

Development and validation of an HPTLC method for simultaneous determination of gliclazide and sitagliptin phosphate monohydrate in tablets

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Abstract

A high-performance thin-layer chromatography (HPTLC) method was developed and validated for the simultaneous determination of gliclazide (GLC) and sitagliptin phosphate monohydrate (STP) in tablet dosage form. Separation was achieved on precoated silica gel G60 F254 aluminum-backed plates using methanol:ethyl acetate:toluene:ammonia (1.5:5:3:0.3, v/v/v/v) as the mobile phase. The chamber was saturated for 20 min and the mobile phase was allowed to migrate to 80 mm. Densitometric detection was performed at 215 nm. Well-resolved bands were obtained with mean retardation factors of approximately 0.32 for GLC and 0.76 for STP. The method showed linear response over 600–6000 ng/band for GLC and 1000–10,000 ng/band for STP. Repeatability, intraday precision and interday precision gave low relative standard deviations. Recovery was 99.41–101.58% for GLC and 99.11–101.72% for STP. The detection limits were 32.7829 and 69.0064 ng/band, while the quantitation limits were 99.3422 and 209.1103 ng/band for GLC and STP, respectively. Assay of the marketed tablet Cyblex S 60 XR gave 99.43% of the GLC label claim and 99.90% of the STP label claim. The developed method therefore provided a practical, rapid and reproducible approach for simultaneous chromatographic determination of the two antidiabetic drugs in pharmaceutical dosage form.

1 Introduction

Gliclazide is a sulfonylurea antidiabetic drug used in the management of type 2 diabetes mellitus, whereas sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor. Combination therapy with agents having complementary mechanisms is used to improve glycemic control in appropriate patients. Consequently, reliable analytical procedures are required for quality control of combination dosage forms.

Chromatographic techniques provide separation of analytes according to their differential interactions with stationary and mobile phases. HPTLC is an optimized form of thin-layer chromatography that permits simultaneous qualitative and quantitative analysis with relatively simple sample preparation and densitometric evaluation. It has been applied to pharmaceutical analysis, including assay, fingerprinting and quantification of active ingredients.

Several analytical procedures have been reported for individual and combined determination of gliclazide and sitagliptin, including UV-visible spectrophotometric and liquid chromatographic methods. The submitted study was therefore undertaken to develop a simple HPTLC method capable of simultaneously separating and quantifying gliclazide and sitagliptin phosphate monohydrate in a marketed tablet formulation, followed by validation of the method with respect to linearity, precision, accuracy, robustness, detection limit and quantitation limit.

2 Materials and methods

2.1 Chemicals and reagents

Gliclazide and sitagliptin phosphate monohydrate working standards were received as gift samples from J B Chemicals, Panoli, Gujarat, India. Methanol, ethyl acetate, toluene and ammonia were used as solvents and mobile-phase components. A marketed tablet formulation, Cyblex S 60 XR, was analyzed for assay.

2.2 Instrumentation and chromatographic materials

UV spectral measurements were performed using a Shimadzu UV-1900 double-beam UV-visible spectrophotometer with UV Probe software. Chromatographic separation was performed on precoated silica gel G60 F254 aluminum sheets (10 × 10 cm; layer thickness 0.2 mm). HPTLC densitometric measurements were performed at 215 nm.

2.3 Preliminary characterization

The APIs were subjected to preliminary characterization by melting-point determination, solubility assessment and infrared spectral comparison before chromatographic method development.

2.4 Selection of detection wavelength

UV spectra of gliclazide and sitagliptin phosphate monohydrate were recorded over 200–400 nm. A common detection wavelength of 215 nm was selected for densitometric analysis because both analytes showed suitable absorption at this wavelength.

2.5 HPTLC method development

Chromatographic conditions were optimized by evaluating mobile-phase composition and development conditions. The final stationary phase was precoated silica gel G60 F254. The optimized mobile phase was methanol:ethyl acetate:toluene:ammonia (1.5:5:3:0.3, v/v/v/v). The chamber was saturated for 20 min, and the mobile phase was developed over a migration distance of 80 mm. Sample bands were applied with a 100 µL syringe using a 6 mm band width. Densitometric scanning was carried out at 215 nm at a scanning speed of 20 mm/s.

