

Content Uniformity Testing of Prasugrel Tablets by HPTLC methods

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Abstract

A new, simple, sensitive, precise and accurate High Performance Thin Layer Chromatographic (HPTLC) method for determination of Prasugrel in their tablet dosage form has been developed, validated. Chromatographic separation was achieved on aluminum plates precoated with silica gel 60 F254 as stationary phase and toluene-methanol-acetic acid (8:2:0.04, v/v/v) as mobile phase. Densitometric measurement of their spots was achieved at 220 nm over the concentration ranges of 0.4-1.4 $\mu\text{g spot}^{-1}$ with mean recoveries of 100.61 ± 1.04 . Limit of detection and Limit of quantification for Prasugrel were found to be 0.08 $\mu\text{g spot}^{-1}$ and 0.26 $\mu\text{g spot}^{-1}$ respectively. The proposed method was successfully applied for analysis of Prasugrel in commercial tablets and applied to the content uniformity testing of tablets.

Keywords:

Prasugrel, High Performance Thin Layer Chromatography, Content Uniformity Testing

INTRODUCTION

Prasugrel (PRA), (*RS*)-5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno [3,2-*c*]pyridin-2-yl acetate is novel third-generation thienopyridine that has been shown to achieve greater, faster and more persistent level of platelet inhibition than clopidogrel. Prasugrel binds to the P2Y₁₂ on the platelet cell surface, leading to the irreversible inhibition of platelet activation and aggregation. Prasugrel is oral antiplatelet agent approved in July 2009 by U.S. FDA for reduction in atherothrombotic cardiovascular events in patients presenting with acute coronary syndrome and undergoing percutaneous coronary intervention. [1] Literature Review reveals that few HPLC/UPLC [2-4], HPTLC [5], LC-MS [6] and UV [7] methods are available for determination of Prasugrel. Based on literature, it was felt that there is a need to develop a new HPTLC method for estimation of Prasugrel in its dosage form and application of developed method for content uniformity testing of Prasugrel Tablets. The proposed method was validated in accordance with International Conference on Harmonization (ICH) guidelines. [8]

EXPERIMENTAL

Chemicals and Reagents

Prasugrel hydrochloride standard was kindly supplied by Torrent Pharmaceuticals Ltd., Ahmedabad, India. Toluene, Methanol, Acetic acid was purchased from Merck Specialties Pvt. Ltd., Mumbai, India. Effient Tablets (Eli Lilly & Company) and Aplet Tablets (Glenmark Pharmaceuticals Ltd.) were purchase form local pharmacy.

HPTLC Instrumentation and Chromatographic Conditions

Chromatography was performed on 10 cm \times 10 cm aluminum HPTLC plates precoated with 250 μm layers of silica gel 60 F254 (E. Merck, Darmstadt, Germany). Before chromatography, the plates were prewashed with methanol and activated at 60 $^{\circ}\text{C}$ for 5 min. Samples were applied as 3

mm wide bands, under a continuous flow of nitrogen, by means of Camag (Muttenez, Switzerland) Linomat V sample applicator fitted with 100 μL Hamilton Syringe (Bonadauz, Switzerland). A constant application rate of 150 nL s^{-1} was used and distance between the adjacent bands was 10 mm. The plates were then conditioned for 20 min in a pre-saturated twin-trough glass chamber (10 \times 10 cm^2) with the mobile phase, toluene-methanol-acetic acid (8:2:0.04, v/v/v), in one trough and plates in other trough. The plates were then placed in the mobile phase and ascending development was performed to a distance of 80 mm from the point of application at ambient temperature. After development, plates were dried in air and densitometric scanning was performed at 220 nm with a Camag TLC scanner III operated in reflectance-absorbance mode and controlled by WinCATS software (V 1.4.). The slit dimensions were 3.0 \times 0.10 mm and the scanning speed was 20 mm s⁻¹. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The concentrations of the compounds chromatographed were determined from the intensity of diffusely reflected light. Evaluation was based on linear regression of peak areas.

Preparation of Standard Solution

Stock standard solution of Prasugrel was prepared by dissolving a quantity of 10.97 mg of Prasugrel hydrochloride equivalent to 10 mg of Prasugrel with 100 mL methanol. Aliquots of stock standard solution of PRA was transferred to a 10 mL volumetric flask and diluted to volume with methanol to get working standard solutions concentration of 20 $\mu\text{g mL}^{-1}$ for PRA.

Method Validation

The analytical method was validated for accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) in accordance with ICH guidelines.

