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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR SIMVASTATIN AND SITAGLIPTIN PHOSPHATE WITH DEGRADATION STUDIES IN MARKETED PHARMACEUTICAL HYPO-CHOLESTEROL AND ANTIDIABETIC TABLETS

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ABSTRACT

Simvastatin is drug used with Sitagliptin Phosphate for a type 2 diabetes treatment. Simvastatin officially got approval from Merck in 1991 which emphasized its mechanism of action as HMG-CoA reductase inhibitor which is useful in reducing cholesterol levels in the body. However Sitagliptin Phosphate works as peptidase inhibitor. Thus Juvisync which was launched into the pharmaceutical market as solid dosage forms of simvastatin and Sitagliptin Phosphate specifically tablets gained popularity due to its dual functional medicinal activity. The combination is useful due to its synergistic effect in the patients for diabetes as well as high cholesterol levels.

The proposed method is developed and validated for these tablets using Reverse phase HPLC by photo diode array detector. The method also indicates stability testing by forced stress degradation studies. Drug discovery and drug development involves essential stage of stability testing during periodic studies of pharmaceutical preparations thus utilizing advanced Pharmaceutical techniques such as stress degradation sheds light on efficacy and safety of proposed and synthesized medicine.

Keywords: Simvastatin, Sitagliptin Phosphate, Stability, Cholesterol, Type 2.

1. INTRODUCTION

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf lives to be established.

Very few analytical methods have been cited in the literature for the estimation of SIM and SGLP individually or in combination with other drugs and also recently to our knowledge optimized UV method for the routine quality control analysis of SIM and SGLP simultaneously from tablets are now reported. Further, no stability-indicating method has been reported in literature for simultaneous determination of SIM and SGLP in presence of their degradants. Therefore, the present study targets the development and subsequent validation of a stability-indicating RP-HPLC-PDA method for the simultaneous determination of SIM and SGLP in presence of their degradants and to develop LC-MS method with MS compatible mobile phase. To establish the stability indicating nature of the method, forced degradation of each API and drug product was performed under stress conditions and stressed samples were analyzed by the proposed method.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Tablets used for analysis were JUVISYNC TABLETS manufactured by Merck &Co. India ltd. Thus, for analysis these tablets containing SIM 20 mg and SGLP100 mg per tablet. Pure drug sample of SIM (99.74%) and SGLP (99.85%) were obtained as a gift sample from Triveni Interchem Pvt. Ltd. Vapi, Gujrat and MSN Laboratories, Hyderabad, India Respectively. HPLC grade Methanol, acetonitrile and tetrahydrofuran (THF) were procured from Merck and Qualigens fine Chemicals, respectively (Mumbai, India). Ammonium Acetate and Acetic Acid was procured from Research Lab Fine Chem. (Mumbai, India). Double distilled water and tablet placebo was made at lab scale only.

2.2 HPLC Instrumentation and Conditions

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower-version 2 software. Separation was achieved on Qualisil BDS C8 column (250 mm × 4.6 mm, 5.0 µ) columns maintained at 60 ^{0}C using column oven. The mobile phase consisting of a buffer mixture 10 mM Ammonium dihydrogen Phosphate and 2mM hexane-1 sulphonic acid sodium salt and Acetonitrile. Thus isocratic elution was (methanol: acetonitrile): Phosphate buffer (64:12:24 v/v/v) pH adjusted to 5.5 with dil. Phosphoric acid at a flow rate of 1 ml/min was carried out. The detection was monitored at 268 nm and injection volume was 20 µL. The peak purity was checked with the photodiode array detector.

2.3 Standard solutions and calibrations graphs

Standard stock solution of SIM and SGLP (1000 µg/ml) were prepared separately in methanol. To study the linearity range of each component, serial dilutions of SIM and SGLP each were made from 0.1 - 100µg/ml and 0.5 - 500µg/ml, respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution. A typical chromatogram obtained from a standard solution is shown in Fig No.1.

2.4 Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg of SIM and 100 mg of SGLP was weighed and dissolved in the 80 ml of methanol with the aid of ultra-sonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 ml volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and volume was made up to the mark with methanol. From the filtrate, appropriate dilution was done in mobile phase to get a solution of 10 µg/ml of SIM and 50 µg/ml of SGLP respectively. A 20µL volume of each sample solution was injected into HPLC, six times, under the conditions described above. The peak area of the spots was measured at 268 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation. Chromatogram was recorded and the amounts of the drugs were calculated, as shown in Fig. No. 2.

Brand : JUVISYNC
Contents : Simvastatin-20mg
Sitagliptin Phosphate-100mg
Manufacturer: Merck & Co. India Pvt. Ltd.

