

## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF S (-) AMLODIPINE BESYLATE AND CLOPIDOGREL BISULPHATE IN TABLET DOSAGE FORM

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### ABSTRACT

The present work represents a reverse phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of S (-) Amlodipine besylate [S (-) AB] and clopidogrel bisulphate [CLOP]. This method was developed with isocratic HPLC system, UV detector (detection wavelength 232 nm) and Hypersil BDS C<sub>8</sub> (250 mm×4.6mm, 5µm) column at 40±5°C, Sodium dihydrogen ortho phosphate Buffer: Methanol: ACN (35:15:50 at pH 3) as mobile phase at 1mL/min. flow rate and 20 µL sample injection volume. Number of theoretical plates for S (-) AB and CLOP were 10310 and 16330, respectively. Tailing Factors for S (-) AB and CLOP were 1.21 and 1.01, respectively. The retention time for S (-) AB and CLOP were 3.9 and 9.6 min., respectively. The described method had excellent linearity in concentration range for 6.37-19.12µg/mL of S (-) AB and 94.27-282.81µg/mL of CLOP. The percentage assay of S (-) AB and CLOP in tablet samples were 100.96% and 101.54%, respectively. Percent recovery for both drugs demonstrated accuracy and low value of % RSD indicated reproducibility of this method. Result suggest the developed method can be used for routine quality control studies for assay of S (-) Amlodipine Besylate and Clopidogrel bisulphate in bulk and tablet dosage forms.

**Keywords:** S (-) Amlodipine besylate, Clopidogrel bisulphate, High Performance Liquid Chromatography, Validation.

### INTRODUCTION

Amlodipine Besylate (AB), a long-acting calcium channel blocker, is widely used in treatment of hypertension, angina pectoris and congestive heart failure [1]. Amlodipine is a racemic mixture, composed of the S (-) enantiomer, which is the pharmacologically active isomer, and the R (+)-enantiomer, which is 1000-fold less active. Cardio selective S (-) Amlodipine enantiomer have better efficacy (half of the racemate dose) and safety profiles i.e. less side effects [2]. Amlodipine besylate is chemically known as (S)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester [3] (Fig 1).

Clopidogrel Bisulphate (CLOP), an anti-platelet agent is used to prevent ischaemic stroke [4]. CLOP is, chemically (+)-(S)-(2-chlorophenyl) - 6, 7-dihydrothieno [3, 2-c] pyridine- 5(4H)-acetic acid methyl ester sulphate (Fig 2). The combination of S (-) Amlodipine besylate and Clopidogrel Bisulphate is used in the management of hypertension which cannot be controlled by single drug therapy alone [5].

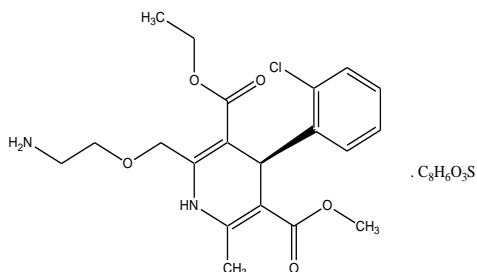


Fig. 1: Chemical structure of S (-) Amlodipine Besylate

Literature survey revealed that S (-) Amlodipine besylate has been estimated by RP-HPLC in bulk and tablet dosage form [6]. Several methods have been reported for determination of Clopidogrel bisulphate by visible [7], derivative U.V. spectrophotometry [8], HPTLC [9] and HPLC method [10]. Simultaneous estimation of racemic Amlodipine besylate and Clopidogrel bisulphate has also been reported by RP-HPLC [11] but there is no report on simultaneous determination of S (-) Amlodipine Besylate enantiomer and Clopidogrel in combined dosage form so far. In this context, the estimation of such enantiomer may play an important role in routine analysis of the formulation. The present paper describes development and validation of RP-HPLC method for simultaneous estimation of S (-) Amlodipine besylate and clopidogrel bisulphate in tablet dosage form

### MATERIAL AND METHODS

#### Chemical and Reagents

Reference Standards of S (-) Amlodipine besylate and Clopidogrel bisulphate were obtained from Emcure Pharmaceuticals Ltd., Bhosari, Pune. Commercially available tablets (Label Claim: 5 mg of S (-) Amlodipine and 75 mg of clopidogrel Bisulphate) of the combined dosage form were procured from local market. HPLC grade of solvents were procured from Lobachemie Pvt. Ltd, Mumbai and AR grade of chemicals were obtained from Merck India Ltd.