Linearity and Range

Aliquots of working standard solution of PRA were spotted on HPTLC plates to obtain concentration in range of 0.4

to $1.6 \mu\text{g spot}^{-1}$ for PRA. Each concentration was spotted six times on TLC plate. The plate were developed, dried and scanned as described in chromatographic conditions. The calibration curves were constructed by plotting peak areas against concentration for each drug. For evaluation of linearity, peak area and concentrations were subjected to least square regression analysis to calculate regression equation and correlation coefficient.

Precision

Precision of the methods was determined by repeatability and intermediate precision and expressed as % RSD which were used as measure of precision. Repeatability of sample application was assessed by spotting PRA ($0.6 \mu\text{g spot}^{-1}$) seven times on an HPTLC plate. Repeatability of measurement of peak area was determined by repeated scanning of the same spot ($n = 7$) of PRA ($0.6 \mu\text{g spot}^{-1}$). Intra-day precision and Inter-day precision was evaluated by assaying freshly prepared solutions of PRA in triplicate on the same day and on three different days respectively at three different concentration levels of 0.6, 1.0 and $1.4 \mu\text{g spot}^{-1}$ for PRA.

Accuracy

The accuracy of the method was determined by recovery studies using standard addition method. Recovery studies were carried out in triplicate by addition of known amounts of standard drug solution to preanalyzed sample at three different levels: 80, 100 and 120 %. Three determinations were performed at each level of recovery.

Analysis of Pharmaceutical Preparations

Effient 5 mg Tablets/Aplet 5 mg Tablets

Twenty tablets were accurately weighed and finely powdered. A quantity of powder equivalent to one tablet containing 5 mg of PRA was transferred in to 50 mL volumetric flask. To this flask, 30 mL of methanol was added, and the solution was sonicated for 30 min with intermittent shaking. Finally volume was made up to 50 mL with methanol and then filtered through $0.45 \mu\text{m}$ membrane filters (Millipore, Milford, MA). From the filtered solution, 2.0 mL of solution was transferred in to a 10 mL volumetric flask and diluted to volume with methanol to obtain concentration of PRA as $20 \mu\text{g mL}^{-1}$ and 25 μL of this solution was spotted on HPTLC plate for analysis.

Effient 10 mg Tablets/ Aplet 10 mg Tablets

Twenty tablets were accurately weighed and finely powdered. A quantity of powder equivalent to one tablet containing 10 mg of PRA was transferred in to 50 mL volumetric flask. To this flask, 30 mL of methanol was added, and the solution was sonicated for 30 min with intermittent shaking. Finally volume was made up to 50 mL with methanol and then filtered through $0.45 \mu\text{m}$ membrane filters (Millipore, Milford, MA). From the filtered solution, 1.0 mL of solution was transferred in to a 10 mL volumetric flask and diluted to volume with methanol to obtain concentration of PRA as $20 \mu\text{g mL}^{-1}$ and 25 μL of this solution was spotted on HPTLC plate for analysis.

Content Uniformity Testing

The same procedure applied for the analysis of Prasugrel in Tablets was followed using one tablet, as a sample. Ten tablets of each dosage were analyzed and the uniformity of

their contents was tested by applying the official USP (The United State Pharmacopoeia 36 NF 31). [9]

RESULT AND DISCUSSIONS

Optimization of Chromatographic Conditions

Selection of best solvent system is the critical step in HPTLC method development. The HPTLC procedure was optimized in view to develop assay method for PRA. Initially toluene, ethyl acetate and methanol were tried in different ratios. Toluene was used to impart the necessary non-polarity to the mobile phase to obtain suitable Rf value. Initially toluene-ethyl acetate-methanol (4:4:2) was selected but Rf was 0.9 and also fronting and tailing with peaks was observed. Then ethyl acetate was completely removed from the mobile phase and volume of toluene in mobile phase was increased from 4 to 9 mL to decrease Rf and acetic acid (0.04 mL) was added to improve fronting and tailing of peaks. The optimized mobile phase was toluene-methanol-acetic acid (8:2:0.04, v/v/v) showed a good resolution and symmetric peak of PRA with Rf value of 0.83 (Figure 1). The chamber saturation time was 20 min and densitometric scanning was performed at 220 nm.

