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A validated high performance thin layer chromatographic method for simultaneous estimation of aspirin and clopidogrel in pharmaceutical dosage form

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ABSTRACT

A simple, accurate, precise and rapid high-performance thin-layer chromatographic method for simultaneous determination of Aspirin and Clopidogrel Bisulphate, both as a bulk drug and in pharmaceutical formulations was developed and validated. The method employed aluminum TLC plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of Toluene: Methanol: Chloroform (5:3:2, v/v/v) as mobile phase. Densitometric analysis of Aspirin and Clopidogrel Bisulphate was carried out at 226 nm. The system was found to give compact spots for Aspirin (R_f 0.4± 0.02) and Clopidogrel Bisulphate (R_f 0.85 ± 0.01). The linear regression analysis data showed good linear relationship in the concentration range 600-1600 ng per spot for Aspirin and 1200-3200 ng per spot for Clopidogrel Bisulphate. The method was validated for precision, accuracy, specificity and robustness. The method has been successfully applied to the analysis of marketed formulation.

KEYWORDS

Aspirin;
Clopidogrel bisulphate;
HPTLC-densitometry;
Simultaneous estimation.

INTRODUCTION

Aspirin (ASP) is analgesic, antipyretic and anti-inflammatory drug and Clopidogrel bisulphate (CLOPI) is an antiplatelet drug[1]. Aspirin is official in I.P. (Indian Pharmacopoeia)[2], both the drugs are official B.P. (British Pharmacopoeia)[3] and U.S.P (U.S.Pharmacopoeia)[4]. Detailed survey of literature revealed some methods reported for the determination of Aspirin and its combinations as Reversed Phase High Performance Liquid chromatography[5-9], LC-MS[10], Near-Infrared Spectroscopy by Radial Basis Function neural networks[11], solid-phase spectrofluorimetry using partial-least squares multivariate calibration method[12] and HPTLC[13]. Methods reported for the determination of Clopidogrel Bisulphate include bioanalytical methods in plasma by LC-Mass spectrometry (MS)[14,15], Gas chromatographic[16] determination of CLOPI from tablets and HPLC technique[17]. Reverse phase High Performance Liquid Chromatography (HPLC)[18,19] methods have been reported for determination of ASP and CLOPI in combination. In the present investigation simple, accurate, sensitive and precise HPTLC method has been developed for simultaneous determination of Aspirin and Clopidogrel Bisulphate in the bulk and marketed formulation.

EXPERIMENTAL

Drugs, reagents and chemicals used

Working standards of Aspirin (Purity -100.99 %) and Clopidogrel Bisulphate (Purity-99.81 %) were provided as a gift sample by Sidmak Laboratories Pvt Ltd, Valsad, India and used without further purification. The drugs were received along with certificate of analysis. All the other reagents used were of analytical grade. Chloroform (AR grade), Toluene (AR grade), Methanol (AR grade), Acetone (A R grade), Acetic acid (A R grade), Dimethyl Sulfoxide (A R grade), Dichloromethane (A R grade), Benzene (A R grade), Triethanolamine (A R grade) were purchased from Thomas Baker Pvt. Ltd., Mumbai. The pharmaceutical dosage form used in this study was a marketed product; labeled to contain 75 mg of Aspirin and 75 mg Clopidogrel Bisulphate of per tablet.

Instrumentation

Chromatographic separation was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 μm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a band with 4 mm width using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Camag Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (10×10 cm). Densitometric scanning was performed using Camag TLC scanner 3 at 226 nm and operated by winCATS software (V 1.4.2, Camag).

Preparation of standard stock solution

Standard stock solution of aspirin

10 mg of Aspirin was weighed and dissolved in 10 ml of methanol to obtain 1000 μg/ml stock solution of Aspirin.

Standard stock solution of Clopidogrel Bisulphate

10 mg of Clopidogrel Bisulphate was weighed and dissolved in 10 ml of methanol to obtain 1000 μg/ml stock solution of Clopidogrel Bisulphate.

