Research Article

SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND SIMVASTATIN IN TABLET DOSAGE FORM BY A VALIDATED RP-HPLC METHOD

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**ABSTRACT**

An economical approach was used to develop and validate a rapid, specific, accurate, and precise reverse phase high performance liquid chromatographic (HPLC) method for the simultaneous estimation of Sitagliptin (SGP) and Simvastatin (SMS) in pharmaceutical dosage forms. The chromatographic separation was achieved on HPLC Column C18 75 x 4.6 mm, 5µ short column; Mobile phase (60:40) was established by using a buffer consisting of 0.05M Ammonium acetate (pH adjusted to 4.0) and Acetonitrile as organic solvent in a gradient program. The flow rate was 1.0 mL.min^{-1} and the detection wavelength was 253 nm. The limit of detection (LOD) for SMS and SGP was 0.305 and 1.156 µg.mL^{-1}, respectively and their limit of quantification (LOQ) for SGP and SMS was 0.952 and 3.608 µg.mL^{-1}, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness.

Keywords: Sitagliptin, Simvastatin, HPLC, Simultaneous, Method Validation, Assay.

INTRODUCTION

Sitagliptinphosphatemonohydrate (**SGP**) chemically, (3R)-3-amino-1-[3(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo [4,3-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluoro phenyl) butan-1-onephosphate hydrate[1] is an oral anti-diabetic, which is available in 25 mg, 50 mg and 100 mg tablets for oral administration. SGP is used for the improvement of glycemic control in patients with type II diabetes mellitus as monotherapy or combination therapy with metformin or a peroxisome proliferator activated receptor gamma (PPAR) agonist (e.g., thiazolidinediones) when the single agent does not provide adequate glycemic control.
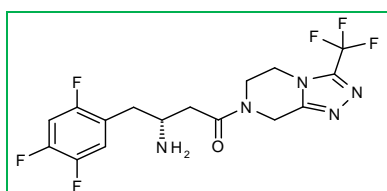
Figure 1: Chemical structure for SGP
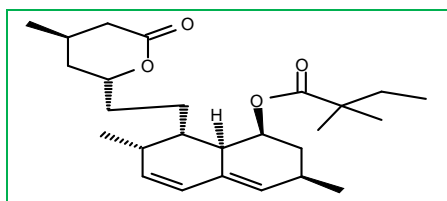
Figure 2: Chemical structure for SMS

Simvastatin (**SMS**) chemically known as (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia. It is a member of the statin class of pharmaceuticals and

is a synthetic derivate of a fermentation product of *Aspergillus terreus.*

The literature reveals that some methods have been reported for SGP and SMS with different drug combinations by using UPLC, UV and RHPLC techniques, Few UPLC[1], UV spectrophotometric methods[3,11-15], HPLC[2,6,8,10,16,17] and ion-pair HPLC[9] methods have been reported for the estimation of SMS. SGP is not yet official in any of the pharmacopoeia but SGP is official in IP, BP and USPNF. Literature survey reveals that only LC-MS[7] methods were reported for the determination of SGP in plasma and urine of Simultaneous Determination of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in 141 Sci Pharm. 2012; 80: 139–152.

MATERIALS AND METHODS

Pharmaceutical grade working standards and all chemicals and reagents were obtained from Chandra Laboratories (A Govt. Approved analytical testing laboratory), Hyderabad, and India.

Instrumentation

The apparatus used in this study were Waters- Alliance E2695 with empower 2 Software and UV-2489 detector with auto sampler HPLC, Micro processor –LP-1395 model pH meter, Biotechnics India-9L250H model Sonicator and Sartorius BSA 2245-CW balance. This method was operated at a wavelength of 253 nm (determined by UV – spectrophotometry). The column used was Short column C18 (75mm×4.6 mm, 5.0µm), different mobile phases were tested in order to find the best conditions for separation of SGP and SMS. The mobile phase contained Acetonitrile, 0.05 M Ammonium acetate (40:60) and the flow rate was maintained at 1.0 ml/min UV detection was

carried out at 253 nm. The mobile phase and samples was filtered using 0.45µm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

