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Development and Validation of HPTLC method for the estimation of Sitagliptin Phosphate and Simvastatin in bulk and Marketed Formulation

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Abstract

Method describes a development and validation of HPTLC method for the estimation of sitagliptin phosphate and simvastatin in bulk and marketed formulation. This employs a precoated silica gel 60 F254 (0.2 mm thickness) on aluminium sheets and mobile phase chloroform: methanol in the ratio of 8:2 v/v, having chamber saturation for 20 min at room temperature. The developing chamber was run up to 8cm. The Rf values were found to be 0.13 and 0.75 for sitagliptin phosphate and simvastatin respectively. The plate was scanned and quantified at 217 nm. The linear detector response was observed between 2000 ng/spot to 7000 ng/spot and 250 ng/spot to 750 ng/spot for sitagliptin phosphate and simvastatin respectively. The method so developed was validated for its accuracy and precision. The LOD and LOQ were found to be 660, 2000 ng/spot and 50, 150 ng/spot, respectively for sitagliptin phosphate and simvastatin .The recovery was carried out by standard addition method. The Average recovery was found to be 92.80 % and 98.01 % for sitagliptin phosphate and simvastatin respectively.

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INTRODUCTION

Sitagliptin chemically 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 ,Fig. No. 1 shows the structure of Sitagliptin Phosphate, an oral Anti-diabetic agent that blocks Dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion [1-3].

Simvastatin (SIMVA) is 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 ;Fig. no: 2 shows the structure of Simvastatin. Simvastatin used as HMG-CoA reductase inhibitors Simvastatin is used in the treatment of primary hypercholesterolemia and is effective in reducing total and LDL-Cholesterol as well as plasma triglycerides and apolipoprotein B [4-6].

The literature reveals that there are some of the methods have been reported for Sitagliptin UV, HPLC, and HPTLC [7-9]. For Simvastatin also have been reported UV, HPLC, and HPTLC [10 12]. As on Sitagliptin and Simvastatin no any method was reported on HPTLC [13].Hence we attempt to developed versatile, accurate, precise and economical method for validation of HPTLC method for the estimation of sitagliptin phosphate and simvastatin in bulk and marketed formulation**.**

MATERIALS AND METHODS

Experimental

Instrumentation and chromatographic conditions:

Instrumentation and chromatographic conditions are given in the following table

Table A Instruments and chromatographic conditions

Sr. no.	Instruments	Descriptions
1	HPTLC system	Camag HPTLC system
2	Sample application	Camag linomate IV automatic sample
3	Scanner	Camag TLC scanner
4	Software	Camag wincats software
5	Saturated chamber	Camag Twin trough chamber (10x10) and (20x20)
6	HPTLC plate	Merch HPTLC plate coated with silica gel 60 F 254(0.2mm thickness) on aluminium sheet
7	Syring	Hamilton syringe (100µl)

Table B Reagents required for study

Reagents	Manufactured by
Methanol A R grade	S. D. Fine chemicals , Mumbai
Chloroform A R grade	S. D. Fine chemicals , Mumbai
Distilled water	S. D. Fine chemicals , Mumbai

Preparation of working standard and sample solutions

Standard stock solutions of SITA and SIMVA were prepared by dissolving 10 mg of drug in 100 ml of methanol: water (8:2) to get a concentration of 100µg/ml for each drug separately. The Standard working solutions were prepared by dilution of the stock standard solution with methanol to reach a concentration of 10µg/ml and 4µg/ml for SITA and SIMVA respectively. Twenty tablets were weighed accurately, finely powdered and powder equivalent to 100 mg of sitagliptin and 40 mg of simvastatin was weighed accurately. The powder was transferred to a 100ml volumetric flask containing 80 ml of methanol, shake for 5 min, to it 20ml of water was added and the solution was sonicated for 20 min, allowed the solution to cool to room temperature, the resulting solution was filtered through Whatmann filter paper 41. Required dilutions were made to get

10 µg/ml and 4 µg/ml for SITA and SIMVA respectively.

Chromatographic conditions and Calibration graphs

The experiment was performed on a silica gel 60 F254 (0.2 mm thickness) HPTLC plates (10x10) and (20×10cm) using a mobile phase chloroform: methanol in the ratio of 8:2 v/v. The plates were prewashed with methanol and activated at 60 °C for 20min. prior to chromatography. Samples were applied as bands 4mm long, at 4mm intervals. Ascending development to distance of 8cm was performed in saturated 20×10cm twin trough TLC developing chamber for 20min at room temperature (camag). The plate was scanned and quantified at 217 nm. Aliquots of 10, 8, 6, 4, 2 µl of working standard solution of SITA and SIMVA were applied on the TLC plate. TLC plate was dried, developed and analyzed photo metrically. The standard calibration curve was generated using regression analysis with Microsoft excel.