Preparation of dilutions

From the stock solution of Aspirin (1000μg/ml), Clopidogrel Bisulphate (1000μg/ml), appropriate dilutions were done to obtain the final concentrations of Aspirin (100μg/ml), Clopidogrel (200 μg/ml). 10μl solutions were applied as bands.

Chromatographic parameters

a. Solvent used: Methanol
b. Stationary phase: TLC plate precoated with silica gel 60 F_{254}
c. Mobile phase: Toluene-Methanol-Chloroform (5: 3: 2v/v/v)
d. Detection wavelength: 226 nm
e. Temperature: Ambient

Preparation of calibration curves

From the respective standard stock solutions, a volume of 6 - 16 μL ASP of and 6 - 16 μL of CLOPI was spotted on the TLC plate to obtain final concentration in the range of 600-1600 and 1200-3200 ng/ spot for ASP and CLOPI respectively. Chromatogram was developed in a twin trough glass chamber, using 15 mins of chamber saturation time in the mobile phase mentioned above. The length of chromatogram run was 90 mm. The developed plates were dried using dryer. Densitometric scanning was performed in the absorbance mode at 226 nm. The slit dimension was kept at 5×0.45 mm at scanning speed of 200 nm/s. After completion of scanning, peak areas of ASP and CLOPI were noted. Peak areas were plotted against corresponding concentrations (ng/ spot) and least square regression analysis was performed to generate the calibration equation for ASP and CLOPI.

Analysis of tablet formulation

Twenty Tablets, each containing 75 mg ASP and 75 mg CLOPI were weighed and finely powdered. A quantity of powder equivalent to 75 mg ASP was weighed and transferred to 25 ml volumetric flask, about 20 ml methanol was added and ultrasonicated for 10 min The volume was made up to 25 ml with methanol. The solution was filtered using Whatmann filter paper No.41. From the filtrate, appropriate dilutions were made with methanol. Suitable volume was spotted to obtain final concentration in the range of 600 ng/spot to 1600 ng/spot for ASP and 1200 ng/spot to 3200 to ng/ spot for CLOPI. The samples were analyzed in the same manner as the standards. The amount of each drug

TABLE 1: Results of analysis of marketed formulation

Sr. no.	Amount present (mg/tablet)		Amount found* (mg/tablet)		% of Label claim	
	ASP	CLOPI	ASP	CLOPI	ASP	COPI
1	75	75	74.72	74.75	99.63	99.67
2	75	75	75.14	75.20	100.19	100.27
3	75	75	74.39	74.77	99.19	99.69

*Average of three determinations

TABLE 2: Intra-day and Inter-day precision studies for Aspirin and Clopidogrel

ASP			CLOPI		
Concentration (ng/spot)	% R.S.D		Concentration (ng/spot)	%R.S.D	
	Intra-day	Inter-day		Intra-day	Inter-day
1000	0.543	1.021	2000	0.682	0.958

TABLE 3: Recovery studies of Aspirin and Clopidogrel

Drug	Level of Recovery (%)	% Recovery			% RSD
		Replica 1	Replica 2	Replica 3	
ASP	80	98.52	100.73	100.24	1.109
	100	99.76	98.82	100.56	0.8734
	120	99.89	100.21	99.68	0.267
CLOPI	80	100.84	98.86	99.54	1.01
	100	100.26	100.84	101.21	0.474
	120	99.26	99.37	100.25	0.544

present per tablet was estimated from the respective calibration curves(TABLE 1).

Method validation

As per the ICH guidelines[20], the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and specificity.

Precision

The repeatability and intermediate precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 repeated measurements of standard and sample solutions were made in a day and percentage RSD values were calculated. In the inter day variation studies, 3 repeated measurements of standard and sample solutions were made on 3 consecutive days and percentage RSD values were calculated. (TABLE 2)

Accuracy

For checking the accuracy of method, recovery studies were carried out by applying the method to drug sample to which known amount of ASP and CLOPI were added at level of 80, 100 and 120% of label claim (standard addition method). Three determinations were performed at each level, and the results obtained were compared with expected results. (TABLE 3)

Limit of detection and limit of quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was determined by visual inspection.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was determined by visual inspection.