2.5 Selection of analytical wavelength

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200-400 nm and the spectra were overlain. λ_{max} observed for SIM and SGLP was 240.2 nm and 268.9 nm respectively (Fig No.3). 268 nm wavelengths was selected for simultaneous determination of SIM and SGLP.
The retention time was found to be:
SIM : 6.217 ± 0.02 min
SGLP: 7.323± 0.02 min

2.6 Method validation

The stability indicating method was validated in terms of precision, accuracy and linearity according to ICH guidelines.

2.6.1 Precision

The precision of repeatability was studied by replicate (n=3) analysis of tablet solutions. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation.

2.6.2 Accuracy

The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Recovery studies were carried out by

applying the method to drug content present in tablet dosage form to which known amount of mix standard of SIM and SGLP was added at 50 %, 100 % and 150 % levels. The technique involves addition of standard drug solution to pre-analyzed sample solution. The resulting sample solutions were injected and chromatograms were recorded. And the concentrations of both the standard drugs from tablet sample were determined using the respective calibration graphs. At each of the levels, three determinations were performed and results were obtained.

2.6.3 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analytes in the sample. For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity test solutions were prepared.

2.6.4 LOD and LOQ

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response and Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOD and LOQ were calculated using the following formula:

- ✓ LOD = (3.3 x σ)/ b
- ✓ LOQ = (10 x σ)/ b

Where σ = Standard deviation of the response
b = Slope of the calibration curve

2.6.5 Robustness

To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 ml/min, the percentage of methanol was varied by (±) 2%, column temperature was varied by (±) 1 ^{0}C, pH of mobile phase was varied by (±) 0.1, salt concentration of buffer was varied by (±) 5 Millimoles, the column was changed from different manufacturer and wavelength of measurement was changed by (±) 1nm.

For method development and optimization, retention factor (*k*) was calculated using the equation: $k = (t_R - t_M)/ t_M$. Where, t_R = retention time, t_M = is the elution time of the solvent front.

2.7 Procedure for forced degradation study

The API and the tablet solution were subjected to various forced degradation conditions to effect partial degradation of the drug preferably in 10 - 30% range, to determine whether any observed degradation occurred because of drug properties or was due to drug–excipient interactions. Moreover, the studies provide information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities. The stability samples were prepared by dissolving each

API or drug product in methanol and later diluted with either distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide or aqueous hydrogen peroxide solution at a concentration of 1000 (SIM) and 5000 (SGLP) µg/ml , separately. After degradation, these samples were diluted with mobile phase to achieve the nominal concentration of 20(SIM) and 100 (SGLP) µg/ml, which was based on their label strength in tablets. The results are shown in Table 6.8. Standard stock solution of SIM and SGLP were prepared by taking 100 mg in 100 ml of methanol. Then from that standard solution, 1 ml was transferred to 10 ml of amber colored flask, to it 2 ml each of 3M HCl and 1M NaOH were added separately and volume was made up to 10 ml with distilled water. It was subjected to selected stress condition and diluted with mobile phase to nominal concentration. Sample solution of SIM and SGLP were also prepared by taking 100 mg in 100 ml of methanol. Then from that sample solution, 2 ml was transferred to 10 ml of amber colored flask, to it 2 ml each of 3M HCl and 1M NaOH were added separately and volume was made up to 10 ml with distilled water. It was subjected to selected stress condition and diluted with mobile phase to nominal concentration.

2.8 Acid and Base Hydrolysis

To 1 ml of methanolic stock solution of SIM and SGLP, 2 ml each of 3M HCl and 1M NaOH were added separately and volume was made up to 10 ml with distilled water. These mixtures were heated at 70 ^{0}C for 6 h for acid degradation and at 80 ^{0}C for 3 hr for alkali degradation and were kept in a water bath then left to equilibrate to ambient temperature. It was observed that both acid and base hydrolysis was a fast reaction for both drugs and almost completed within 6 hrs of the sample preparation, therefore the samples were analyzed after this period of time, therefore the samples were analyzed after this period of time. The solution was then adjusted to neutralize the solution with 1 M NaOH and 3M HCL and then diluted to 10 ml with mobile phase to get concentration of

20 µg/ml SIM and 100 µg/ml SGLP and 20 µL was injected into the system. The same condition was applied to tablet solution and was further diluted with mobile phase to get

concentration of 20µg/ml SIM and 100µg/ml SGLP and 20 µL was injected into the system.