Validation of the method

The developed method was validated as per ICH guidelines for specificity, linearity, repeatability, Limit of detection, limit of quantitation and accuracy. (Table I)

Assay

From the prepared sample solution 5µl was spotted in duplicate along with same concentration of standard solution on pre-coated silica gel 60 F254 TLC plate. The plate was developed and scanned as mentioned earlier. Analysis was repeated in triplicate. Peak area was recorded and the amount of SITA and SIMVA present in formulation was estimated. (Table II)

RESULTS AND DISCUSSION

HPTLC method was optimized with a view to develop a simple, accurate method for estimation of drug in pharmaceutical formulation and in bulk drug. UV scanning at 190-450 nm for both SITA and SIMVA show that 217 nm is the suitable wavelength for detection of drugs. (Fig. I) The mobile phase of chloroform: methanol in the ratio of 8:2 v/v was selected because it gave highest resolution, minimum tailing and Rf values 0.13 and 0.75 for SITA and SIMVA respectively (Fig. II). SITA and SIMVA showed linearity in the concentration range of 2000 ng/spot to 7000 ng/spot (r2 =0.998) and 250 ng/spot to 750 ng/spot (r2 =0.997) respectively (Fig.III and IV). For HPTLC method the linearity of calibration graphs and adherence of the system to Beer's law was validated by higher value of correlation coefficient. The LOD and LOQ were found to be 660, 2000 ng/spot and 50, 150 ng/spot, respectively for SITA and SIMVA. Repeatability of measurement of peak area was determined by six replicate spotting and six time measurement of working standard of SITA and SIMVA and percentage relative standard deviation (%RSD) was found to be 0.0077 and 0.0015 for SITA and SIMVA respectively. To confirm the specificity, the solution of formulation was spotted on the TLC plate, developed and scanned. Complete separation of SITA and SIMVA in presence of common tablet Excipients was noticed, indicating the specificity of the method. Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels (80%, 100%, and 120%) by standard addition method and mean recovery was found to be 92.80 % and 98.01 % for SITA and SIMVA respectively. (Table III).