#### Equipment

The HPLC system (Waters Alliance, 2695) consisted of a pump with auto injection facility, UV-VIS spectrophotometer/photodiode array detector and Empower 2 software. Hypersil BDS C<sub>8</sub> (250 mm×4.6mm, 5µm) column was used. Absorbance measurements were made on a double beam spectrophotometer (Shimadzu UV-1800) for selection of wavelength for detection. Weighing of the drug was performed using an Electronic Balance (Mettler Toledo AG 245). Sonications of standard and sample solutions were carried out using an Ultrasonic Cleaning Bath (Leela sonic 250).

#### Chromatographic Conditions

Isocratic HPLC system was used with following optimized conditions. The flow rate of mobile phase was set at 1mL/min. and column temperature was maintained at 40°C ± 5° C. The detection was carried out at 232 nm. The injection volume was taken 20µl and

Fig. 2: Chemical structure Clopidogrel Bisulphate

runtime of the analysis was kept 15 minutes. The mobile phase selected for analysis was Sodium dihydrogen ortho phosphate Buffer: Methanol: ACN (35:15:50), pH adjusted to 3 with O-phosphoric acid.

#### Standard preparation

Accurately weighed S(-) Amlodipine besylate (38 mg) and clopidogrel bisulphate (25 mg) reference standards were transferred into a volumetric flask (100 ml) followed by dissolution in methanol (20 ml) by sonication for 10 min. The volume of this solution was made up to mark with methanol, which was used as standard stock solution. This stock solution was further diluted with mobile phase for preparation of working standard solutions.

#### Sample solution

Twenty tablets were accurately weighed and average weight of a tablet was calculated. These tablets were crushed and powdered in glass mortar. Tablet Powder equivalent to 190 mg of CLOP was weighed accurately and transferred into a 100 ml volumetric flask. It was dissolved with about 70 ml methanol. The contents of volumetric flask were sonicated for 10 minutes and volume was made up to the mark with same solvent. The solution was filtered using Whatmann filter paper No. 41. This solution was further diluted with mobile phase to obtain final concentration of S (-) AB (13 µg/ml) and CLOP (189 µg/ml). The final sample solution was filtered (Whatmann filter paper No. 41) and injected. Run time of analysis was kept 15 min and detection is carried out at 232nm. Aliquots of sample solution were injected under the operating chromatographic condition. All the determinations were carried out at three times and the percentage assay of S (-) AB and CLOP in tablet samples were calculated [12, 13].

#### Method Validation

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The developed chromatographic method was validated for system suitability, linearity & range, accuracy, precision, and robustness, solution stability, specificity, as per ICH guidelines [14].

#### System suitability test

The system suitability test was performed by five replicate analyses of working standard solution. Results of %RSD, retention time, theoretical plates and tailing factor (peak symmetry) were presented in Table 2.

#### Linearity and range

Working solutions were injected under the operating chromatographic conditions and peak areas for each drug were calculated at 232 nm. The calibration curve was plotted between areas against corresponding concentrations of each drug. Linear regression data for calibration curves were shown in Table 3. The range of solution has been decided according to correlation coefficient of regression equation.

#### Accuracy (% recovery)

The accuracy of the method was determined by calculating % recovery of each drug by standard addition method. Percent recovery of S (-) AB and CLOP was determined at three different level 50%, 100% and 150% of the target concentration in triplicate (Table 4).

#### Precision

Method repeatability (intra-day precision) was evaluated by assaying six samples, prepared as described in the sample preparation. The intermediate precision (inter-day precision) was performed by assaying six samples prepared by different analyst, different HPLC system and different HPLC column in different days as described in the sample preparation. The relative standard deviation (RSD) values for inter-day and intra-day precision for S (-) AB and CLOP were mentioned in Table 5.

