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Analytical method development and validation for the estimation of Dipyridamole in pharmaceutical dosage form by HPLC

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ABSTRACT

A simple, rapid, accurate and reproducible reverse phase high performance liquid chromatography method for the quantitative determination of Dipyridamole in tablet dosage form was studied in this experiment. With the optimized chromatographic conditions, the drugs were linear in the concentration range of 10 µg/ml to 30 of µg/ml Dipyridamole, methyl paraben and propyl paraben. The correlation co – efficient was found to be above 0.999 for all three drugs. The percentage purity was found to be 99.9 and 100.1 for Dipyridamole.

1. INTRODUCTION

Dipyridamole is a phosphodiesterase inhibitor that blocks uptake and metabolism of adenosine by erythrocytes and vascular endothelial cells. Dipyridamole also potentiates the antiaggregating action of prostacyclin. (From AMA Drug Evaluations Annual, 1994, p752)

Dipyridamole likely inhibits both adenosine deaminase and phosphodiesterase, preventing the degradation of cAMP, an inhibitor of platelet function. This elevation in cAMP blocks the release of arachidonic acid from membrane phospholipids and reduces thromboxane A2 activity. Dipyridamole also directly stimulates the release of prostacyclin, which induces adenylate cyclase activity, thereby raising the intraplatelet concentration of cAMP and further inhibiting platelet aggregation.

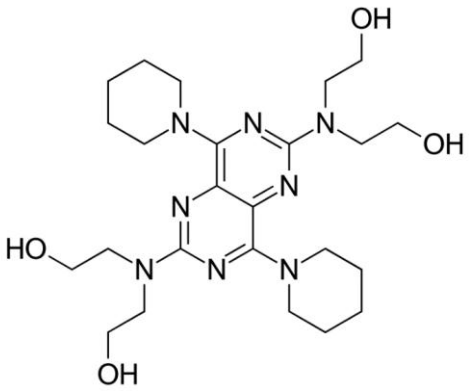

Figure.1.Molecular structure Dipyridamole

2. MATERIALS AND METHODS

Dipyridamole API, Methyl Paraben and Propyl Paraben samples were procured from Chandra labs, Hyderabad. All the chemicals used were of analytical grade and HPLC grade procured from Qualigens, India Ltd. The chemicals used for the study were Acetonitrile (HPLC grade), Water (HPLC grade) and Ortho phosphoric acid (Analytical grade). Waters HPLC 2695 with UV detector was used for the analysis.

Method development and optimization of chromatographic conditions:

Selection of mobile phase and λ_{max}: Solution of Dipyridamole (20 µg/ml) were prepared in the mobile phase [Acetonitrile: Phosphate Buffer (75:25 v/v)] and scanned in the UV region of 200 – 400 nm and recorded the spectrums. It was found that the drug has marked absorbance at 254 nm 288 nm can be effectively used for estimation. Therefore 254 nm 288 nm was selected

as detection wavelength for estimation of three drugs by RP – HPLC method with an Isocratic elution technique.

Stability of sample solutions: Solution of Dipyridamole (20 µg/ml) absorbance was checked for the stability at 254 nm and 288 nm and it was found that the drug was stable for approximately two and half hour.

Optimization of chromatographic conditions:

Initial separation conditions: The following chromatographic conditions were preset initially to get better resolution of Dipyridamole.

Mode of operation: Isocratic
Stationary phase: Inertsil ODS-3 (250 mm × 4.6 mm i.d. 5µ)
Mobile phase: Acetonitrile : Phosphate Buffer
Proportion of mobile phase: 75:25
Detection wavelength: 258 nm and 288 nm
Flow rate: 1.3 ml/min
Temperature: Ambient
Sample load: 20 µl
Operating pressure: 1200 psi
Method : External Standard Calibration method.

The mobile phase was primarily allowed to run for 15 minutes to record a sturdy baseline. Solutions of Dipyridamole was injected and the respective chromatogram was recorded.

Selection of mobile phase: Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following:

S.No	Mobile phase	Observation
1	Acetonitrile : Dibasic sodium phosphate Buffer (50:50)	Undetectable retention time
2	Acetonitrile : Dibasic sodium phosphate Buffer (25:75)	Peak area was not constant
3	Acetonitrile : Dibasic sodium phosphate Buffer (75:25)	Retention time and peak area was constant

From the above information, in the mobile phase of Acetonitrile : Buffer (75:25), these two drugs were eluted with sharp peak and better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

Effect of ratio of mobile phase p^H: The different p^H were tried. They were 1M Dibasic sodium phosphate Buffer : Acetonitrile (25:75 % v/v) p^H 4.60 and 4.40, At p^H 4.60, the peak obtained was very sharp with better resolution. Hence this p^H was selected for further analysis.

Optimized chromatographic conditions: The following optimized conditions were employed for analysis of Dipyridamole by Isocratic RP – HPLC method.

