Research Article

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Sitagliptin Phosphate and Lobeglitazone Sulphate in Synthetic Mixture

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ABSTRACT

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Sitagliptin Phosphate and Lobeglitazone Sulphate in a Synthetic Mixture. A robust and stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Sitagliptin Phosphate and Lobeglitazone Sulphate in a synthetic mixture. The method was optimized using an Xselect C18 column (150 mm × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of Buffer and Methanol in a 50:50 (% v/v) ratio. Chromatographic analysis was performed at a flow rate of 0.8 mL/min, with detection set at 268 nm. The method was validated following ICH guidelines, confirming its suitability for pharmaceutical analysis. Forced degradation studies demonstrated its stability-indicating capability, as degradation was observed under various stress conditions, including acidic, basic, oxidative, photolytic, and thermal environments. The developed RP-HPLC method exhibited linearity within the concentration range of 100–300 µg/mL for Sitagliptin Phosphate and 0.5–1.5 µg/mL for Lobeglitazone Sulphate. The correlation coefficients were determined to be 0.9995 for Sitagliptin Phosphate and 0.9994 for Lobeglitazone Sulphate, confirming an excellent linear relationship. Precision and Robustness. The method demonstrated high reliability and reproducibility, with the %RSD for both precision and robustness studies remaining below 2%.

Keywords: RP-HPLC, Method Development, Validation, Forced degradation, Sitagliptin Phosphate, Lobeglitazone Sulphate.

INTRODUCTION

Diabetes mellitus is a chronic medical condition that arises when the body either fails to produce sufficient insulin or is unable to use it effectively. This leads to abnormally high blood sugar levels. Under normal circumstances, the body converts food into glucose, which serves as a primary energy source. Insulin, a hormone present in the bloodstream, enables cells to absorb glucose and utilize it for energy. [1-2]

Sitagliptin Phosphate

Sitagliptin Phosphate functions as a selective inhibitor of the enzyme **dipeptidyl peptidase-4 (DPP-4)**. By inhibiting DPP-4, Sitagliptin prolongs the action of these hormones, leading to increased pancreatic glucose-dependent insulin secretion and reduced hepatic glucose production, thereby helping to regulate blood sugar levels effectively. [3]

Lobeglitazone Sulphate

One of the key effects of Lobeglitazone Sulphate-induced PPAR-γ activation is the upregulation of genes involved in glucose absorption and utilization. This includes the enhanced expression of glucose transporter type 4 (GLUT4) in muscle and adipocyte cells, facilitating increased glucose uptake and thereby lowering blood glucose levels. [4]

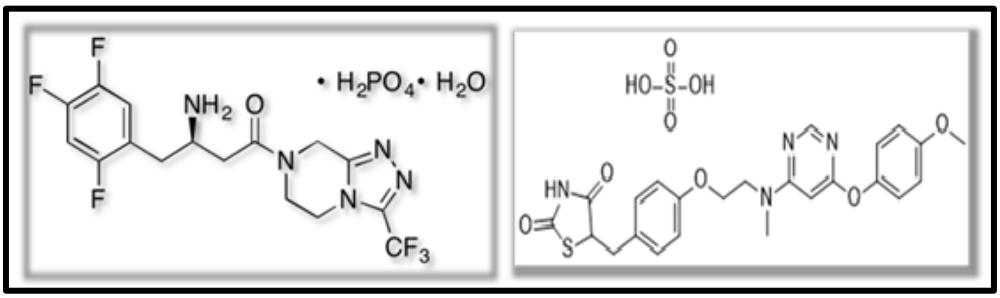

Figure 1: Structure of Sitagliptin Phosphate and Lobeglitazone Sulphate

The literature review reveals that few analytical methods were reported like RP-HPLC methods[5], Spectrophotometric method[6], LC/MS[7] and Stability study[8-10] in single or in Combination with other drug in bulk and pharmaceutical dosage form. But no method is reported for stability study. Hence the present study aimed to develop a new Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Sitagliptin Phosphate and Lobeglitazone Sulphate in Synthetic Mixture suitable for routine quality control analysis.