Table 1
Optimized chromatographic conditions

Parameter	Optimized condition
Stationary phase	Precoated silica gel G60 F254, aluminum sheet, 10 × 10 cm, 0.2 mm
Mobile phase	Methanol:ethyl acetate:toluene:ammonia (1.5:5:3:0.3, v/v/v/v)
Chamber saturation	20 min
Migration distance	80 mm
Detection wavelength	215 nm
Slit dimension	6 × 0.1 mm
Scanning speed	20 mm/s
Band width	6 mm

2.6 Standard solutions

A gliclazide stock solution corresponding to 600 ppm was prepared by accurately weighing 10 mg of gliclazide into a 10 mL volumetric flask and making up to volume with methanol; 1 mL was subsequently diluted to 10 mL with methanol. A sitagliptin phosphate monohydrate stock solution corresponding to 1000 ppm was prepared by weighing 128 mg of sitagliptin phosphate monohydrate, equivalent to 100 mg of sitagliptin, into a 10 mL volumetric flask and diluting with methanol; 1 mL was then diluted to 10 mL.

2.7 Validation procedure

The method was validated according to ICH Q2(R2). Linearity was evaluated at six concentration levels covering 600–6000 ng/band for gliclazide and 1000–10,000 ng/band for sitagliptin phosphate monohydrate. Precision was assessed by repeatability and by intraday and interday studies. Accuracy was evaluated through standard addition at 80%, 100% and 120% levels. Robustness was studied by deliberate small changes in mobile-phase composition, chamber saturation time and detection wavelength. Detection and quantitation limits were calculated from the standard deviation of the response and the calibration slope using $DL = 3.3\sigma/S$ and $QL = 10\sigma/S$, respectively. Solution stability was examined for 24 h.

3 Results and discussion

3.1 Chromatographic separation

Systematic optimization produced a compact and well-resolved chromatographic profile. Under the final conditions, the mean R_f values were approximately 0.32 for gliclazide and 0.76 for sitagliptin phosphate monohydrate. The individual peak maxima in the finalized chromatogram were approximately 0.323 for

STP and 0.768 for GLC. The selected Rf values were within the practical separation range reported in the study.

3.2 Linearity

Table 2
Linearity data for gliclazide and sitagliptin phosphate monohydrate

Analyte	Concentration (ng/band)	Mean area ± SD (n = 5)	%RSD
GLC	600	0.001100 ± 0.0000141	1.2856
GLC	1200	0.002334 ± 0.0000344	1.4742
GLC	2400	0.004902 ± 0.0000232	0.4722
GLC	3600	0.007084 ± 0.0000413	0.5827
GLC	4800	0.008794 ± 0.0000459	0.5215
GLC	6000	0.010628 ± 0.0000271	0.2552
STP	1000	0.000840 ± 0.0000141	1.6835
STP	2000	0.002008 ± 0.0000279	1.3872
STP	4000	0.003400 ± 0.0000400	1.1764
STP	6000	0.004608 ± 0.0000299	0.6495
STP	8000	0.006190 ± 0.0000395	0.6380
STP	10000	0.007892 ± 0.0000312	0.3958

The calibration response increased with concentration across the studied ranges. The calibration equation for gliclazide was $y = 0.00000177x + 0.00032934$ with $R^2 = 0.99339731$, while that for sitagliptin was $y = 0.00000075x + 0.00027590$ with $R^2 = 0.99551162$. These results demonstrate a strong linear relationship between applied amount and densitometric response over the selected working ranges.