2.9 Oxidation Studies

To 1 ml of methanolic stock solution of SIM and SGLP, 3 ml of 30% w/v hydrogen peroxide (H_2O_2) was added. The mixture was left for 8 h at ambient temperature then diluted to 10 ml with mobile phase to get concentration of 20µg/ml SIM and 100µg/ml SGLP and 20 µL was injected into the system. The same condition was applied to tablet solution and was further diluted with mobile phase to get concentration of 20 µg/ml SIM and 100 µg/ml SGLP and 20 µL was injected into the system.

2.10 Dry heat Degradation

Approximately 10 mg drug product powder SIM and SGLP was spread in a flat-bottomed tube to give a homogeneous layer (<5mm thick) and subjected to dry heat degradation. Also the tablet powder equivalent to 0.1 mg of SIM (20 mg of SGLP) was left at same condition. The sample was then diluted to obtain solution containing 20 µg/ml of SIM & 100 µg/ml of SGLP.

2.11 UV Degradation

Samples were exposed to short (in Pyrex container) and long UV radiations (in quartz container) for 48 hrs and were used. Solutions of SIM and SGLP (100mg/ml) in distilled water were placed in Pyrex (visible and long-wavelength UV-light) or quartz vessels (short wavelength UV-light) and exposed to forced irradiation (at 15 cm from the sources) in a 40 cm×30cm×30cm chamber fitted with either four Philips F4T5/D daylight fluorescent lamps (6500K) or four Philips G4T5 short-wavelength UV-lamps (4Weach). Irradiation with long-wavelength UV-light was carried out with a Philips ML Wblack-light lamp (160W). For chromatographic analyses, 20 µL of the solution was injected into the system.

2.12 Neutral Hydrolysis

For Neutral Hydrolysis study, the solution of SIM and SGLP separately and in mixture prepared using distilled water were kept at 70ºC for 1 hour. The sample was then diluted to obtain solution containing 20 µg/ml of SIM & 100 µg/ml of SGLP and then 20 µL of the solution was injected into the system. Stress condition should be uniform for both the drugs. So as per ICH guidelines the study was extended to formulation also.

3. Method Optimization

The HPLC method was optimized with a view to develop a reversed-phase HPLC method for SIM and SGLP in tablet dosage form. A well-defined symmetrical peak was obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials that can be summarized. Two columns were used for performance investigations, including Kromasil C_{18} (5 micron 4.6×250mm) and Qualisil C_8 (5 micron 4.6×250mm), the second column was the most suitable one since it produced symmetrical peaks with high resolution.

The UV detector response of SIM and SGLP was studied and the best wavelength was found to be 268 nm showing highest sensitivity. Development studies revealed that Methanol: ACN: phosphate buffer pH 5.5 (64:12:24v/v/v) mobile phase at the flow rate of 1 ml/min were suitable conditions for a stability-indicating method for study of the degradation of SIM and SGLP. Our objectives in chromatographic method development were to achieve a peak tailing factor <2 and retention times from 3 to 10 min.

Under the optimized conditions SIM, SGLP and its degradation products were well separated. Although the conditions used for forced degradation were attenuated to achieve degradation in the range 10–30%, this could not be achieved for oxidative condition, thermal and photolytic degradation even after prolonged exposure. The drug was extensively degraded by acid hydrolysis and alkaline hydrolysis condition.

4. RESULTS AND DISCUSSION

4.1 Method Validation

The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness. System suitability was established by injecting standard solution and results are given in Table No. 1.

The chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from the tablet excipients was found. Peak purity was verified by confirming homogeneous spectral data for SIM and SGLP.

4.2 Linearity

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity was determined for SIM in the range of 0.1-100 µg/ml and for SGLP 0.5-500 µg/ml. The correlation coefficient ('r') values were >0.999(n = 6). Typically, the regression equations for the calibration curve were found to be y = 44508.02 X + 20986.82 for SIM, y=19221.30 X + 64211.84

for SGLP. The results are shown in Table No. 2 & 3.

4.3 Formulation Analysis

The assay for the marketed tablets was established with present chromatographic condition developed. The average drug content was found to be 99.65 % for SIM and 99.98 % for SGLP of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results are given in Table No. 4A & B.

4.4 Precision

The precision of the method was done by replicate (n=3) analysis of tablet preparations. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table No. 5A& 5B

4.5 Accuracy

The accuracy of the assay method was evaluated with the recovery of the standards from excipients. The mean percentage recoveries obtained for SIM and SGLP were 99.76% and 99.81%, respectively, reported in Table No.6.

Limit of Detection (LOD)

SIM : 0.03 µg/ml
SGL : 0.2 µg/ml

Limit of Quantification (LOQ)

SIM : 0.1 µg/ml
SGLP : 0.6 µg/ml

4.6 Specificity

A blend of commonly used tablet excipients was treated as per developed procedure and the chromatogram shows no interfering peaks at retention time of the drug. In Stress Degradation studies all degradation peaks were well separated and well resolved.