MATERIALS AND METHODS

Chemicals and Reagents

The Sitagliptin (SITA) API and Lobeglitazone Sulphate (LOBE) API were generously provided as a gift sample by Leaf Pharma, Gujarat. Other common reagents such as methanol, acetonitrile provided as Rankem (HPLC grade) Pvt. Ltd and Potassium dihydrogen phosphate, dipotassium hydrogen phosphate and HPLC grade water provided in a merck life science Pvt.LTD.

Preparation of standard Stock solution

Preparation of Standard Stock Solution of sitagliptin phosphate monohydrate (2000 µg/ml):

Accurately weight and transferred about 100 mg of sitagliptin phosphate monohydrate into 50 ml volumetric flask and diluted with methanol.

Preparation of Standard Stock Solution of Lobeglitazone sulfate (10 µg/ml):

Accurately weight and transferred about 5 mg of Linagliptin WS into 500 ml volumetric flask and diluted with methanol.

Preparation of standard solution of binary mixtures of sitagliptin phosphate monohydrate and Lobeglitazone sulfate (200 µg/ml + 1 µg/ml):

Pipette out 1 ml of SITA stock solution and 1 ml of LOBE stock solution into 10 ml volumetric flask. Volume was made upto the mark with methanol.

Preparation of mobile phase

3.45 mg of di-potassium hydrogen phosphate has been accurately weighed and dissolved in 1000 mL of water by intermittent shaking. pH was then adjusted to 3.0 by diluted ortho phosphoric acid.

Mobile phase: Buffer 500 mL and Methanol 500 mL has been mixed to make 1 L mobile phase. Mobile phase was then filtered under vacuum by 0.45 micron membrane filter.

Forced Degradation Study

1) Acid Degradation

A 1 mL aliquot of the stock solution of Sitagliptin and Lobeglitazone was transferred into a 10 mL volumetric flask. Then, 1 mL of 0.1N HCl solution was added, mixed thoroughly, and kept at room temperature (25°C) for 12 hours. The solution was then neutralized with 1 mL of 0.1N NaOH solution, and the volume was adjusted with the diluent to prepare the sample solution.

2) Base Degradation

A 1 mL aliquot of the stock solution of Sitagliptin and Lobeglitazone was transferred into a 10 mL volumetric flask. Then, 1 mL of 0.1N NaOH solution was added, mixed thoroughly, and kept at room temperature (25°C) for 12 hours. The solution was then neutralized with 1 mL of 0.1N HCl solution, and the volume was adjusted with the diluent to prepare the sample solution.

3) Oxidation Degradation

A 1 mL aliquot of the stock solution of Sitagliptin and Lobeglitazone was transferred into a 10 mL volumetric flask. Then, 1 mL of 3% H_2O_2 solution was added, mixed thoroughly, and kept at room temperature (25°C) for 12 hours. Afterward, the volume was adjusted with the diluent to prepare the sample solution.

4) Photolytic Degradation

A 1 mL aliquot of the stock solution of Sitagliptin and Lobeglitazone was transferred into a 10 mL volumetric flask. The solution was then exposed to sunlight for 2 days. Afterward, the volume was adjusted and diluted with the diluent to prepare the sample solution.

5) Thermal Degradation

Sitagliptin and Lobeglitazone were placed in a petri dish and kept in an oven at 70°C for 12 hours. After the exposure, the samples were prepared for further analysis.

***Method Validation*[11]**

1) Linearity and Range (n=3)

The linearity for Sitagliptin Phosphate Monohydrate and Lobeglitazone Sulfate were assessed by analysis of combined standard solution in range of 100-3000 µg/ml and 0.5-1.50 µg/ml respectively. Correlation co-efficient for calibration curve Sitagliptin Phosphate Monohydrate and Lobeglitazone Sulfate was found to be 0.9995 and 0.9994.