3.3 Precision

Table 3
Precision results

Study	GLC	STP
Repeatability, %RSD (n = 6)	0.4352	0.7125
Intraday, %RSD (n = 3)	0.3084–1.1304	0.5239–1.4730
Interday, %RSD (n = 3)	0.3628–1.4737	0.6123–1.8770

Repeatability at 2400 ng/band for GLC and 4000 ng/band for STP showed %RSD values of 0.4352 and 0.7125, respectively. Intraday precision at low, medium and high levels gave %RSD ranges of 0.3084–

1.1304 for GLC and 0.5239–1.4730 for STP. Interday precision gave ranges of 0.3628–1.4737 and 0.6123–1.8770, respectively. The low variability indicates satisfactory precision of the procedure.

3.4 Accuracy

Table 4
Recovery study at 80%, 100% and 120% levels

Analyte	Level	Sample (ng/band)	Standard added (ng/band)	Mean recovery (%)
GLC	80%	2400	1920	100.27
GLC	100%	2400	2400	101.58
GLC	120%	2400	2880	99.41
STP	80%	4000	3200	99.82
STP	100%	4000	4000	101.72
STP	120%	4000	4800	101.37

Recovery results were 99.41–101.58% for gliclazide and 99.11–101.72% for sitagliptin phosphate monohydrate when the complete study range, including the unspiked reference, was considered. The observed recoveries support the accuracy of the developed method.

3.5 Robustness

Table 5
Robustness results

Analyte	Parameter varied	Observed %RSD range
GLC	Mobile-phase ratio, chamber saturation, wavelength	0.6635–1.3579
STP	Mobile-phase ratio, chamber saturation, wavelength	0.5352–1.8827

Robustness was assessed by small deliberate changes in mobile-phase ratio, chamber saturation time and wavelength. The reported %RSD values remained low for both analytes, indicating that the analytical response was not unduly affected by the investigated minor changes.

3.6 Detection and quantitation limits

Table 6
Detection and quantitation limits

Parameter	GLC (ng/band)	STP (ng/band)
Detection limit	32.7829	69.0064
Quantitation limit	99.3422	209.1103

The calculated detection limits were 32.7829 ng/band for GLC and 69.0064 ng/band for STP. The corresponding quantitation limits were 99.3422 and 209.1103 ng/band, respectively, demonstrating suitable sensitivity for the intended dosage-form analysis.

3.7 Assay of marketed formulation

Table 7
Assay results for the marketed tablet formulation (n = 3)

Formulation	GLC label amount (ng/band)	GLC found (ng/band)	GLC assay (%)	STP label amount (ng/band)	STP found (ng/band)	STP assay (%)
Cyblex S 60 XR	2400	2386.43 ± 7.0467	99.43 ± 0.2913	4000	3996.22 ± 22.6612	99.90 ± 0.5654

The developed method was applied to Cyblex S 60 XR tablets. The assay was 99.43% for gliclazide and 99.90% for sitagliptin phosphate monohydrate. The results were close to the respective label claims, indicating applicability of the method for routine pharmaceutical quality-control analysis.

4 Conclusion

A simple HPTLC-densitometric method was developed for simultaneous determination of gliclazide and sitagliptin phosphate monohydrate in tablet dosage form. The optimized system using silica gel G60 F254 and methanol:ethyl acetate:toluene:ammonia (1.5:5:3:0.3, v/v/v/v), with densitometric detection at 215 nm, provided well-resolved bands with R_f values of approximately 0.32 and 0.76. The method exhibited linear response, satisfactory precision and accuracy, robustness to small experimental variations, and useful sensitivity. Assay results for the marketed formulation were 99.43% and 99.90% for GLC and STP, respectively. The validated procedure is therefore suitable for simultaneous chromatographic estimation of these drugs in pharmaceutical dosage forms.

Declarations

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Ethics approval

Not applicable. The study did not involve human participants, animals, human data or biological materials.

Competing interests

The authors declare that they have no competing financial or non-financial interests.