4.7 Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, and System suitability parameters were found to be within acceptable limits. Results were shown in Table 8.7 indicating that the test method was robust for all variable conditions.

4.8 Stress Degradation

The API and the tablet solution were subjected to various forced degradation conditions to effect partial degradation of the drug preferably in 20–80% range, to determine whether any observed degradation occurred because of drug properties or was due to drug–excipients interactions. The results are shown in fig. 4, 5, and 6

4.9 Acid and Base induced

The chromatogram of the SIM acid degraded sample showed one additional peak. The degradation peak was observed at t_R 6.2 min and the chromatogram of the SIM base degraded samples showed one additional peak at t_R 6.3 min

The chromatogram of the SGLP acid degraded sample showed two additional peaks at t_R 8.10 & 9.21 min and the chromatogram of the SGLP base degraded samples showed one additional peak at t_R 8.80 min. The t_R at 8.80 min, which was the major degradation peak of SGLP and was identified as Aceclofenac free acid.

4.10 Hydrogen Peroxide induced

SIM and SGLP, 30% H_2O_2 degraded samples did not show any degradation peak under H_2O_2.

4.11 Dry heat Degradation Product

SIM did not show any degradation peak but SGLP showed one additional peak at peak at t_R 10.00 min under dry heat condition.

4.12 UV Degradation Product

In HPLC, sample showed no degradation for short and long UV radiation.

4.13 Neutral Hydrolysis

The samples showed no degradation under Neutral Hydrolysis conditions.

5. CONCLUSION

The developed methods were found to be simple, sensitive, accurate, precise and reproducible and can be used for the routine quality control analysis of SIM and SGLP in bulk drug and marketed formulation. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one. The method is sensitive enough for quantitative detection of the analytes in pharmaceutical preparations and can thus be used for routine analysis, quality control, and for stability studies of analytes. This method can be applied for LC-MS and Bio-analytical studies.

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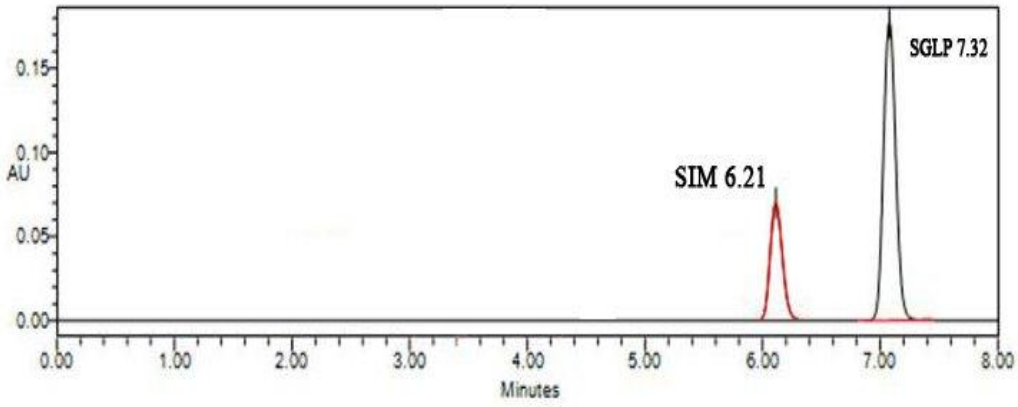

Fig. 1: Chromatogram of working standard SIM and SGLP

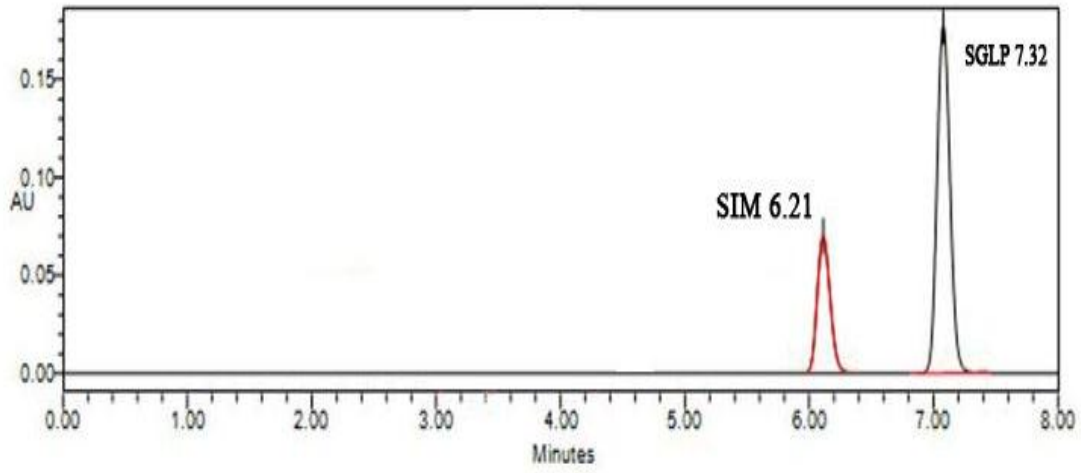

Fig. 2: Chromatogram of formulation consisting SIM (20 µg/ml) and SGLP (100 µg/ml)