#### Robustness

Robustness of the method was studied by changing flow rate ( $\pm 0.1$  ml/min), change in temperature ( $\pm 5^\circ\text{C}$ ), and change in wavelength ( $\pm 2$  nm) during analysis. Sample solution of 100% concentration is prepared and injected in triplicate for every condition and % Assay was calculated for each condition (Table 6).

#### Specificity

Specificity was performed by separately injecting blank, placebo sample solution of S (-) AB and CLOP. The retention time of S (-) AB does not interfere with CLOP. Hence, the method was found to be specific, chromatogram were shown in Fig 7.

#### RESULTS AND DISCUSSION

The composition, flow rate of mobile phase and column as well as column temperature was suitably optimized for better separation of these drugs. Finally, sodium dihydrogen ortho phosphate Buffer: Methanol: ACN (35:15:50 at pH 3), at 1 mL/min. flow rate, Hypersil BDS C8 column at  $40 \pm 5^\circ\text{C}$  was selected. These optimized conditions had following system suitability parameters. The value of % RSD was 0.27 and 0.16 for S (-) AB and CLOP, respectively, Number of theoretical plates for S (-) AB and CLOP were 10310 and 16330, respectively. Tailing Factors for S (-) AB and CLOP were 1.21 and 1.01, respectively. These values were acceptable and therefore the chosen optimized conditions were used further analysis. The retention time for S (-) AB and CLOP were 3.9 and 9.6 min., respectively. The values of correlation coefficient for S (-) AB and CLOP (Table 2) demonstrated the good relationship between peak area and concentration. Therefore, the developed method was linear in concentration range for 6.37-19.12 µg/mL of S (-) AB and 94.27-282.81 µg/mL of CLOP. The percentage assay of S (-) AB and CLOP in tablet samples were 100.96% and 101.54%, respectively (Table 1). Percent recovery was 99.60-100.48 for S (-) AB and 101.2-101.4% for CLOP demonstrated accuracy. The low value of % RSD in intra-day and inter-day precision (Table 5) indicated reproducibility of this method. Finally, deliberate variations were made to check the significant variations in experimental conditions (Table 6) suggested robustness of developed method.