Author contributions

Dr. Drashti Mandale: Conceptualization, methodology, investigation, data curation, formal analysis, and writing original draft. Ms. Priyanshiben Chandrakant Patel: Investigation, data collection, validation and writing review and editing. Mrs. Nishi Desai: Supervision, methodological guidance, data review and critical revision of the manuscript. Dr. Nidhi Chauhan: Co-supervision and critical review of the manuscript. Dr. Megha Shah: Review and editing of the manuscript. Ms. Dutiya Rumana: Writing review and editing. All authors reviewed and approved the final manuscript.

Data availability

The data supporting the findings of this study are contained within the article. Additional raw analytical records are available from the corresponding author on reasonable request.

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Consent to Public Declaration

Not applicable

Consent to Participate Declaration

Not applicable

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Figures

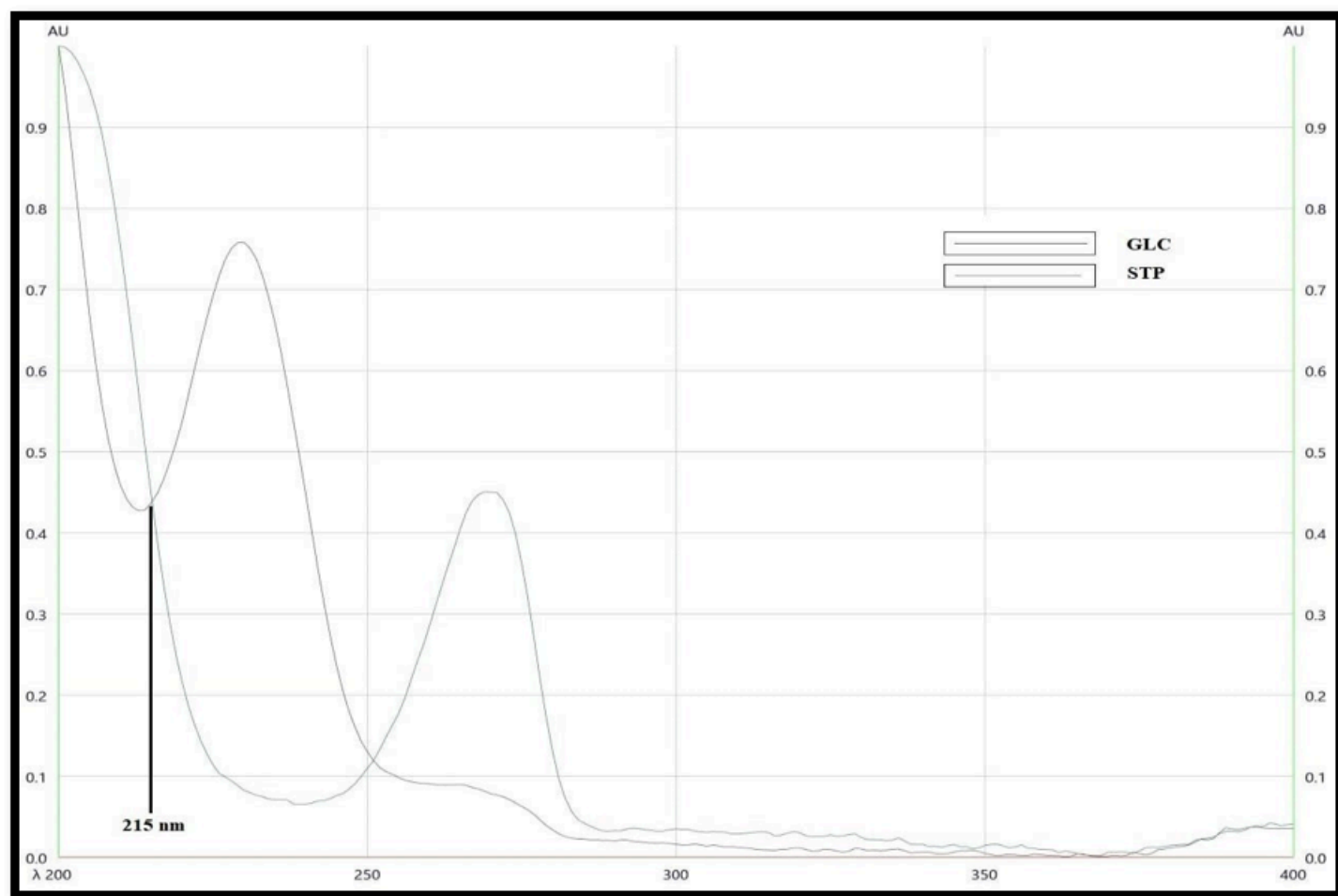


Figure 1

UV spectra of gliclazide and sitagliptin phosphate monohydrate showing selection of the common detection wavelength at 215 nm