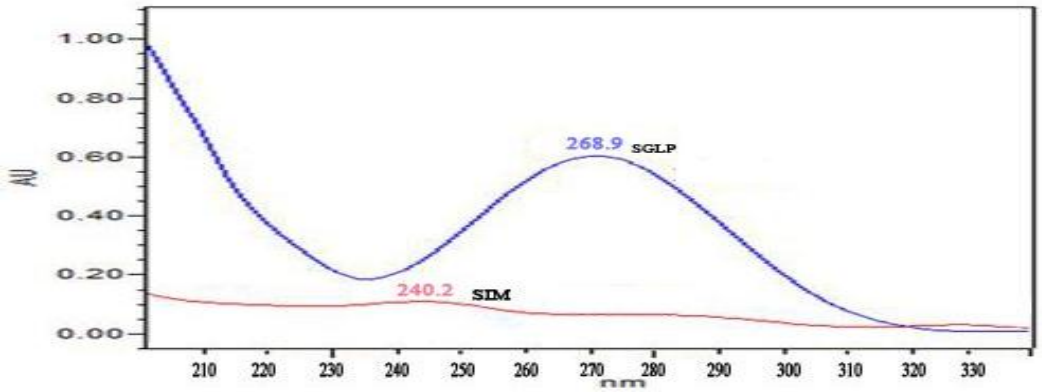

Fig. 3: Online Overlain PDA spectrum of SIM (20 µg/ml) and SGLP (100 µg/ml)

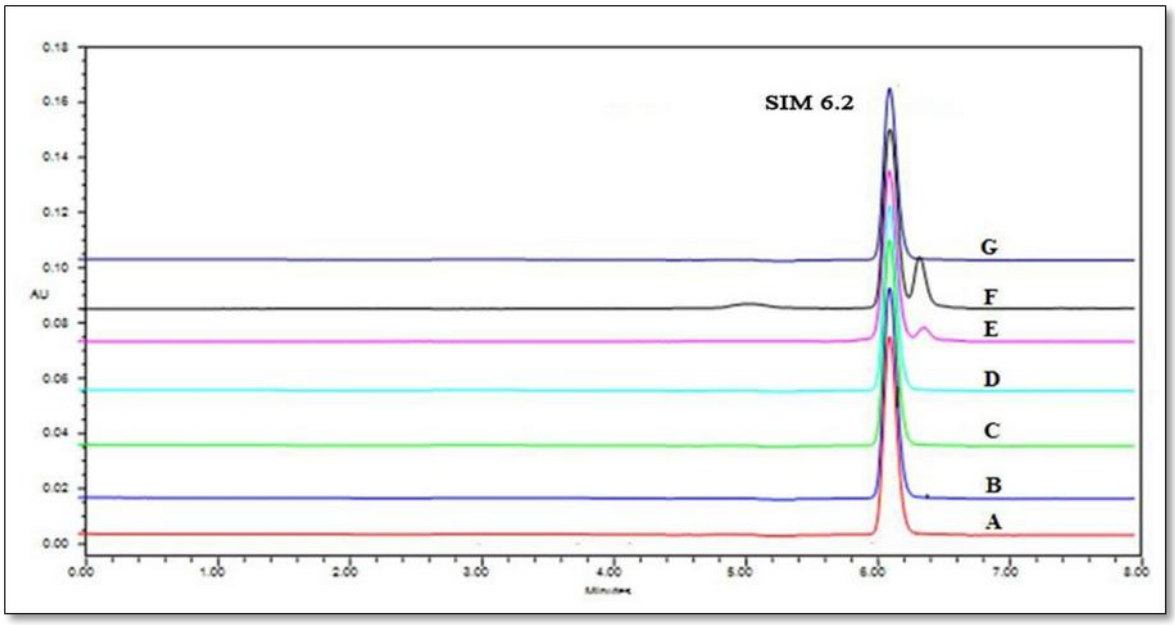

Fig. 4: Overlain Chromatogram consists of A) Long UV, B) Short UV, C) Moist Heat D) Dry Heat E)1 M NaOH F) 3n Hcl G) 30%H202 Degradation Of Sim (20 µg/Ml)