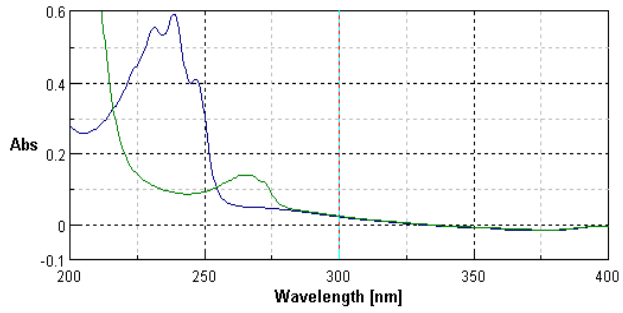

Fig. I Overlay spectra of standard SITA and SIMVA at 217 nm

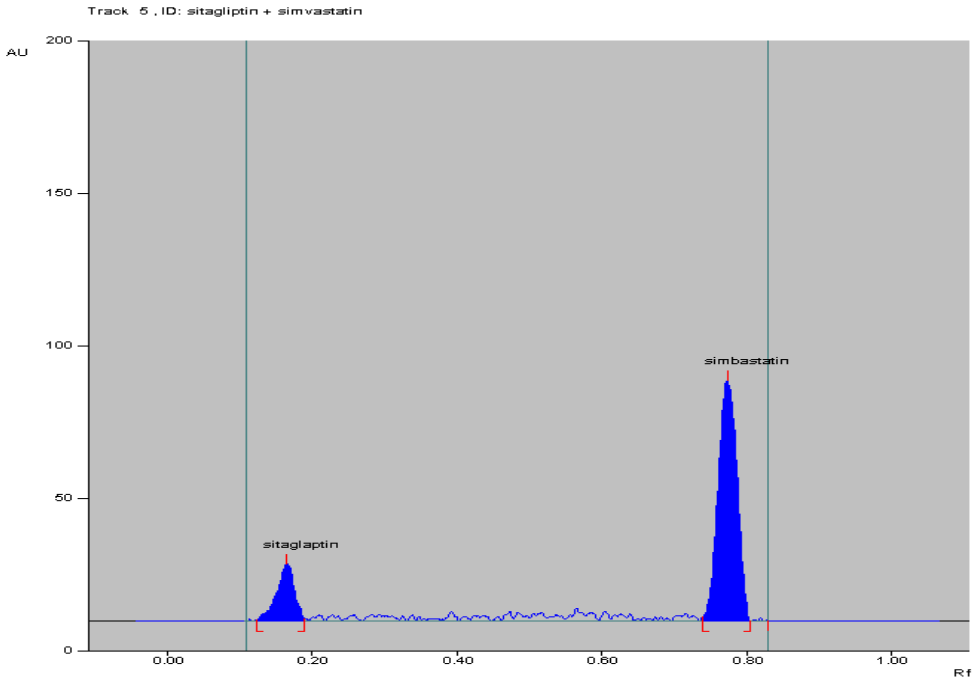

Fig. II Chromatogram of standard SITA and SIMVA

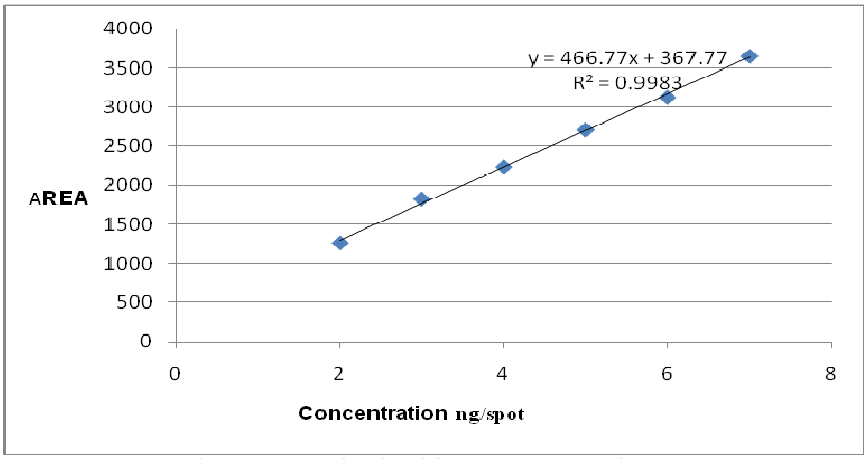

Fig. III Standard calibration curve of SITA

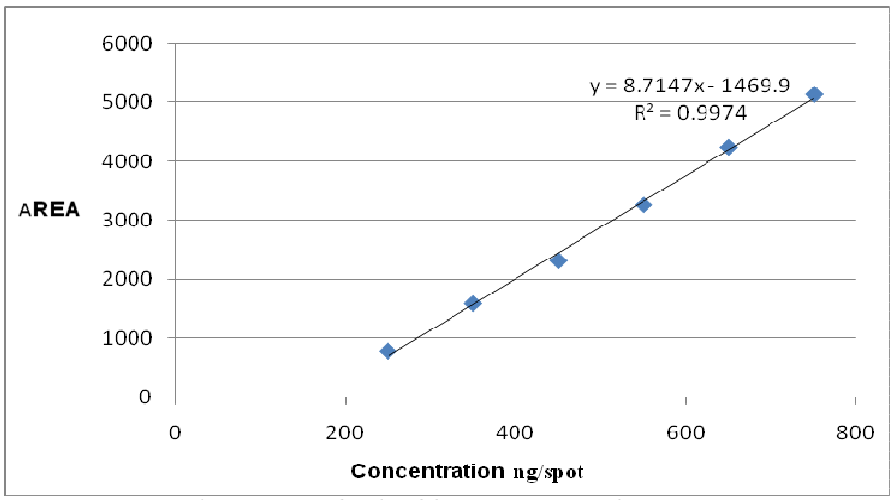

Fig. IV Standard calibration curve of SIMVA

Table I Method Validation Parameters

Sr. no.	Parameter	SITA	SIMVA
1	Linearity Range (ng/spot)	2000-7000	250-750
2	Correlation Coefficient	0.998	0.997
3	Limit of Detection (ng/spot)	660	50
4	Limit of Quantitation (ng/spot)	2000	150
5	Precision % RSD	0.043	1.13
6	Specificity	Specific	Specific

Table II Analysis of the marketed formulation

Sr.no.	Parameter	SITA	SIMVA
1	Label claim (mg/tab.)	100	40
2	Amount found (mg/tab.)	99.97	39.95
3	Drug content (%)	99.97	99.89
4	% R.S.D.	0.013	0.0015

Table III Recovery results of SITA and SIMVA

Level of recovery %	Mean of % recovery	
	SITA	SIMVA
80%	87.18	84.91
100%	92.80	98.01
120%	92.99	99.07

CONCLUSION:

The developed HPTLC method was found to be simple, precise, specific and accurate. Therefore this method can be applied for routine analysis of drugs in formulation and in bulk drug. The relative std. deviation (RSD) for all parameters was found to be less than two, which indicates the validity of method and assay results are also within the limit so the proposed method can be used for routine quantitative simultaneous estimation of both the drugs in multi-component pharmaceutical preparation.

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