2) Precision

Precision was evaluated at three levels: intermediate precision (intraday precision), reproducibility (interday precision), and repeatability. The solution containing 200µg/ml of Sitagliptin Phosphate Monohydrate and 1µg/ml of Lobeglitazone Sulfate was injected six times for repeatability study. Intermediate precision study was performed by injecting 100, 200, 300 µg/ml of Sitagliptin Phosphate Monohydrate and 0.5, 1.0, 1.5 µg/ml of Lobeglitazone Sulfate solutions three times for each aliquot. The %RSD for precision was calculated.

3) Limit of Detection and Limit of Quantitation

The LOD and LOQ were separately determined from calibration curve. Calibration curve was repeated for three times and the standard deviation (SD) of the intercept was calculated. Then LOD and LOQ were calculated using following equation:

$$LOD = 3.3 * \sigma/S \text{ and } LOQ = 10 * \sigma/S$$

Where, σ = Standard deviation of Y-intercepts, S = Mean slope of calibration curve.

4) Accuracy

The accuracy of the method was assessed using the standard addition technique, where a known amount of the working standard was spiked into a placebo at three concentration levels: 50%, 100%, and 150% of the standard

concentration. Each solution was injected in triplicate and the recovery was calculated by measuring peak areas.

5) Robustness

The robustness of the analytical procedure was evaluated to assess its ability to remain unaffected by minor but deliberate variations in method parameters, ensuring its reliability during routine use. Robustness testing was conducted (n=3) by altering key parameters, including:

Flow rate of the mobile phase (± 0.2 ml/min)

Detection wavelength (± 2 nm)

Column temperature (± 5°C).

RESULT AND DISCUSSION

Optimized Chromatographic Conditions

Shimadzu HPLC system was used for method development, degradation studies and validation. Data acquisition was performed on HPLC. The separations were achieved on Xselect C18 (150 mm × 4.6 mm × 5 µ), column. The column was maintained at Room temperature and the eluent was monitored at 268 nm using detector. The mobile phase of Buffer: Methanol (50:50 % v/v) mixture at a flow rate of 0.9ml/min was used as a mobile phase. The injection volume was 50µl.

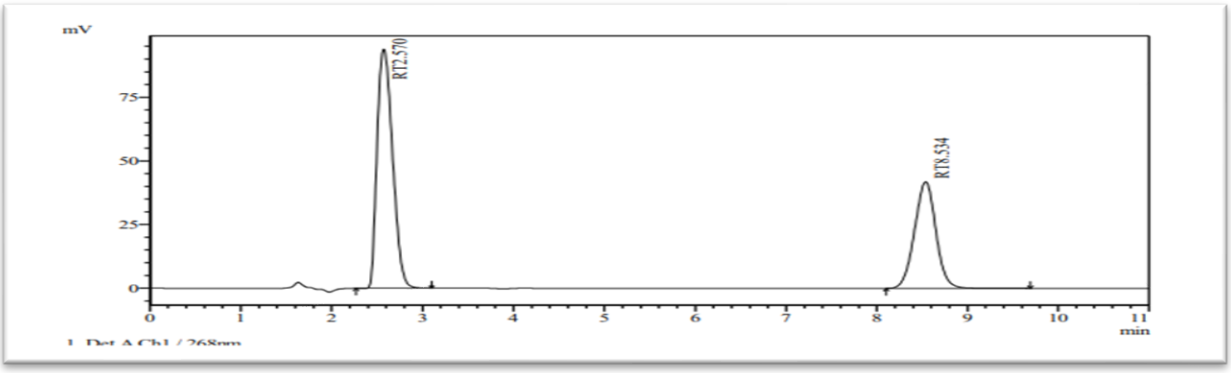

Figure 2: Optimized Chromatogram of SITA and LOBE Buffer: Methanol (50:50 % v/v)