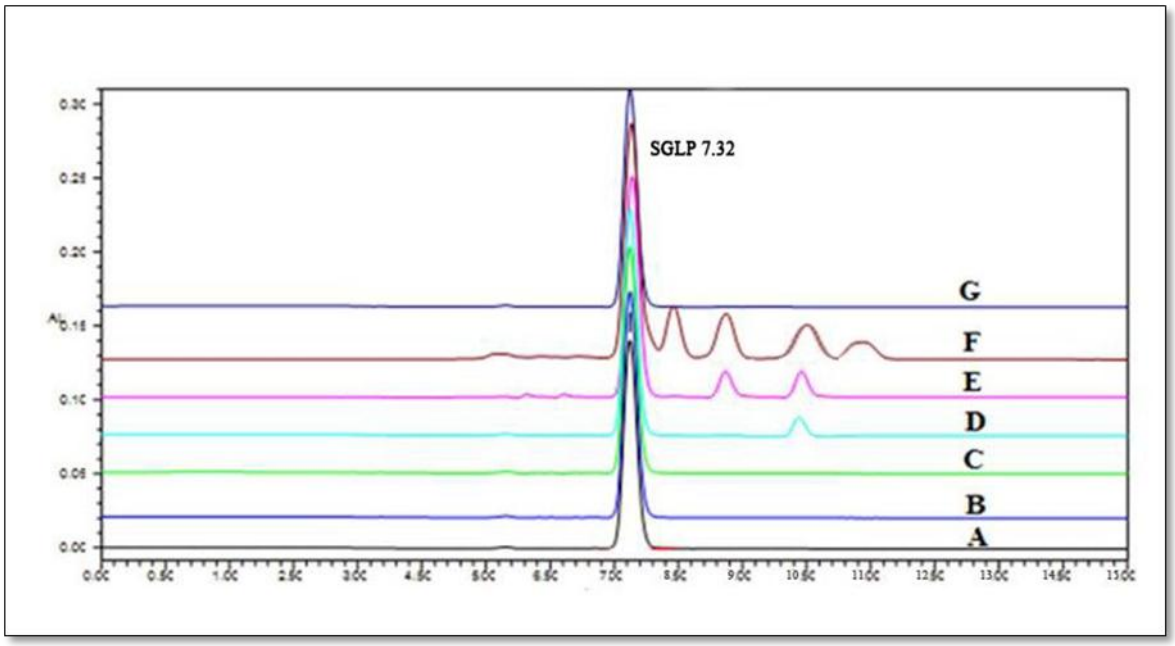

Fig. 5: Overlain Chromatogram consists of A) LONG UV, B) SHORT UV, C) MOIST HEAT, D) DRY HEAT E) 1 M NAOH F) 3N HCL G) 30%H202 degradation of SGLP (100 µg/ml)