Robustness

Robustness is checked by making slight deliberate change in the experimental procedures. Mobile phases having different compositions like toluene-methanol-chloroform (5:2.5:2.5 v/v/v) and (4.5:2:3.5 v/v/v) were tried and chromatograms were run. Robustness of the method was checked at three different concentration levels 600, 800, 1200 ng/spot and 1200, 1600, 2400 ng/spot for ASP and CLOPI, respectively.

Specificity

The specificity of the method was ascertained by analyzing standard drug and commonly used tablet excipients like starch and lactose. The absorption spectra of the resolved spots for the two drugs were compared with the spectra of reference standards.

RESULT AND DISCUSSION

Optimization of solvent system and chromatographic conditions

Chromatographic separation studies were carried out on the stock solution of ASP and CLOPI. Initially the plates spotted with 10µL of stock solution were developed by linear ascending development method using neat solvents like toluene, methanol, chloroform, dimethyl sulfoxide, and acetone, with chamber saturation. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum resolution between ASP and CLOPI. The final mobile phase consisting of toluene: methanol: chloroform in the ratio of (5:3:2 v/v/v) was considered optimum since good resolution with R_f values of 0.4 ±

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0.02 for Asp and 0.85 ± 0.01 for CLOPI were obtained as shown in figure 1. The samples were applied in form of bands of width 4mm on precoated aluminum sheets of silica gel 60 $F_{254.}$ The application position (X) and (Y) were kept at 10mm and 15 mm respectively to avoid edge effect.

Linearity

ASP showed good correlation coefficient when peak area of the resolved spot was plotted against concentration in the range of 600-1600 ng/spot and CLOPI in the concentration range of 1200-3200 ng/spot. Linearity was determined by evaluating five working standards.

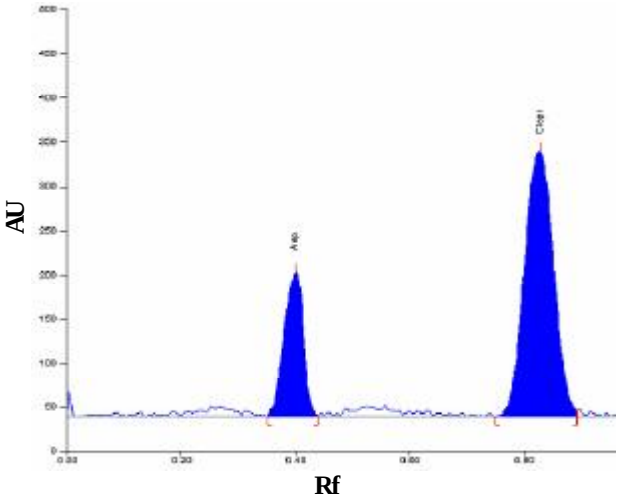

Figure 1: Representative HPTLC densitogram of Aspirin ((600 ng/spot, R_f 0. 4) and clopidogeral (1200 ng/spot, R_f 0.85)

The equations of the regression lines are

For ASP $y = 3.1355x - 765.89$ ($r^2 = 0.9948$)

For CLOPI $y = 7.1348x - 894.19$ ($r^2 = 0.9971$)

Analysis of tablet formulation

The proposed methods were also evaluated in terms of assay of commercially available tablets containing ASP and CLOPI. Three replicate determinations were performed on the accurately weighed amounts of tablets. The results obtained are shown in TABLE 1.

Precision

The proposed method was found to be precise as indicated by percent RSD (Relative Standard Deviation) not more than 1.5%. The intra-day and inter-day precision results are shown in TABLE 2.

Accuracy

The proposed method when used for estimation of ASP and CLOPI from pharmaceutical dosage form after spiking with working standard afforded recovery of 98-102% and result of recovery for ASP and CLOPI from the marketed formulation are listed in TABLE 3.

Limit of detection and limit of quantification

The limit of detection was found to be 50 ng/spot for ASP and 170 ng/spot for CLOPI, while the limit of quantitation of ASP was found to be 165ng/spot and for CLOPI it was 510 ng/spot.

