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Novel HPTLC Technique for Accurate Measurement of Sitagliptin and Simvastatin in Combined Tablet Formulation

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This method offers a fast, specific and reliable way to measure both Sitagliptin and Simvastatin in a single tablet dosage form. It utilizes a plate with a pre-applied silica gel coating & a unique mobile phase composition for separation. Chloroform, methanol, toluene, and glacial acetic acid were combined in the ratio of 3:4:2:1 (v/v/v/v) and utilized as mobile phase. Exposure is achieved at 210 nm. This technique is linear between 62.5-700 nano gram per spot for Sitagliptin Phosphate & 25-300 nano gram per spot for Simvastatin. The LOD & the LOQ were established to be 10 nano gram per spot & 30 nano gram per spot; 8 nano gram per spot & 25 nano gram per spot respectively. Validation following ICH guidelines confirmed parameters to be within the limits. The method can detect & quantify Sitagliptin & Simvastatin within defined ranges. This approach has the potential for detection with confidence.

Keywords: Estimation; Formulation; HPTLC; Simvastatin; Sitagliptin.

Sitagliptin (Figure 1) is a medication taken by mouth that helps regulate blood sugar. It works by blocking an enzyme called DPP-4, which increases the levels of hormones that stimulate insulin production & decrease glucagon levels, results in better blood sugar control for people with type 2 diabetes.[1,2]

Simvastatin (Figure 2) is a medication that helps lower cholesterol. It works by blocking an enzyme in the liver which is essential for cholesterol production, reduces amount of cholesterol made by the liver &lowers overall cholesterol levels. Simvastatin may also help lower triglycerides & increase HDL, the good cholesterol.[3,4]

Building on existing research,[5-15] this study developed & validated a novel HPTLC method for precisely measuring Sitagliptin & Simvastatin together in a single medication. This method adheres to strict quality standards set by ICH guidelines.[16-17]

MATERIALS AND METHODS

Chemicals & solvents

Sitagliptin and Simvastatin standard samples were given as gift samples. Tablets of 100 milli gram Sitagliptin and 40 milli gram Simvastatin were purchased from a local vendor. All chemicals, including chloroform, methanol, toluene, and glacial acetic acid, were as per analytical standards.

Instrumentation

The experiment utilized a plate with a pre-applied silica gel coating (twenty x twenty cm with a 200 micro meter layer thickness) from E. Merck (Mumbai, India) as the stationary phase. A Camag HPTLC system was employed, consisting of an automatic sample applicator (Camag Linomat V), and accessories as of an automated instrument.

Preparation of standard solution

Weigh 10 milli gram of Sitagliptin & 4 milli gram of Simvastatin into a 100 milli litre volumetric flask. Add 10 milli litre of diluent, & then fill to the mark with diluent. Dilute 1 milli litre of this solution in another 100 milli litre volumetric flask. From this, dilute 6.25 milli litre in a third 100 milli litre flask to prepare the final solution for calibration curve.

Sample solution preparation

Crush tablets count of twenty & weigh a portion equivalent to 100 milli gram of Sitagliptin & 40 milli gram of Simvastatin. Transfer to a hundred milli litre volumetric flask, add fifty milli litre of diluent & sonicate for 20 minutes. Fill to the mark with diluent & filter. Dilute 1 milli litre of filtrate in another 100 milli litre flask, dilute 5 milli litre of this solution in a third 100 milli litre flask for a final concentration of 500 ng/b & Sitagliptin & 200 ng/b& Simvastatin.

HPTLC Method optimization

Wash silica gel plates (20 x 20 cm) with methanol & activate at 110°C for 5 minutes. Use these plates with mobile phase of chloroform, methanol, toluene, & glacial acetic acid (3:4:2:1, v/v/v/v). Pre-conditioning of plate & chamber should be done for 20 minutes. Detect at 210 nm using deuterium lamp. Spot standard solutions on plate & develop. Use Camag TLC scanner III with WinCATS software for measurements.

Procedure

Wash silica gel plates with methanol & activate at 110°C for 5 minutes. Use these plates with mobile phase of chloroform, methanol, toluene, & glacial acetic acid (3:4:2:1, v/v/v/v). Condition chamber & plate for 20 minutes. Detect at 210 nm using deuterium lamp. Spot standard solutions on plate & develop. Use Camag TLC scanner III with WinCATS software for measurements.

RESULTS

The optimization of the separation for Sitagliptin and Simvastatin was conducted by exploring various mobile phase compositions. After testing different solvent combinations, the mixture of chloroform, methanol, toluene, and glacial acetic acid (3:4:2:1, v/v/v/v) was found to be the optimal mobile phase. This solvent system produced sharp, compact peaks with suitable retention factors (Rf values) of 0.64 for Sitagliptin and 0.74 for Simvastatin, ensuring accurate quantification of both drugs in the pharmaceutical formulation. The densitogram at 210 nm demonstrated clear peak separation for both drugs.