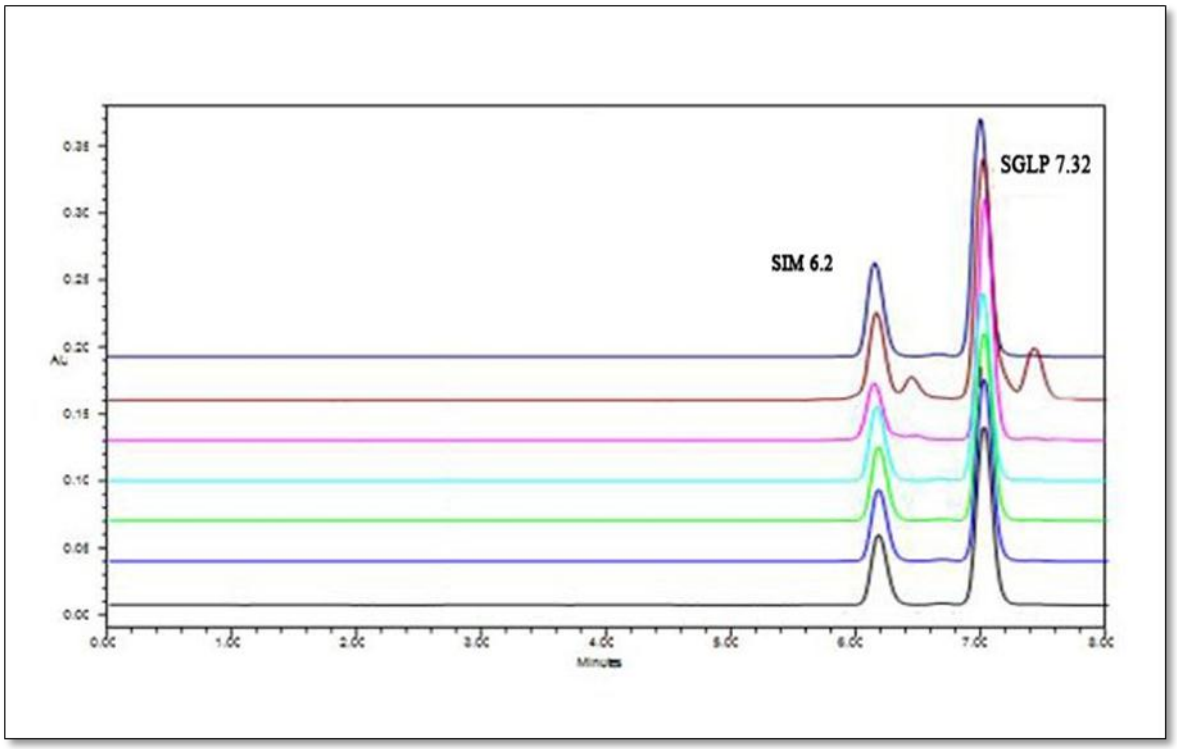

Fig. 6: Overlain Chromatogram consists of A) LONG UV, B) SHORT UV, C) MOIST HEAT, D) DRY HEAT E) 1 M NAOH F) 3N HCL G) 30%H_2O_2 degradation of SIM (20 µg/ml) and SGLP (100 µg/ml)

Table 1: System suitability parameters and peak purity data of SIM and SGLP (n=6)

COMPOUND	SYSTEM SUITABILITY AND PEAK PURITY	
	PARAMETER	**VALUE**
SIM	Area	504195
	Theoretical plates (T.P.)	4394
	USP resolution[a]	-
	Peak Tailing[a]	1.15
	K prime	2.16
	% R.S.D.(T.P.)	0.86
	Purity Angle	0.24
	Purity Threshold	3.63
SGLP	Area	1276402
	Theoretical plates (T.P.)	6566
	USP resolution[a]	3.51
	Peak Tailing[a]	1.09
	K prime	2.84
	% R.S.D.(T.P.)	0.79
	Purity Angle	0.16
	Purity Threshold	0.27

[a]USP-NF 29 section 621, pp. 2135

Table 2: Linearity of SIM (n=6)

Standard ⇨ Concentrations	0.1 µg/ml	0.8 µg/ml	8 µg/ml	20 µg/ml	40 µg/ml	100 µg/ml
Replicates ⇩	Peak Area					
1	6988	41061	341978	932310	1871435	4420149
2	6999	41069	341989	942311	1871887	4420178
3	6890	42078	341987	932319	1872996	4520158
4	6979	41189	351013	932379	1824435	4438198
5	6996	41098	341998	942315	1872789	4420199
6	6994	41099	341567	932398	1871998	4421185
Mean	6974.333	41265.67	343422	935672	1864257	4440011
SD	41.91738	400.5544	3722.632	5144.207	19517.28	39903.91
% RSD	0.601023	0.970672	1.08204	0.549787	1.04692	0.898734

Regression Equation : Y= 44508.02 X + 20986.82
Coefficient of correlation: 0.9997

Table 3: Linearity of SGLP (n=6)

Standard ⇨ Concentrations	0.6 µg/ml	5 µg/ml	50 µg/ml	125 µg/ml	250 µg/ml	625 µg/ml
Replicates ⇩	Peak Area					
1	19311	111260	901871	2424159	4856106	11459020
2	19290	112378	903689	2526178	4866147	1167898
3	19487	111258	914867	2424175	4866259	1145968
4	19299	112789	901879	2424186	4956199	1145978
5	19497	111288	901890	2424197	4856256	1145989
6	19490	111295	911898	2424199	4856189	1145996
Mean	19395.67	111711.3	906015.7	2441182	4876193	1149622
SD	105.0593	688.1231	5825.336	41639.21	39501.13	8953.52
% RSD	0.541664	0.615983	0.642962	1.705698	0.810081	0.778823

Regression Equation : Y= 19221.30 X + 64211.84
Coefficient of correlation: 0.9997

Table 4A: Analysis of Tablet Formulation I (n=6)

Sr. No.	Label Claim (mg/tab)		Amount found (mg/tab)		% of Label claim determine	
	SIM	SGLP	SIM	SGLP	SIM	SGLP
1	20	100	19.79	100.42	98.49	99.42
2	20	100	19.78	99.91	99.38	100.21
3	20	100	19.98	99.99	100.69	100.99
4	20	100	20.12	100.89	100.08	99.89
5	20	100	20.01	99.50	99.25	99.80
6	20	100	19.98	99.87	100.06	99.57
Mean			7.96333	50.0966	99.65833	99.98
SD			0.15266	0.48737	0.776232	0.56483
%RSD			1.21710	0.97281	0.778894	0.56494