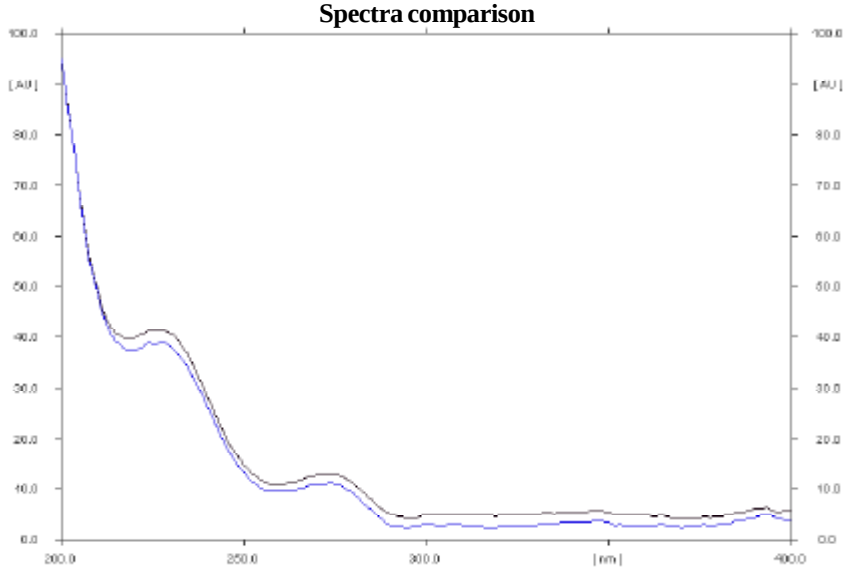

Figure 2 : Overlain spectra of Aspirin at R_f 0.40 from standard and sample

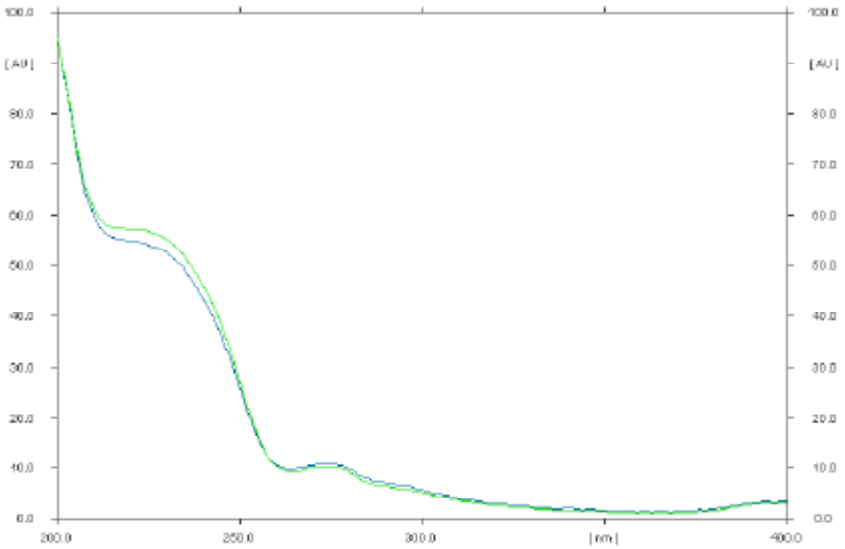

Figure 3 : Overlain spectra of spot at R_f 0.85 from standard and sample

Robustness

Robustness is checked by making slight deliberate change in the experimental procedures. The method was found to be robust for CLOPI since the peak area values were not significantly affected. The signal for ASP was found to be very sensitive to changes in mobile phase composition.

Specificity

The method was found to be specific since no interfering spots were seen when various excipients were tried by the same procedure. The spectra of working standards and the resolved bands at the respective R_f values from the marketed sample matched exactly (Figures 2 and 3) indicating no interference by the matrix.

CONCLUSION

The validated HPTLC-densitometric method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of Aspirin and Clopidogrel Bisulphate in combined tablet dosage form.

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