

# Ion-Pairing RP-HPLC Method for Simultaneous determination of Aspirin and Clopidogrel bisulphate in Tablet and Capsule Dosage Form

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**ABSTRACT:** A simple and accurate Ion-Pairing RP-HPLC method was developed and subsequently validated for the simultaneous determination of Aspirin and Clopidogrel bisulphate in commercial formulation. The proposed RP-HPLC method utilized LiChroCART-LiChrospher 100;C<sub>18</sub> column(250mm × 4mm i.d.,5μm) and mobile phase consisting of acetonitrile:0.01M TBAHS (50:50% v/v) at a flow rate of 1.0ml/ min.Quantitation was achieved with UV detection at 240 nm .The retention time were 3.167min and 5.758min for Aspirin and Clopidogrel respectively. The linearity ranges were 1-250 μg/ml for Aspirin and 0.5-125 μg/ml for Clopidogrel ( $R^2 > 0.999$  for both drugs).The method was validated for accuracy, precision, linearity, LOD, LOQ and system suitability. This method was successfully applied to pharmaceutical dosage forms and no interference from the excipients was found.

**Keywords:** Aspirin, Clopidogrel, Liquid Chromatography, Validation.

## INTRODUCTION

Aspirin is an antipyretic, analgesic agent<sup>1</sup>. Chemically it is 2-acetoxy benzoic acid<sup>1</sup>. Clopidogrel bisulphate is an antiplatelet agent<sup>2</sup>. Chemically it is Methyl-2-chlorophenyl-(4,5,6,7-tetrahydrothieno[3,2-c]pyridine-5yl)acetate bisulphate<sup>2</sup>.

Literature survey shows that several Spectrophotometric<sup>3,4,5</sup>, Spectrofluorimetric<sup>6</sup>, HPLC<sup>7,8,9,10,11,12,13,16,17</sup>, LC-MS<sup>14,15</sup>, HPTLC<sup>17,18</sup>, Potentiometric<sup>8</sup> and GC<sup>19</sup> methods have been reported .But no method has been reported so far for simultaneous estimation of both the drugs in this particular mobile phase composition by RP-HPLC. So an attempt was made to develop a novel, simple, accurate, rapid and precise RP-HPLC method for simultaneous determination of Aspirin and Clopidogrel in both tablet and capsule dosage form.

## MATERIALS AND METHODS

### Experimental

#### Reagents and Standards

Pure standard drugs Aspirin and Clopidogrel bisulphate were procured as gift samples from Zydus Healthcare (Sikkim, India). HPLC grade Acetonitrile was purchased from Merck Ltd. (Mumbai, India). Tetra butyl ammonium hydrogen sulphate (TBAHS) of analytical grade was purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Tablets and Capsules containing Aspirin (150mg) and Clopidogrel bisulphate (75mg), were purchased from the local market.

#### Instrumentation and Chromatographic Conditions

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT VP Series HPLC pumps, with a 20μl sample

injection loop (manual) and SPD 10A VP series UV-Visible detector. The output signal was monitored and integrated using Shimadzu Class-VP Version 6.12 SP1 Software. A LiChroCART LiChrospher 100 C<sub>18</sub> column (250mm × 4mm i.d., 5µm) was used for separation. Separation was achieved at ambient temperature on the column using the acetonitrile: 0.01M TBAHS (50:50, v/v) as mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The detector wavelength was set at 240 nm. The System Suitability Parameters are mentioned in Table 1.

#### **Preparation of Mobile phase and Standard Stock Solution**

0.01M TBAHS was prepared by dissolving 3.3954 gm of TBAHS salt in 1000ml Triple Distilled Water. The prepared mobile phase was ultrasonicated for 15 min. Also the acetonitrile was ultrasonicated for 15 min. Both the phases were filtered through a 0.45µ membrane filter.

Standard stock solutions for both the drugs were prepared separately by dissolving 25mg of the drugs in mobile phase up to 25ml. From there a mixed standard stock solution was so prepared that the drugs Aspirin and Clopidogrel will be in the ratio same as that of the marketed formulations available (2:1).