Table 4B: Analysis of Tablet Formulation II

Sr. No.	Label Claim (mg/tab)		Amount found (mg/tab)		% of Label claim determine	
	SIM	SGLP	SIM	SGLP	SIM	SGLP
1	20	100	20.12	100.12	99.49	99.42
2	20	100	20.09	99.39	98.45	98.56
3	20	100	19.88	99.87	98.67	99.06
4	20	100	19.86	99.89	99.12	99.04
5	20	100	20.11	99.50	100.05	100.02
6	20	100	20.09	100.93	99.78	99.89
Mean			8.025	49.95	99.26	99.33167
SD			0.120789	0.55052	0.573178	0.507885
%RSD			1.50516	1.1021	0.577451	0.511302

Table 5A: Intraday and Inter day precision of SIM (n=3)

SIM	Measured concentration (µg/ml), % R.S.D	
Conc. (µg/ml)	Intra day	Inter day
4	3.08, 1.05	3.99, 0.71
8	8.05, 0.86	8.01, 0.58
12	11.95, 0.68	12.02, 0.95

Table 5B: Intraday and Inter day precision of SGLP (n=3)

SGLP	Measured concentration (µg/ml), % R.S.D	
Conc. (µg/ml)	Intra day	Inter day
25	24.08, 1.04	25.09, 1.07
50	49.07, 0.73	50.01, 0.59
75	74.09, 0.69	75.02, 0.88

Table 6: Results of the recovery analysis of SIM and SGLP (n=3)

Compound	Recovery Level (%)	Qty. spiked (µg/ml)	Qty. recovered (µg/ml)	Recovery (%)	R.S.D (%)
SIM	50	25	25.08	99.62	0.69
	100	50	49.96	99.88	0.87
	150	75	74.12	99.78	0.61
SGLP	50	156	155.14	99.55	0.52
	100	312	312.06	100.01	0.77
	150	468	467.99	99.89	0.98

Table 7: Results of robustness study (n=3)

Parameter (Limit)	Level	Analyte Name	System Suitability Parameters (SD) n=3				% Assay, % RSD, n=3
			t_R	N	Rs	K	
Flow rate ml/min (± 0.1 mL)	0.9(-)	SIM	3.16	4394	---	2.16	99.69, 0.46
	1.1(+)	SGLP	3.84	6566	3.51	2.84	100.02, 0.57
% of Organic (± 2%)	62(-)	SIM	3.11	4392	---	2.12	99.46, 0.42
	66(+)	SGLP	3.83	6564	3.57	2.87	99.88, 0.40
pH of Mobile Phase (±0.1mL)	6.4(-)	SIM	3.10	4390	---	2.13	99.58, 0.28
	6.6(+)	SGLP	3.81	6568	3.50	2.88	100.01, 0.55
Separation column	Column I[a]	SIM	3.08	4387	---	2.15	99.75, 0.77
	Column II[b]	SGLP	3.78	6564	3.49	2.88	99.84, 0.38
Measurement Wavelength (± 1nm)	257(-)	SIM	3.12	4395	---	2.17	99.69, 0.25
	259(+)	SGLP	3.79	6569	3.46	2.82	99.86, 0.95
Buffer strength (± 5milimoles)	20(-)	SIM	3.16	4391	---	2.11	99.98, 0.87
	30(+)	SGLP	3.85	6562	3.50	2.84	99.78, 0.92
Column Temp. (± 1 ^{o}C)	59(-)	SIM	3.10	4393	---	2.10	100.02, 0.16
	61(+)	SGLP	3.81	6566	3.52	2.89	99.48, 098

Table 8: Degradation studies of SIM and SGLP by using HPLC

Sr.No	Exposure Condition	SIMVASTATIN		SITAGLIPTIN PHOSPHATE	
		t_r (min)of degradation product	%Deg.	t_r (min)of degradation product	%Deg.
1	Acid, 3N HCl (70º C)	6.02	19.94%	7.17,5.43	22.66%
2	Base1M NaOH (70º C)	6.18	21.49%	7.32	23.25%
3	H_2O_2 (30%, v/v)	---	---	No deg. peaks	---
4	Dry Heat 80 ^{0}C	No deg. peaks	---	5.43	19.98%
5	Short Wavelength UV	No deg. peaks	---	No deg. peaks	---
6	Long Wavelength UV	No deg. peaks	---	No deg. peaks	---
7	Neutral hydrolysis	No deg. peaks	---	No deg. peaks	---

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