The analysis showed no interfering peaks from excipients at the Rf values of Sitagliptin and

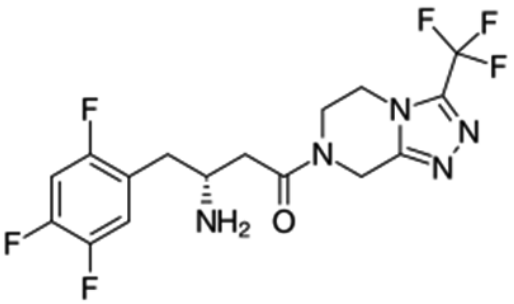

Fig. 1. Chemical structure of Sitagliptin

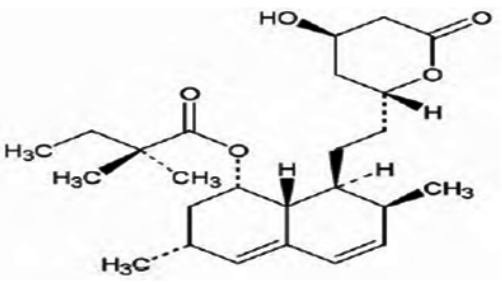

Fig. 2. Chemical structure of Simvastatin

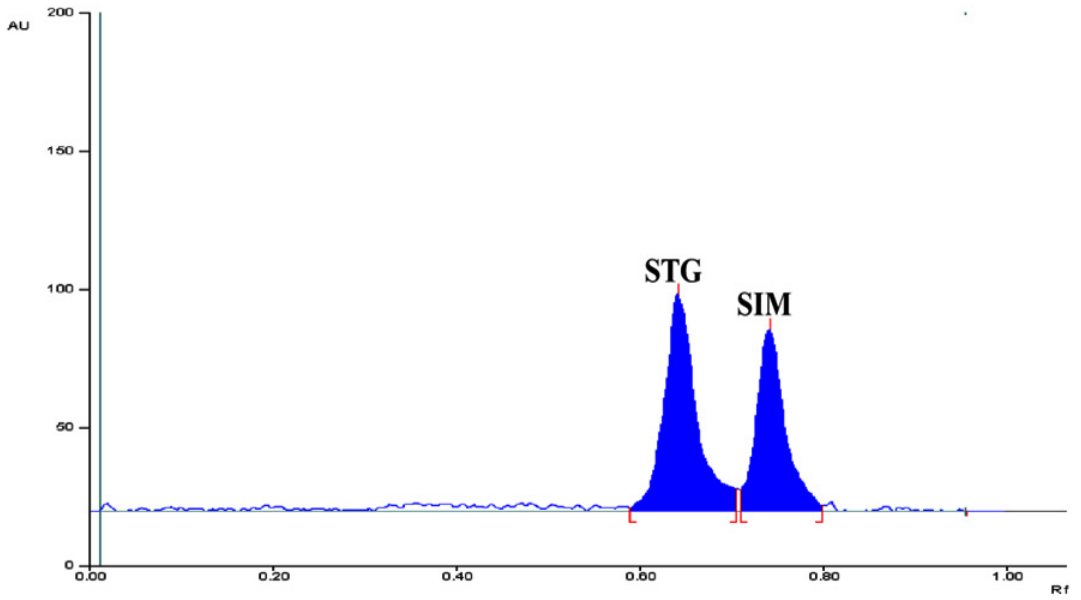

Fig. 3. Typical HPTLC chromatogram of Sitagliptin & Simvastatin

Table 1. Method validation parameters of proposed method

Parameters	Sitagliptin	Simvastatin
Linearity range (ng/spot)	62.5-700	25-300
Correlation coefficient (r^2)	0.999	0.999
Slope (m)	0.953	4.928
Intercept (c)	17496	304.4
Detection Limit	10	8
Quantitation Limit	30	25

Simvastatin. The densitograms of the drugs in the sample solution closely matched those of the standard solution, confirming the specificity of the method. Additionally, the validation results are summarized in Table 1.

DISCUSSION

The method developed for the separation of Sitagliptin and Simvastatin using high-performance thin-layer chromatography (HPTLC) demonstrated excellent peak resolution and specificity. The optimal mobile phase composition of chloroform, methanol, toluene, and glacial acetic acid ensured suitable Rf values for both drugs, making it highly effective for accurate analysis. The Rf values of Sitagliptin (0.64) and Simvastatin (0.74) allowed for good separation, which is critical for quantification in pharmaceutical formulations.

The absence of interfering peaks from excipients further emphasizes the method's specificity, which is essential when analyzing complex pharmaceutical matrices. The comparison of densitograms for the sample solution and standard solution also confirms the robustness of the method.

The validation parameters indicated strong linearity, with correlation coefficients of 0.999 for both Sitagliptin and Simvastatin, showcasing the method's precision across the tested range. The detection and quantitation limits were also sufficiently low, ensuring that the method could detect and quantify both drugs accurately at trace levels.

Overall, the proposed method is reliable, sensitive, and specific for the analysis of Sitagliptin and Simvastatin in pharmaceutical formulations, making it suitable for routine quality control in pharmaceutical industries.

CONCLUSION

This HPTLC method offers a novel, reliable, & specific approach for simultaneous

analysis of Sitagliptin & Simvastatin. Importantly, method is not affected by presence of excipients commonly found in tablets. This makes it well-suited for quantifying both drugs in their pure form (bulk drug) & in commercially available tablet formulations.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

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Not Applicable.

Author's Contribution

Lakshmana Rao Atmakuri: Conceptualization, Methodology, Writing – Original Draft; Prasanthi Thayi: Data Collection, Analysis, Writing – Review & Editing; Haritha Potluri: Visualization, Supervision, Project Administration; Ramesh Alluri: Funding Acquisition, Resources, Supervision; Satya Venkata Sakuntala Mamidi: Conceptualization, Data Interpretation, Writing – Review & Editing; Vijaya Kumar Ghanta: Project Administration, Supervision, Final Draft Approval.

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