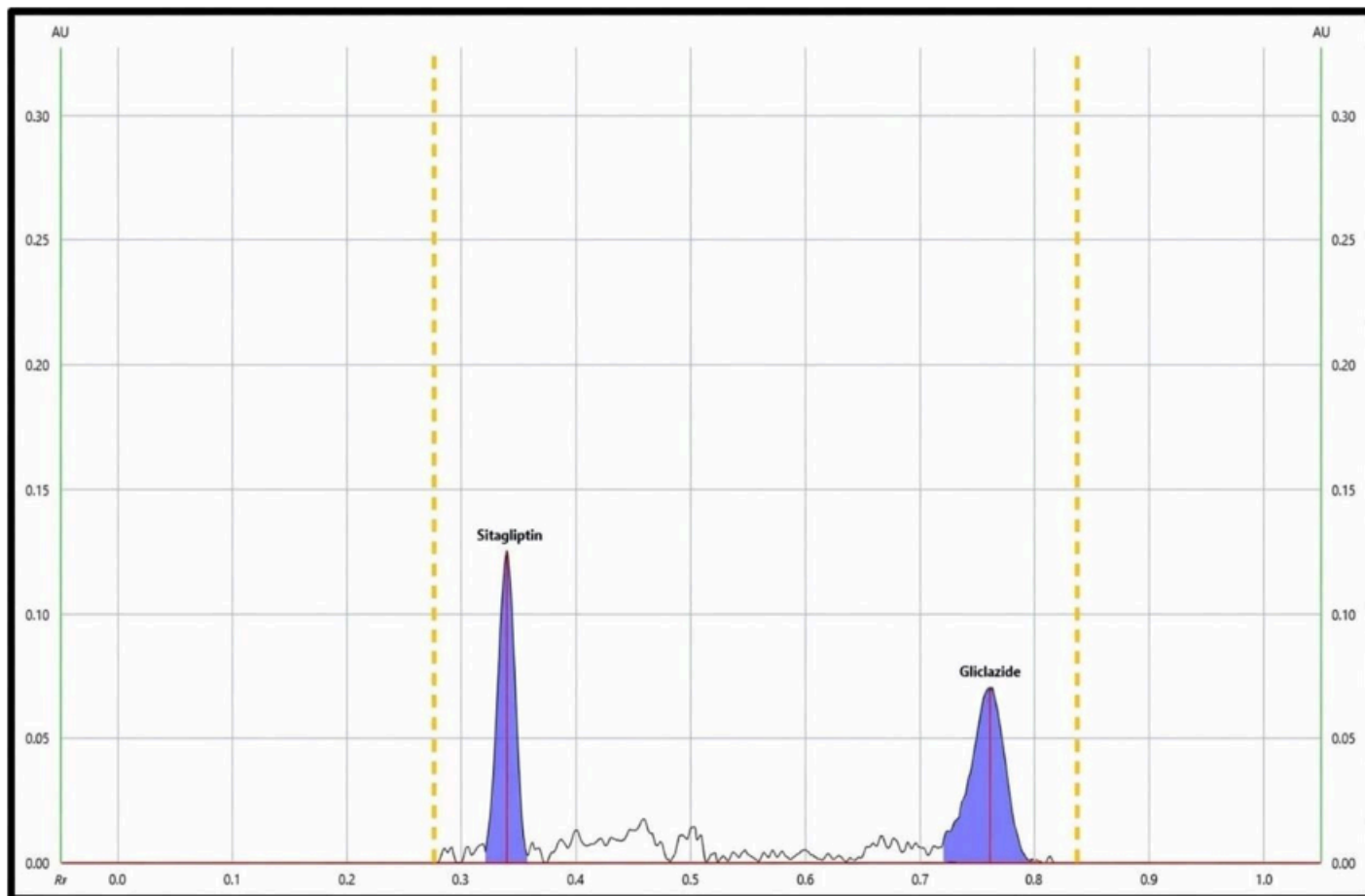


Figure 2

Densitogram obtained with the optimized chromatographic system showing separated sitagliptin and glimepiride bands