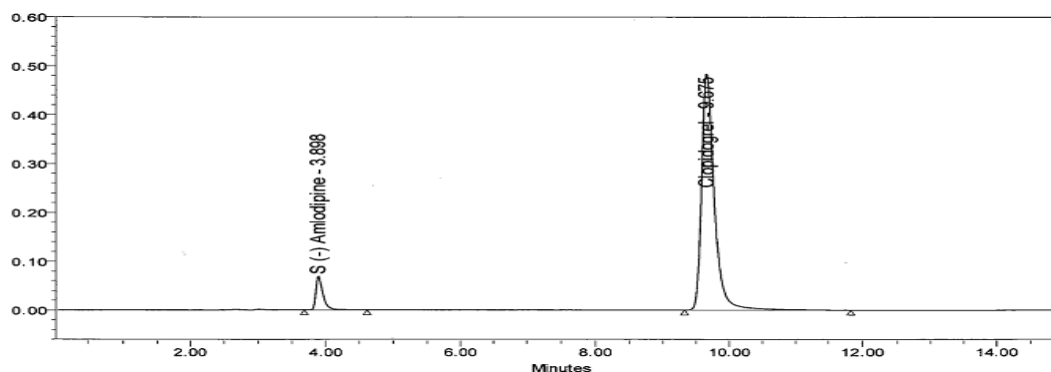


Fig. 3: Standard Solution of S (-) AB and CLOP

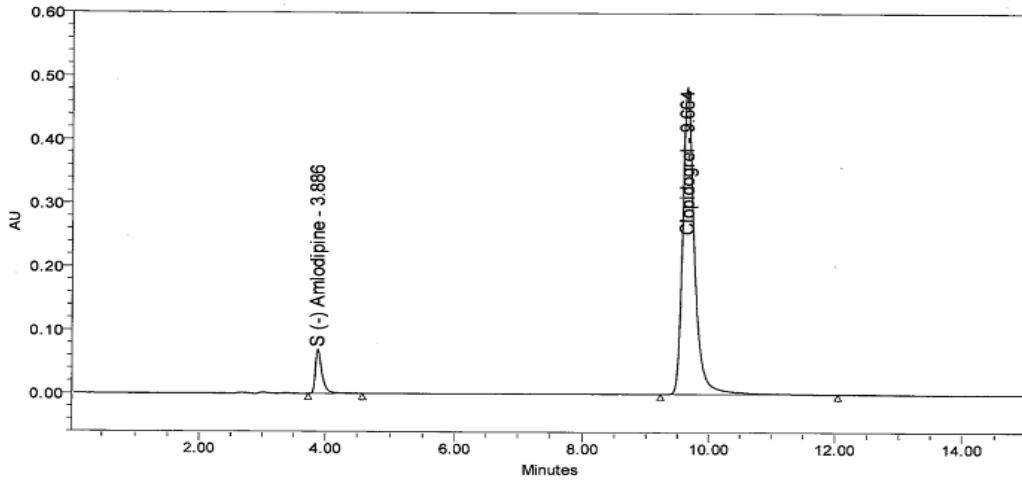


Fig. 4: Sample solution of S (-) AB and CLOP