RESULTS AND DISCUSSION

Standard solutions preparation

100 mg SGP and 3mg SMS were weighed accurately and transferred to 50 ml volumetric flasks. All the drugs were dissolved in Mobile phase to prepare 2000 µg/ml of SGP and 60 µg/ml of SMS standard stock solutions. Calibration standards at six levels were prepared from this standard stock solution (the concentrations were 25, 50, 75, 100, 125, 150 µg/ml for SGP (Linearity range was 25 – 150 µg/ml) and 10, 20, 30, 40, 50, 60 µg/ml SMS (linearity range was 10 – 60 µg/ml) and peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample Preparation

For the analysis of a tablet dosage form, 10 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder amount equivalent to 100 mg of SGP and 3mg of SMS were transferred to a 50 ml volumetric flask and dissolved in 50 ml of mobile phase, sonication was done for 10 min with swirling. After sonication, the solution was filtered through a Whatman filter paper. Before the assay of tablet formulations, 6

replicate aliquots (Each 20 ml in volume) of the appropriately diluted tablet stock solution were sonicated for 8 min, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times. The possibility of excipient interference with the analysis was examined.

Method development

Optimization of chromatographic method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for SGP and SMS respectively. The mixed standard stock solution injected in HPLC with Different Mobile phase ratio's (seven trials) was tried.

Herein the present work, Author and his overall focus is to develop a suitable HPLC method for the analysis of SGP and SMS in fixed dose, combined dosage form. Initially Acetonitrile and water in different ratios were tried, but unacceptable retention times and no asymmetry in peaks., so water was replaced by potassium acetate, Ammonium Acetate (0.05 M) and by Ammonium acetate buffer (0.05 M; pH~4 in different ratios were tried (table 1). It was found that Acetonitrile : Ammonium Acetate in ratio of 40: 60(v/v) gave acceptable retention time, (RT 2.125 min for SGP and RT 5.044 min for SMS), plates, and good resolution for SGP and SMS at the flow rate of 1 ml/min)

Table 1: Selection of chromatographic conditions

S. No	COLUMN	MOBILE PHASE
1	INERTSIL ODS-3	METHANOL:WATER, 80:20
2	INERTSIL ODS-3	ACN :WATER, 60:40
3	INERTSIL ODS-3	ACN: WATER, 80:20
4	SYMMETRY C18	ACN: WATER, 80:20
5	SYMMETRY C18	0.01M POTASSIUM PHOSPHATE BUFFER :ACN 30: 70
6	INERTSIL ODS-3	0.01M POTASSIUM PHOSPHATE BUFFER: ACN 20: 80
7	**C 18 (75mm*4.6mm) 5µ, S.C**	**0.05M AMMONIUM ACETATE : ACN 60: 40 (pH~4)**

Table 2: Linearity results for SGP and SMS

S. No	Sitagliptin (SGP)		Simvastatin (SMS)	
	Concentration µg/ml	Peak area	Concentration µg/ml	Peak area
1.	25	440023	10	797145
2.	50	886133	20	1595582
3.	75	1312724	30	2400233
4.	100	1752182	40	3192209
5.	125	2188862	50	3978418
6.	150	2632535	60	4753369

Table 3: Accuracy studies results

Mixture of pure and formulation	Concentration of pure drug, µg/ml	Concentration of Formulation, µg/ml	% Recovery of pure drug
50+50	50	50.11	100.22
50+100	100	101.51	101.51
50+150	150	148.7	99.13

Table 4: Precision studies, each values mentioned above are mean of five replicates

Concentration µg/ml	Peak area	% RSD
40	3202109	0.23

Table 5: Statistical analysis of parameters required for system suitability

S. No.	Parameters	Values (SMS)	Values (SGP)
1	Theoretical Plates (N)	6637.000	3522
2	LOD, µg/ml	0.30520	1.15620
3	LOQ, µg/ml	0.95270	3.60890
4	Resolution	1.06067	1.420