Forced Degradation Study

1) Acid Degradation

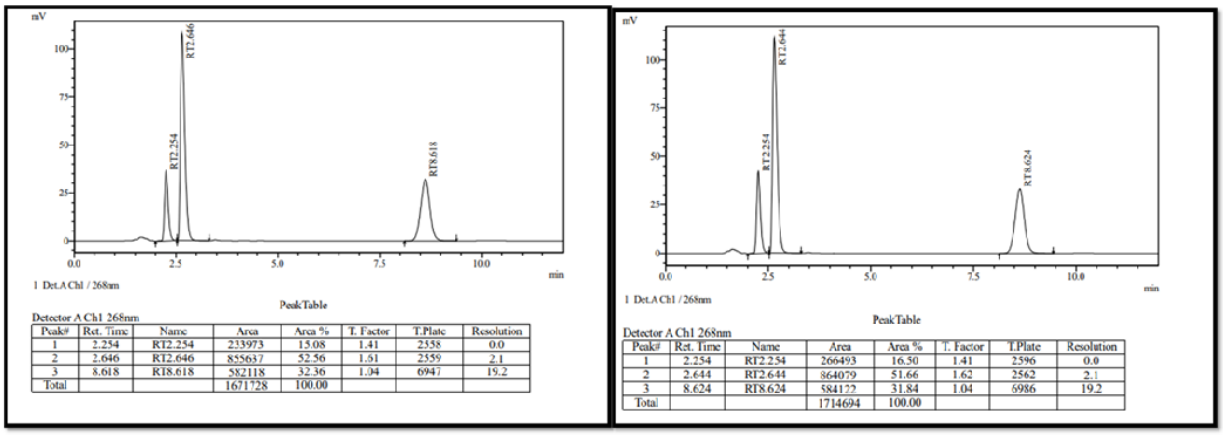

Figure 3: Chromatogram of Acid Degradation Standard and Sample

2) Base Degradation

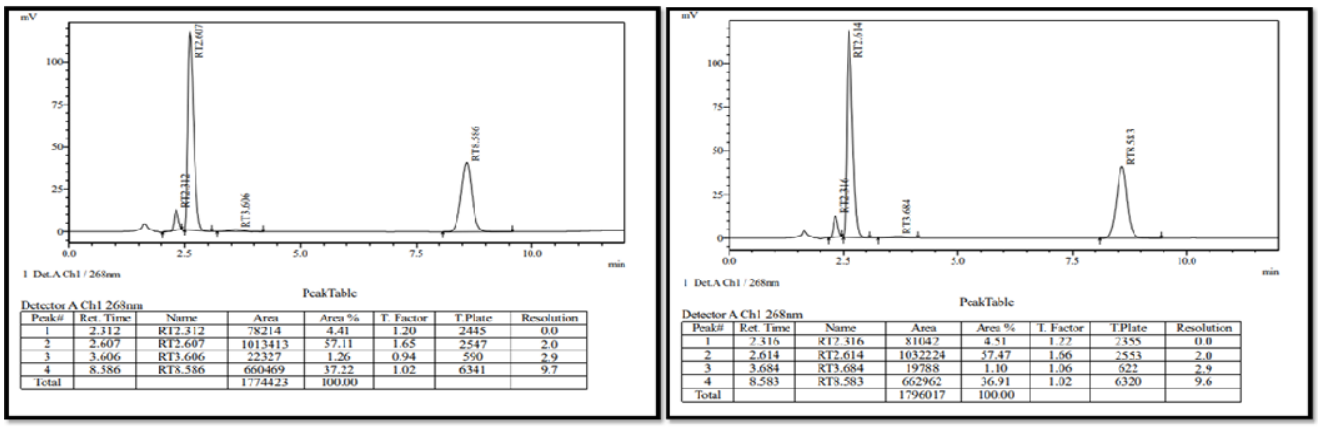

Figure 4: Chromatogram of Base Degradation Standard and Sample

3) Oxidation Degradation

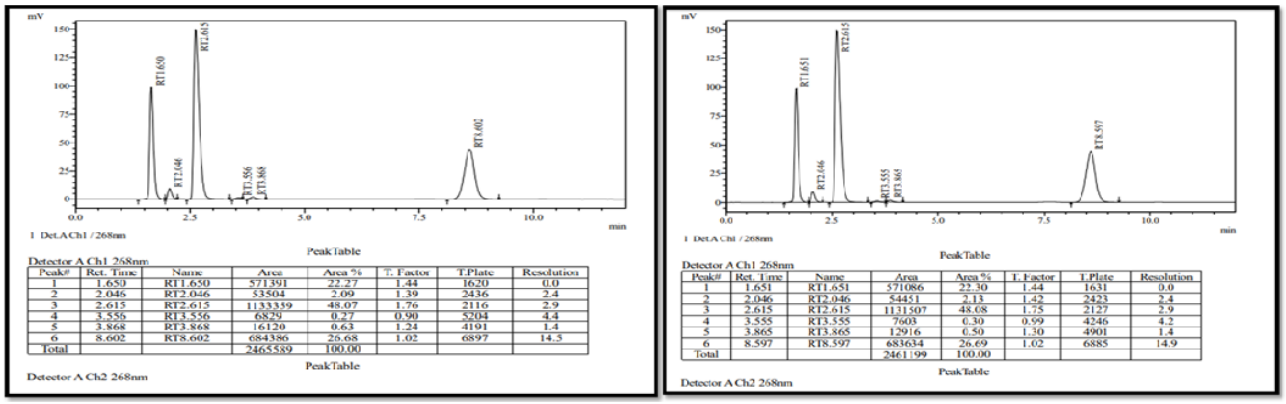

Figure 5: Chromatogram of Oxidative Degradation Standard and Sample

4) Photolytic Degradation

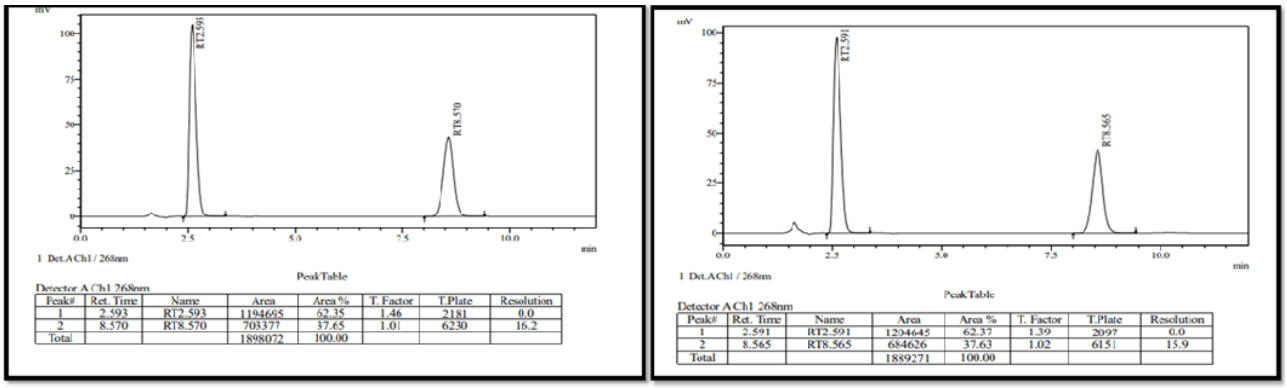

Figure 6: Chromatogram of Photolytic Degradation Standard and Sample

5) Thermal Degradation

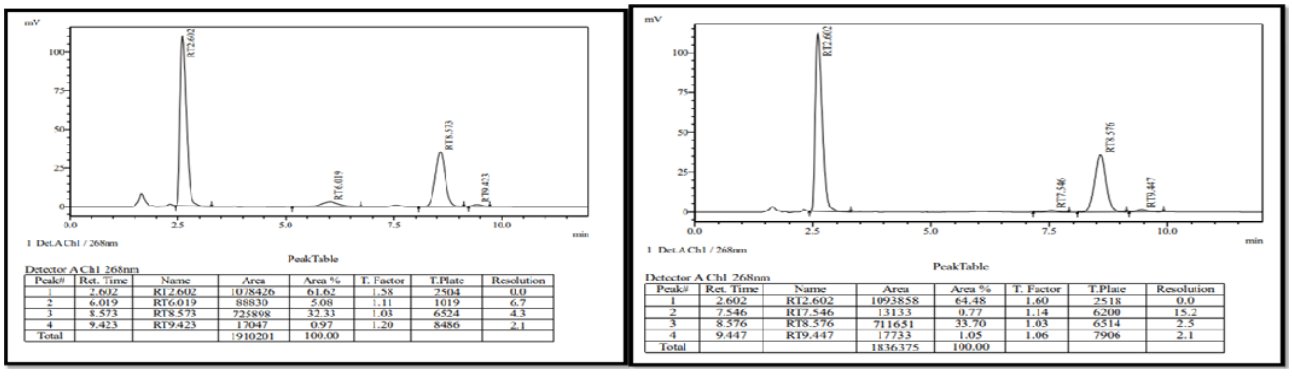

Figure 7: Chromatogram of Thermal Degradation Standard and Sample

Table 1: Forced degradation summary of SITA and LOBE in API and Sample

SITAGLIPTIN					
Initial Sample Area		1227360	**Initial API Area**		1213139