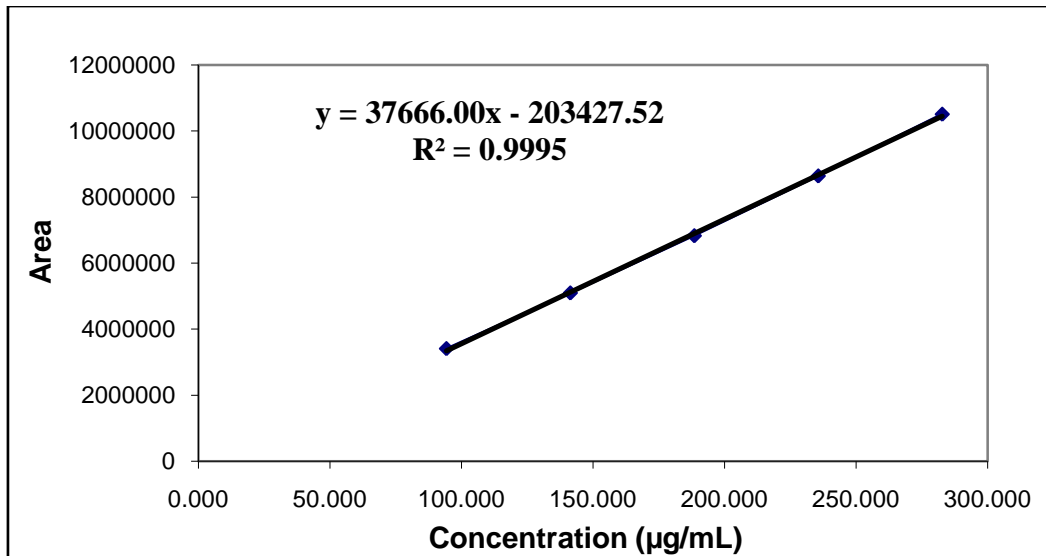


Fig. 5: Calibration curve of Clopidogrel Bisulphate

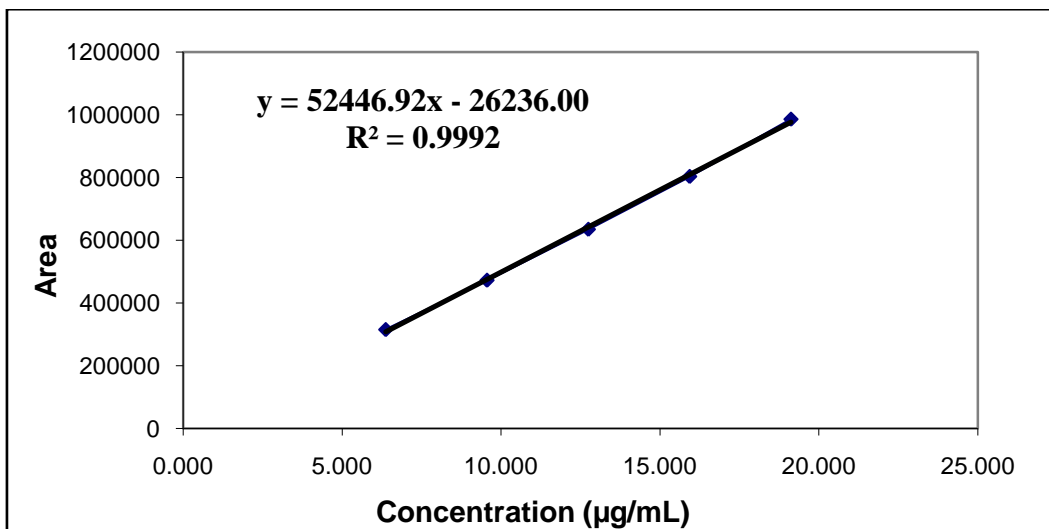
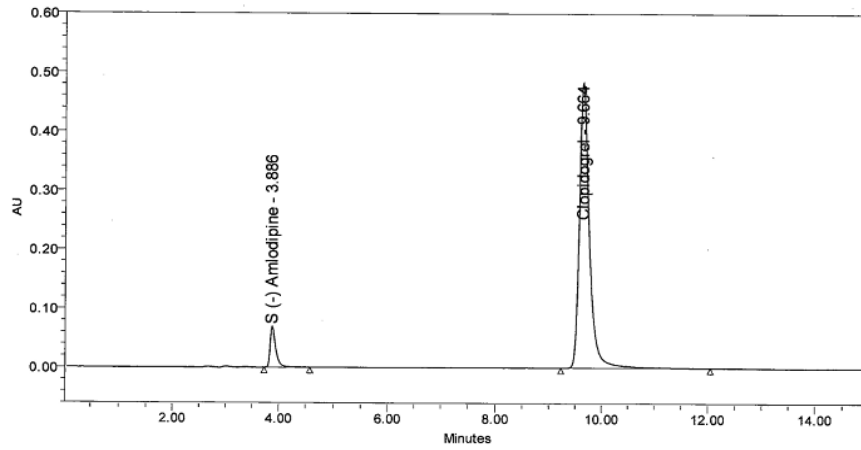
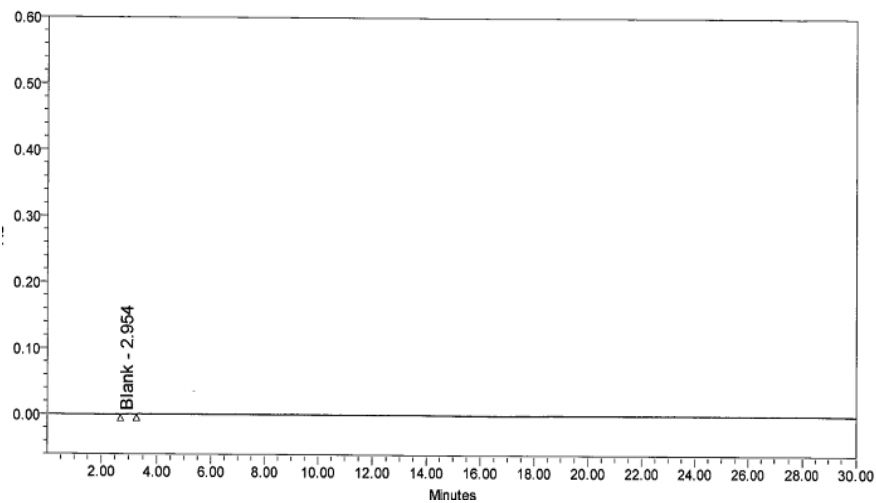