Table 6: Recovery Studies

Formulation Injection	Labeled amount in mg.	Amount recovered in mg	% Recovery
1	40mg	39.81mg	99.50%

*Each value is determination of five replicates
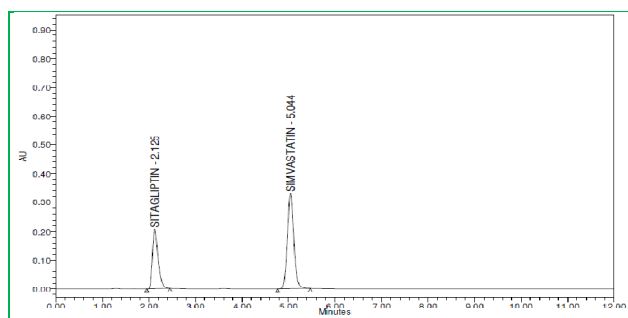
Figure 3: HPLC chromatogram for SGP and SMS
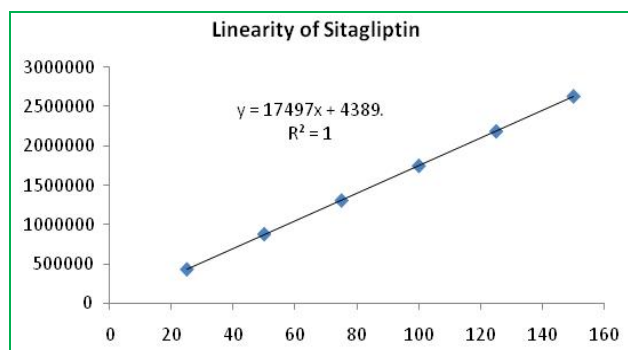
Figure 4: Linearity graph for SGP
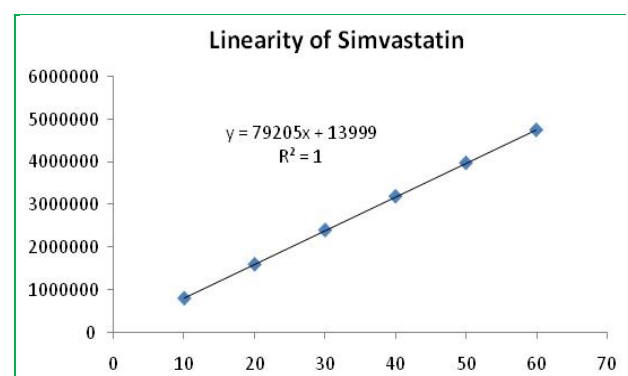
Figure 5: Linearity graph for SMS

Linearity

Linearity was evaluated by analysis of working standard solutions of SGP and SMS of six different concentrations. The range of linearity was from 25 -150 µg/ml for SGP and 10-60 µg/ ml for SMS.

The regression data obtained are represented in table 2. The results show that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration of each drug as shown as in figure 4 & 5.

Accuracy

Accuracy is the parameter of recovery, which involves quantitating the amount of analyte that can be retrieved, the accuracy of SGP and SMS was determined in three concentrations (50, 100 and 150 µg/ml) of each four replicates and one can see in table 3.

Precision

The results of the repeat of experiments in the same conditions to several times (5 times) are shown in Table-4. The developed method was found to be precise, with RSD values for repeatability and intermediate precision <2%, as recommended by ICH guidelines. Separation of the drugs was found to be similar when analysis was performed on different times **LOD** and **LOQ.**

System suitability

System suitability parameters such as the number of theoretical plates, Resolution, LOD, and LOQ are determined. The results obtained are shown in table 5.

Recovery studies

Good recoveries of the SGP and SMS were obtained at various added concentrations for the tablets as shown in table-6.

CONCLUSION

The new HPLC method described in this paper provides a simple, convenient, and reproducible approach for the simultaneous identification and quantification that can be used to determine sitagliptin, simvastatin in routine quality control.

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