Degradation Condition	**Area after degradation**	**% Degradation**	**Degradation condition**	**Area after degradation**	**% Degradation**
Acid	864079	29.6	Acid	855637	29.5
Base	1032224	15.9	Base	1013413	16.5
Peroxide	1131507	7.8	Peroxide	1133359	6.6
Thermal	1093858	10.9	Thermal	1078426	11.1
Photo	1204645	1.9	Photo	1194695	1.5
LOBEGLITAZONE					
Initial Sample Area		720701	**Initial API Area**		735509
Degradation Condition	**Area after degradation**	**% Degradation**	**Degradation condition**	**Area after degradation**	**% Degradation**
Acid	584122	19.0	Acid	582118	20.9
Base	662962	8.0	Base	660469	10.2
Peroxide	683634	5.1	Peroxide	684386	7.0
Thermal	711651	1.3	Thermal	725898	1.3
Photo	684626	5.0	Photo	703377	4.4

Validation of RP-HPLC method

Linearity

Linearity was assessed by preparing five standard solutions of SITA and LOBE. The method demonstrated linearity over the concentration range of 100-300 µg/ml for SITA with a correlation coefficient (R^2 = 0.9995) and 0.5-1.5 µg/ml for LOBE with a correlation coefficient (R^2 = 0.9994). The results of the linearity study for SITA and LOBE are presented in Table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Using slope and Y-intercept, the determined values of LOD and LOQ were evaluated. LOD and LOQ value for SITA was found to be 3.02 µg/ml and 9.17 µg/ml respectively. LOD and LOQ value for LOBE was found to be 0.001 µg/ml and 0.004 µg/ml respectively.

Accuracy (n=3)

Accuracy of the method was confirmed by recovery study at three levels (50%, 100% and 150%) of placebo addition.

Table 2: Linearity Data for SITA and LOBE

Sitagliptin		Lobeglitazone	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
100.00	551838	0.50	339172
150.00	822892	0.75	515434
200.00	1115577	1.00	666985
250.00	1367341	1.25	847699
300.00	1663307	1.50	1007556