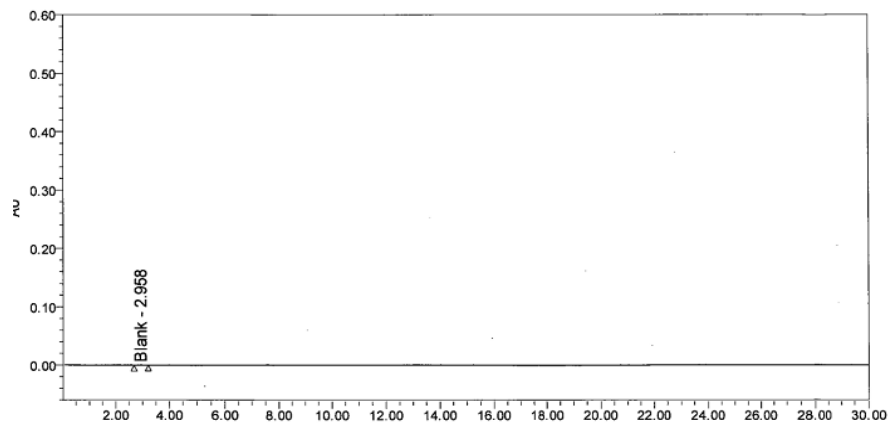
Fig. 6: Calibration curve of S (-) Amlodipine besylate



Sample solution of S (-) AB and CLOP



Blank solution



Placebo solution

Fig. 7: Specificity

Table 1: Results of Assay of tablet formulation

Parameters	Amlodipine besylate	Clopidogrel bisulphate
Label Claim (mg)	5	7
Actual content found (mg)	5.048	76.15
% Assay	100.96	101.54

\*Three replicates injections (\*n=3)

Table 2: Results of system suitability test

Parameter	S (-) Amlodipine Besylate	Clopidogrel bisulphate
SD*	1707.16	11086.93
%RSD*	0.27	0.16
RT(min)	3.967	8.984
Theoretical Plate	10310	16330
Tailing Factor	1.21	1.01

\*n=5

Table 3: Linear regression data for calibration curves of S (-) Amlodipine Besylate and Clopidogrel bisulphate

Parameters	S(-)Amlodipine Besylate	Clopidogrel bisulphate
Linearity range (µg/ml)	6.37 – 19.11	94.27 – 282.81
r <sup>2</sup>	0.9992	0.9995
Slope	52447.71	37666.00
Intercept	26236.00	203427.53

Table 4: Accuracy data of S (-) AB and CLOP

Drug	Amount Added (µg/ml)	Amount recovered (µg/ml)	Mean % Recovery ± SD*	% RSD*
S (-) AB	50% (6.6 µg/ml)	6.57	99.60% ± 0.81	0.82
	100% (13 µg/ml)	12.94	99.57% ± 0.29	0.29
	150% (19.6 µg/ml)	19.69	100.48% ± 1.22	1.21
CLOP	50% (94.5 µg/ml)	96.63	101.21% ± 0.29	0.29
	100% (189 µg/ml)	192.45	101.83% ± 0.16	0.15
	150% (283 µg/ml)	286.96	101.40% ± 0.61	0.59

\*n=3

Table 5: Precision data of S (-) Amlodipine Besylate and Clopidogrel bisulphate

Parameter	S(-) Amlodipine besylate	Clopidogrel bisulphate
Inter-day (%RSD)*	0.615	0.344
Intra-day (%RSD)*	0.597	0.3437

\*Six replicates Injections (\*n=6)

Table 6: Robustness studies of S (-) AB and CLOP

Parameter	Variation	S(-)AB			CLOP		
		Tailing factor	% RSD of Area	%Assay	Tailing factor	% RSD of Area	%Assay
Flow Rate	0.9 ml/min	1.16	0.60	101.24	1.11	0.18	101.65
	1.1 ml/min	1.11	0.48	100.82	1.03	0.11	102.43
Column Temperature	35°C	1.11	0.19	97.64	1.01	0.06	99.41
	45°C	0.29	0.29	97.13	1.02	0.21	99.13
Wavelength	230 nm	1.18	0.37	100.48	1.01	0.11	97.72
	234 nm	1.17	0.42	100.27	1.04	0.25	101.05

\*n=3

## CONCLUSION

It can be concluded from the results that the proposed RP-HPLC method was found to be simple, accurate, robust & precise for the analysis of S (-) Amlodipine Besylate and Clopidogrel bisulphate in bulk and tablet dosage forms. This method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of S (-) Amlodipine Besylate and Clopidogrel bisulphate.

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