#### **Preparation of Calibration Curve**

Working standard solutions were so prepared that each concentration contained Aspirin and Clopidogrel in the ratio 2:1. The mixed working solutions of concentrations ranging from 1 to 250 µg/ml for Aspirin and 0.5 to 125 µg/ml for Clopidogrel were prepared by taking appropriate aliquots of the standard stock solution and diluting it with mobile phase. Each solution was injected into the column and the chromatograms were recorded. Two Calibration curves were prepared by plotting the peak area vs. concentrations. The chromatogram of standard drugs is shown in Figure 1.

#### **Analysis of Commercial Dosage Forms**

Twenty tablets were weighed accurately and powdered finely. A quantity of tablet powder equivalent to 25 mg of Aspirin and 12.5mg of Clopidogrel was accurately weighed into a 25 ml calibrated flask, 10 ml of diluent solution was added and the content was ultrasonicated for 20 min; the volume was then diluted to the mark and mixed well. A small portion of the extract was withdrawn and filtered through a 0.2µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent mobile phase for analysis as already described. Similar procedure was repeated for Capsules also. A representative chromatogram of both the drugs in dosage form has been given in Figure 2. The amount of drug present in the sample solution was determined

using the calibration curve of standard drug. The results are shown in Table 2.

## **RESULTS AND DISCUSSION**

To develop a RP-HPLC method different mobile phases, flow rates were tested. The different mobile phase compositions were methanol: water (50:50), acetonitrile: water (50:50), methanol: 0.01M TBAHS (50:50), acetonitrile: 0.01M TBAHS (50:50).

Also different flow rates like 0.8ml/min, 1.0ml/min and 1.2ml/min were tried. A concentration of the mixed drug solution was injected into the column and was monitored by UV-detection at 240 nm. An isocratic method was selected. The optimum flow rate was 1.0 ml/min. However under the described experimental conditions, the peaks were well defined and with good resolution. The proposed LC method was also validated for different parameters like linearity, accuracy, precision, LOD, LOQ and stability.

#### **Linearity**

The drugs followed a linearity of peak area *versus* concentrations ranging from 1-250 µg/ml for Aspirin and 0.5-125µg/ml for Clopidogrel respectively. A linear response was observed over the examined concentration range. The slope, intercept were found to be 45523, 60944 and 18767, 20722 for Aspirin and Clopidogrel respectively. The correlation coefficients were found to be 0.9996 and 0.9993.

#### **Accuracy**

To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120% of the test concentration as per ICH guidelines<sup>20</sup>. The recovery study was performed three times at each level. The results of recovery study are given in Table 1.

#### **Precision**

The precision of the method was ascertained separately from the peak areas obtained by actual determination of eight replicates of a fixed amount of drug. The percent RSD values for precision were calculated. The results are shown in Table 3.

#### **Limit of detection and Limit of quantization**

The LOD and LOQ were separately determined based on the Signal to Noise ratio. For LOD the S/N ratio is 3:1. For LOQ the S/N ratio is 10:1.

#### **Stability**

Stability of the drugs in the proposed mobile phase was checked at ambient conditions by storing it up to 48hrs. No significant loss of the active constituents was monitored also no interfering peaks of degraded products were observed at the retention times of the drugs.

**CONCLUSION**

A reversed-phase Ion-Pairing Liquid chromatographic method was developed for the simultaneous determination of Aspirin and Clopidogrel, and validated. The method is novel, simple, precise, accurate, and sensitive and has greater range of linearity. The good % recovery in tablet and capsule dosage forms suggests that the excipients

present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method.

So, the method is suitable for the determination of the drugs in tablets and capsules without interference from commonly used excipients, and could be used in a quality control laboratory for routine drug analysis.

**Table no.1. System Suitability Parameters**

Parameters	Obtained Values		
	Aspirin	Clopidogrel bisulphate	
Theoretical Plates(N)	2235.6	6877.4	
Resolution( $R_s$ )	6.09		
Tailing factor(T)	1.19	1.2	
LOD ( $\mu\text{g/ml}$ )	0.1218	0.2436	
LOQ ( $\mu\text{g/ml}$ )	0.406	0.812	
Retention Time(min)	3.167	5.758	
Accuracy* (Recovery Studies)	80%	101.82 $\pm$ 0.88	100.33 $\pm$ 0.62
	100%	102.56 $\pm$ 0.68	102.0 $\pm$ 0.87
	120%	100.13 $\pm$ 0.31	100.33 $\pm$ 0.60

\*Average of three readings  $\pm$  S.D.