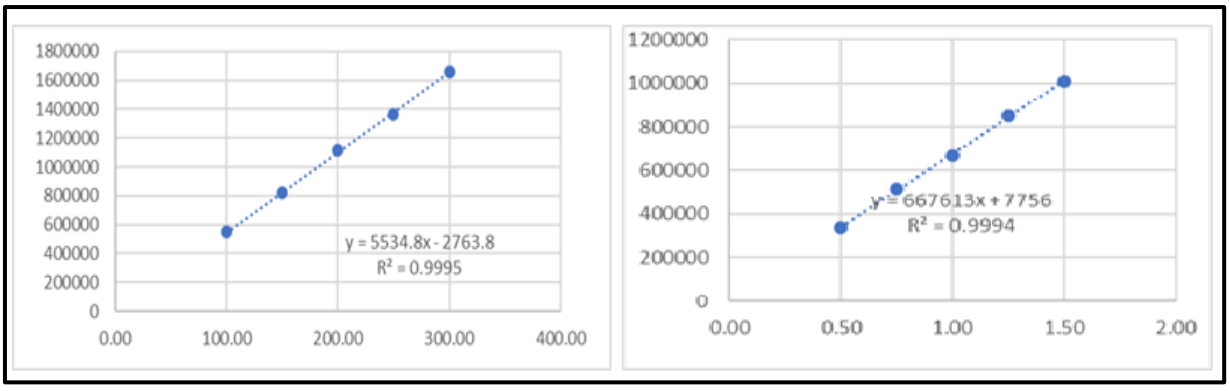

Figure 8: Calibration Curve of SITA and LOBE

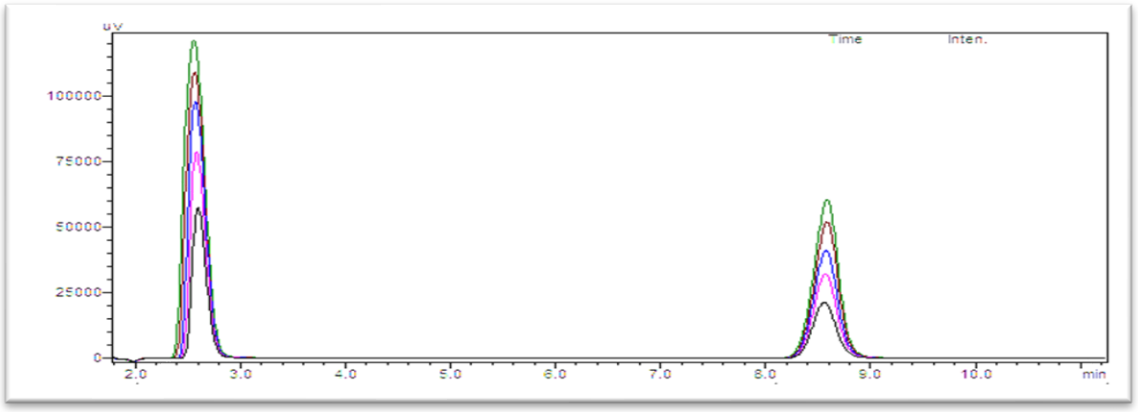

Figure 9: Overlay of Linearity Chromatogram of SITA and LOBE

Precision

Table 3: Repeatability Data of SITA and LOBE

Conc of SITA	Peak area	Conc of LOBE	Peak area
200 µg/ml	1121297	**1 µg/ml**	685755
	1122790		685866
	1123590		686124
	1124793		687203
	1125527		686810
	1125466		687856
Mean (n = 6)	1123910.5	**Mean (n = 6)**	686602
SD	1673.324	**SD**	832.414
% RSD	**0.14**	**% RSD**	**0.12**

Table 4: Intraday and Inter Day Precision Data of SITA and LOBE

Precision	Intraday		Interday	
Drug	**Sitagliptin**	**Lobeglitazone**	**Sitagliptin**	**Lobeglitazone**
Concentration (µg/ml)	100	0.5	100	0.5
	200	1	200	1
	300	1.5	300	1.5
Mean peak area ± SD (n = 3)	559075.45 ± 6734.08	337590.68 ± 4136.68	560290.66 ± 7980.58	338881.66 ± 4716.06
	1156408 ± 8817.63	686741 ± 10900.54	1174898 ± 8817.83	697123 ± 11974.18
	1668472.8 ± 25636.24	1164604 ± 18404.41	1684978.3 ± 22676.42	1033240 ± 19499.24
% RSD	0.51	1.09	1.42	1.39
	0.81	1.02	0.75	1.72
	1.03	0.99	1.35	1.89

