	5.61	100.21
	14	14.07	100.48	5.6	5.53	98.70

*n = 6. SIT – Sitagliptin; SIM – Simvastatin

Table 5: Results of LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
SIT	0.6	0.7
SIM	0.1	0.2

LOD – Limit of detection; LOQ – Limit of quantitation; SIT – Sitagliptin; SIM – Simvastatin

Table 6: Results of robustness

Change in	% Assay of SIT	% Assay of SIM
Flow rate (ml)		
0.9	100.58	107.31
1.0	99.59	100.51
1.1	97.30	95.23
Mobile phase ration (ACN:MeOH:buffer)		
(43:9:48)	97.67	94.46
(45:10:45)	99.79	100.51
(47:11:42)	96.46	97.97
Wavelength in nm		
252	96.31	95.05
254	99.95	101.98
256	95.65	95.58

SIT – Sitagliptin; SIM – Simvastatin; ACN:MeOH:buffer – Acetonitrile:methanol:phosphate buffer

Table 7: Assay results of tablet formulation

Amount of drug recovered SIT (µg/ml)	Amount of drug recovered SIM (µg/ml)	% Assay for SIT	% Assay for SIM
9.87	3.98	98.73	99.42

SIT – Sitagliptin; SIM – Simvastatin

Accuracy

Accuracy is expressed as percentage recovery by the assay of known added amounts of analyte carried out at three different levels (80%, 100%, and 120%). The results are tabulated in Table 4.

Limit of detection and limit of quantification

For estimation of limit of detection (LOD) and limit of quantification (LOQ), visualization method was followed. In visualization method lower dilutions of the standard drugs of SIT and SIM were successively prepared, injected in to the chromatograph and response obtained was recorded and presented in Table 5.

Robustness

The robustness of the method was carried out by small, but deliberate variations in the flow rate, mobile phase ratio and detection wavelength. Results are presented in Table 6.

Assay

The proposed method was also evaluated by the assay of SIT and SIM in their combined dosage formulation. The results are mentioned in Table 7.

Results and Discussion

Literature review reveals only few UV and HPLC methods have been reported for the simultaneous estimation of SIT and SIM in bulk and tablet dosage formulation till date. So, new simple, rapid, and accurate RP-HPLC method has been developed and validated for the simultaneous estimation of SIT and SIM in bulk and tablet dosage formulation. The separation was carried out by using Prontosil 120-5-CN (250 mm × 4.6 mm, 5 µm) column with a mobile phase consisting of acetonitrile:methanol:phosphate buffer (45:10:45 v/v). The mobile phase was delivered at a flow rate of 1.0 ml/min with detection at 254 nm. The retention times of SIT and SIM were found to be 4.8 and 6.0 min and the calibration curves were linear (R^2 = 0.999) over a concentration range from 1 to 30 µg/ml for SIT and SIM, respectively. LOD and LOQ were 0.6 µg/ml and 0.7 µg/ml for SIT and 0.1 µg/ml and 0.2 µg/ml for SIM, respectively. The developed method was validated for parameters such as system suitability, specificity, linearity, accuracy, precision, ruggedness, and robustness as per ICH guidelines and the results were found to be within the limits.

Conclusion

Good agreement was seen in the assay results of tablet formulation as well as in bulk by developed method. It can be concluded that the proposed method was good approach for obtaining reliable results and were found to be suitable for the routine estimation of SIT and SIM in bulk and tablet formulation.

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References

1. Available from: http://www.en.wikipedia.org/wiki/sitagliptin.svs. [Last accessed on 2011 Dec 19].
2. Available from: http://www.drug bank.ca/drugs/DB0126. [Last accessed on 2011 Dec 19].
3. Available from: http://www.merck.com/product/usa/pi_circulars/j/juvisync/juvisync_pi.pdf. [Last accessed on 2011 Dec 19].
4. Indian Pharmacopoeia. Vol. 3. Ghaziabad: The Indian Pharmacopoeia Commission; 2010. p. 2103-5.
5. United States of Pharmacopoeia and National Formulary. United States Pharmacopeial; 26th edition, November, 2002 p. 1571-2.
6. Harry GB. Analytical Profiles of Drug Substances and Excipients. Vol. 22. Academic Press, USA. The Academic Press; 2005. p. 359-88.
7. Phaneemdra D, Venkatesh V, Ramarao N. Simultaneous estimation of simvastatin and sitagliptin by using different analytical methods. Int J Adv Pharm Anal 2012;2:19-23.
8. Sonali DR, Pallavi MP, Vitthal VC, Pravin DC. Q-absorbance ration spectrophotometric method for the simultaneous estimation of sitagliptin phosphate and simvasatatin in their combined dosage form. Asian J Biochem Pharm Res 2012;2:162-9.
9. Sheetal S, Nimita M, Priya B, Sohail H, Prabhat J. Development of UV-spectrophotometry and RP-HPLC method and its validation for simultaneous estimation of sitagliptin phosphate and simvastatin in marketed formulation. Int J Pharm Bio Arch 2012;3:673-8.
10. Anilkumar V, Arunakumari V, Saikumar VS, Srinivasarao A, Santoshkumar T. Validated RP-HPLC method for the simultaneous estimation of sitagliptin and simvastatin in dosage forms. Int J Chem Anal Sci 2012;3:1611-4.
11. Kavitha KY, Geetha G, Hariprasad R, Venkatanarayana R, Subramanian G. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of sitagliptin and simvastatin. Int Res J Pharm 2012;3:123-7.
12. Burugula L, Mullangi R, Pilli NR, Makula A, Lodagala DS, Kandhagatla R. Simultaneous determination of sitagliptin and simvastatin in human plasma by LC-MS/MS and its application to a human pharmacokinetic study. Biomed Chromatogr 2013;27:80-7.
13. Rathod S, Patil P, Chopade V. Development and validation of HPTLC method for the estimation of sitagliptin phosphate and simvastatin in bulk and marketed formulation. Int J Drug Dev Res 2012;4:292-7.
14. ICH Guidance. Validation of Analytical Methods: Definition and Terminology. Geneva: International Conference on Harmonization, Q2A; Nov., 2005.
15. ICH Guidance. Validation of Analytical Procedures: Methodology. Geneva: International Conference on Harmonization, Q2B; Nov., 2005.

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