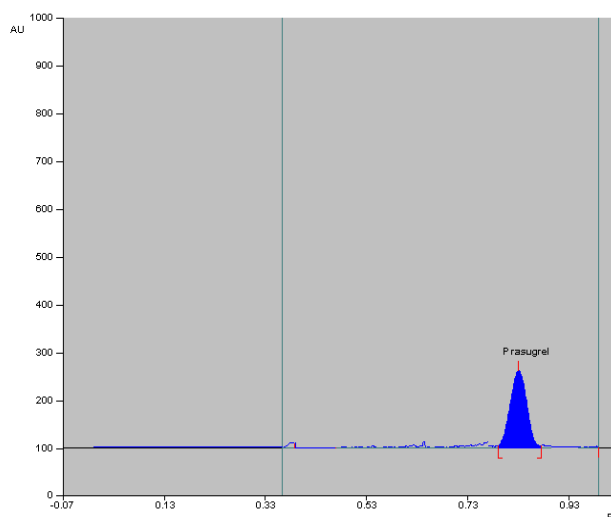


Figure 1 HPTLC Chromatograph of Prasugrel

Validation of Method

Linearity

To assess linearity, standard calibration curves for PRA were constructed by plotting mean peak areas versus concentrations. The calibration curves showed good linearity over the concentration range of $0.4\text{--}1.4 \mu\text{g spot}^{-1}$ for PRA.

Precision

Repeatability of sample application, Repeatability of measurement of peak area, Intra-day precision and Inter-day precision were expressed in terms of relative standard deviation (% RSD). The % RSD values for Repeatability of sample application were found to be 0.72 % for PRA. The % RSD values for Repeatability of measurement of peak area were found to be 0.22 % for PRA. The % RSD values for Intra-day precision was found to be 0.34-0.92 % for PRA. The % RSD values for Inter-day precision was found

to be 0.67-1.54 % for PRA. Low values of RSD (less than 2%) indicate that the proposed method is precise and reproducible.

Accuracy

The recovery experiments were carried out by standard addition method. The percentage recoveries obtained were found to be 100.61 ± 1.04 for PRA.

Analysis of Pharmaceutical Preparations

The proposed method was successfully applied to determine PRA in tablet dosage form. The results are shown in Table 1.

Table 1 Assay of Pharmaceutical Preparations

Pharmaceutical Preparations	Drug	Label Claim (mg)	Recovery (%) Mean \pm S.D.
Effient	PRA	5 mg	100.47 \pm 1.21
Effient	PRA	10 mg	99.27 \pm 0.83
APLET	PRA	5 mg	101.67 \pm 1.30
APLET	PRA	10 mg	97.87 \pm 0.99

Content Uniformity Testing

Due to high precision of the proposed method and its ability to rapidly estimate the concentration of drug in a single tablet extract with sufficient accuracy, the method is suited for content uniformity testing which is a time-consuming process when using conventional assay techniques. Content uniformity testing was done according to the USP procedure. The acceptance value (AV) was calculated for each of the commercially available tablets and found to be smaller than the maximum allowed acceptance value (LI). The results are shown in Table 2.

Table 2 Results of content uniformity testing of Prasugrel Tablets using the proposed method

PARAMETERS	Label Claim (%)			
	Effient 5 mg	Effient 10 mg	Aplet 5 mg	Aplet 10 mg
	100.89	101.36	101.18	97.11
	98.48	100.75	103.43	98.20
	102.46	104.11	97.53	101.67
	101.48	97.45	101.89	97.42
	99.05	103.78	100.40	96.36
	98.38	98.79	98.26	100.66
	96.84	102.37	103.46	101.49
	103.41	101.21	99.57	96.14
	102.68	103.28	102.31	99.54
	104.43	102.98	104.73	96.26
Mean	100.81	101.61	101.28	98.49
% RSD	2.49	2.14	2.32	2.22
Acceptance Value (AV)	6.03	5.32	5.63	5.27
Maximum allowed AV (LI)	15	15	15	15

Table 3 Summary of Validation parameters for proposed HPTLC method

Parameters	HPTLC method
	PRA
Linearity	0.4-1.4 $\mu\text{g spot}^{-1}$
Correlation coefficient	0.9998
Precision (% RSD)	
Repeatability of Sample Application ($n = 7$)	0.72
Repeatability of measurement of peak area ($n = 7$)	0.22
Intraday Precision ($n = 3$)	0.34-0.92
Interday Precision ($n = 3$)	0.67-1.54
Accuracy ($n = 3$)	100.61 \pm 1.04
Limit of Detection	0.08 $\mu\text{g spot}^{-1}$
Limit of Quantitation	0.26 $\mu\text{g spot}^{-1}$
Specificity	Specific

CONCLUSION

A new simple, sensitive, accurate, reproducible and precise HPTLC method for assay of Prasugrel in pharmaceutical formulations has been developed and validated. Statistical analysis proves that the method is suitable for analysis of Prasugrel in pharmaceutical formulations without any interference from excipients. The proposed HPTLC method is less expensive, simple, rapid, and more flexible than HPLC. Thus, it can be used for quality control of Prasugrel tablets with excellent application to content uniformity test.

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