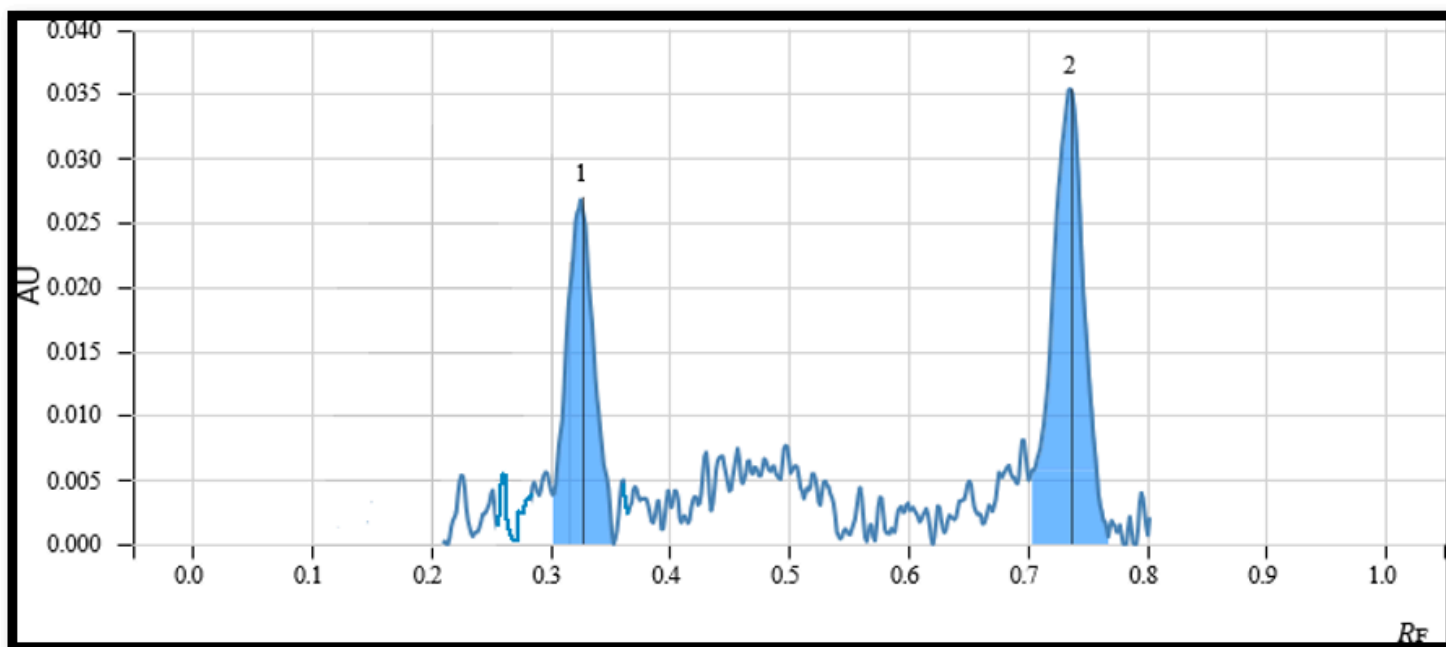


Figure 3

Representative densitogram obtained during linearity evaluation of gliclazide and sitagliptin phosphate monohydrate