**Table no.2. Analysis of Commercial Dosage Forms**

Brand Name	Labeled Amount (mg)		Observed Amount* $\pm$ S.D.		%RSD <sup>†</sup>	
	Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel
Pidlet Plus Tablet	150	75	149.05 $\pm$ 1.01	74.36 $\pm$ 0.15	0.67	0.20
Clopitab A 150 Capsule	150	75	150.75 $\pm$ 1.06	74.77 $\pm$ 0.10	0.70	0.13

\*Average of six determinations, S.D. is Standard deviation, <sup>†</sup> is the Relative Standard Deviation

**Table no.3. Statistical Analysis of Precision**

Drug	Mean Peak Area* $\pm$ S.D.	%RSD
Aspirin	659673.5 $\pm$ 5001.2	0.75
Clopidogrel bisulphate	262172 $\pm$ 2096.23	0.79

\*Average of eight determinations, S.D. is Standard deviation, <sup>†</sup> is the Relative Standard Deviation

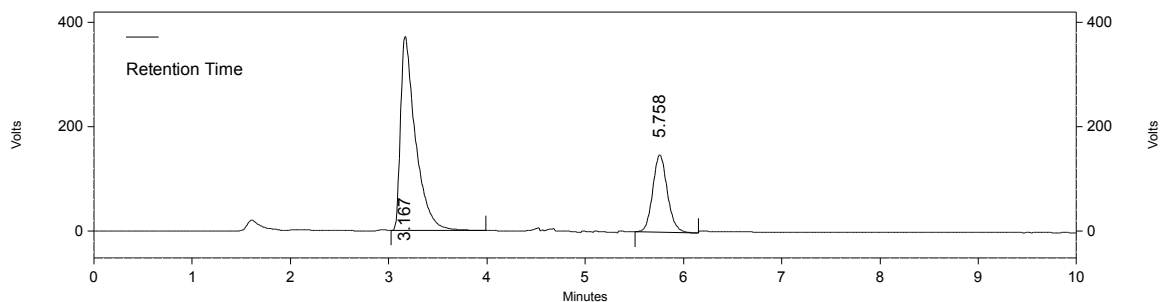


FIG-1. Typical Chromatogram of Aspirin (3.167min) & Clopidogrel bisulphate (5.758min) in Pure drug

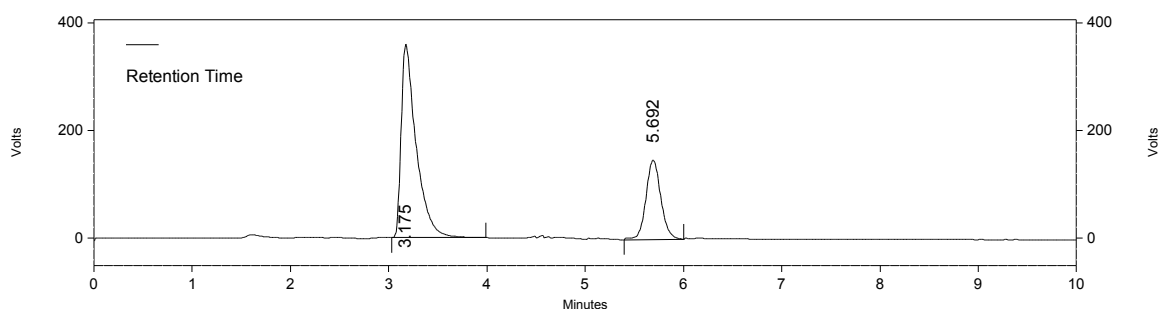


FIG-2. Typical Chromatogram of Aspirin (3.175min) & Clopidogrel bisulphate (5.692min) in Dosage Forms

#### ACKNOWLEDGMENTS

The authors are thankful to Zydus Healthcare for providing the gift samples of Aspirin and Clopidogrel bisulphate standard drug and M/S Roland Institute of Pharmaceutical Sciences, Berhampur-10, Orissa, India for providing the research facilities.

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