Table 5: Accuracy Data of SITA and LOBE

Name of drug	Conc Level (%)	Added (ml)	Amount Added (mg)	Amount Recovered (mg)	%Mean Recovery	% RSD
SITA	50 %	0.500	1.000	0.980	98.0	0.11
		0.500	1.000	0.981	98.1	0.9
		0.500	1.000	0.980	98.0	0.27
	100%	1.000	2.000	1.981	99.0	0.76
		1.000	2.000	1.985	99.3	1.73
		1.000	2.000	1.983	99.1	1.07
	150 %	1.500	3.000	2.961	98.7	1.39
		1.500	3.000	2.963	98.8	0.80
		1.500	3.000	2.963	98.8	0.16

LOBE	50 %	0.005	0.0049	0.005	98.0	1.56
		0.005	0.0050	0.005	100.0	0.29
		0.005	0.0050	0.005	100.0	0.17
	100%	0.010	0.0098	0.010	98.0	0.12
		0.010	0.0099	0.010	99.0	0.22
		0.010	0.0099	0.010	99.0	0.11
	150 %	0.015	0.0147	0.015	98.0	0.98
		0.015	0.0151	0.015	100.7	0.12
		0.015	0.0150	0.015	100.0	0.65

Robustness

Table 6: Robustness Data of SITA and LOBE

SITA						
Sr. No.	**Area at Temp. -5°C**	**Area at Temp. +5°C**	**Area at Flow Rate -10%**	**Area at Flow Rate +10%**	**Area at Organic Phase 2%**	**Area at Organic Phase +2%**
1	1116787	1116124	1248880	1032732	1119355	1131992
2	1117091	1119273	1251703	1033255	1121033	1133235
3	1119990	1113105	1250561	1031317	1119576	1133336
Mean	1117956	1116167	1250381	1032435	1119988	1132854
% RSD	0.2	0.3	0.1	0.1	0.1	0.1
Theoretical Plates	2113.00	2077.00	2134.00	2080.00	2055.00	2170.00
Tailing Factor	1.37	1.32	1.38	1.36	1.32	1.41
LOBE						
Sr. No.	**Area atTemp. -5°C**	**Area atTemp. +5°C**	**Area at Flow Rate -10%**	**Area at Flow Rate +10%**	**Area at Organic Phase -2%**	**Area at Organic Phase +2%**
1	675339	674569	753402	623997	678656	676895
2	674449	672833	755943	624827	682725	680737
3	673762	672855	756336	624994	681709	678316
Mean	674517	673419	755227	624606	681030	678649
% RSD	0.1	0.1	0.2	0.1	0.3	0.3
Theoretical Plates	6063	6676	6666	6025	6369	6181
Tailing Factor	1.02	1.01	1.01	1.02	1.02	1.01

Table 7: Assay of Synthetic Mixture

Drug	Label Claim	Amount Found	Assay (%) Mean ± SD (n = 3), % RSD
SITA	100 mg	99.6 mg	99.8 ± 0.002, 0.34
LOBE	0.5 mg	0.49 mg	99.6 ± 0.005, 1.02

Assay of Synthetic Mixture

Applicability of the proposed method was tested by analyzing the Synthetic Mixture. Results as % Assay is shown in Table 7.

These results indicates that the developed method is specific, accurate, precise, simple, sensitive, robust and rapid.

CONCLUSION

Based on the experimental results, the proposed method is accurate, novel, simple, precise, linear, sensitive, robust, and stable for the simultaneous estimation of SITA and LOBE in both raw materials and synthetic mixtures. This method is suitable for stability and quality control analyses of synthetic formulations.

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