Mode of operation: Isocratic
Stationary phase: Inertsil ODS-3 (250 mm × 4.6 mm i.d. 5µ)
Mobile phase: Acetonitrile : Dibasic sodium phosphate Buffer at pH 4.60
Proportion of mobile phase: 75:25 % v/v
Detection wavelength: 254 nm and 288 nm
Flow rate: 1.30 ml/min
Temperature: Ambient
Sample load: 20 µl
Operating pressure: 1200 psi
Method: External Standard Calibration method.

Preparation of standard Dipyridamole: 50 mg of Dipyridamole was weighed accurately and transferred into 50 ml volumetric flask and dissolved in mobile phase, after dissolution the volume was made up to the mark with mobile phase (1000 µg/ml). Further dilution was made by pipetting 5 ml of standard stock solution into 50 ml to acquire 100 µg/ml solution.

Preparation of solutions for Linearity of detector response: In this progression, the aliquots of stock solution of Dipyridamole were taken to prepare 5µg/ml to 30 µg/ml solution of Dipyridamole. The solutions were injected and the chromatograms were recorded at 254 nm and 288 nm. The above concentration range was found to be linear. The procedure was repeated for six times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Dipyridamole in tablet dosage form: 20 tablets of dipyridamole 20 mg were crushed and an equivalent of 50 mg was taken in a 500 ml volumetric flask and added 300 ml of mobile phase and sonicated for 60 minutes. Finally the solution was made up to the mark with mobile phase. Filtered with 0.45µ nylon filter. The solution was taken in vials and injected to HPLC.

Assay Procedure: 5ml of Dipyridamole suspension was taken in 500 ml volumetric flask. 300 of mobile phase was added and sonicated for 60 minutes. The

solution was made up to the mark with mobile phase. The above solution was filtered with 0.45µ nylon filter. The solution was taken in vials and injected to HPLC.

3. RESULTS AND DISCUSSION

The solutions of 10 µg/ml of Dipyridamole in mobile phase (Acetonitrile: Dibasic sodium phosphate buffer 75:25% v/v) were prepared and the solutions were scanned in the range of 200 – 400 nm. It was found that Dipyridamole has marked absorbance at 254 nm and 288 nm, therefore 254 nm and 288 was selected as detection wavelengths for estimation of two drugs by RP – HPLC method with an isocratic elution technique.

The optimization was done by changing the composition of mobile phase, ratio and flow rate. The mobile phase consists of Acetonitrile : Dibasic sodium phosphate buffer (50:50% v/v) was initially tried and chromatograms were recorded. Followed by Acetonitrile : Dibasic sodium phosphate buffer 25:75% v/v and 75:25% v/v was taken and chromatograms were recorded. Finally the mobile phase consists of 1M Dibasic sodium phosphate buffer :Acetonitrile (25:75% v/v) with pH 4.60 was taken as mobile phase for the estimation Dipyridamole in suspension formulation. The retention time of Dipyridamole was found to be 9 minutes and methyl paraben retention time was about 3 minutes and propyl paraben retention time was 4 minutes.

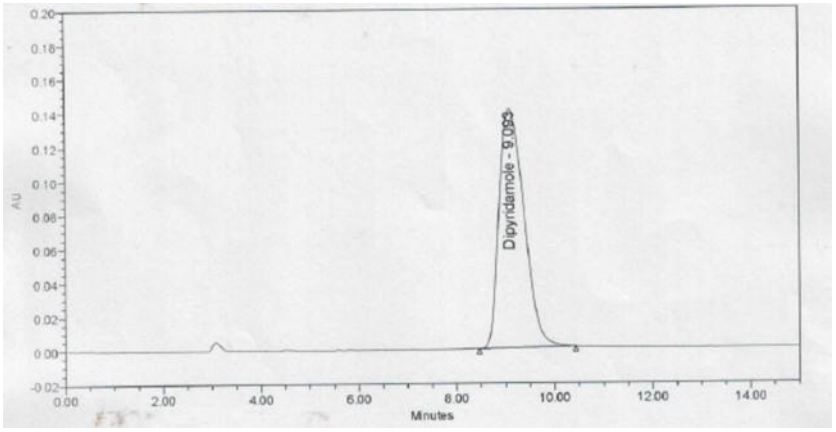

Figure.2. Typical chromatogram of standard solution

Table.1.Linearity of detector response for Dipyridamole

% Linearity level	Concentration (ppm)	Response	Acceptance criteria
20	20.096	1000121	Square of Correlation co-efficient should not be less than 0.999
50	50.24	2532528	
70	70.336	3532848	
100	100.48	5065572	
150	150.72	7644405	
Square of correlation coefficient (r^2): 0.999 Slope :50834.85484 Intercept: -29056.44737 Residual sum of squares : 14290.09833			

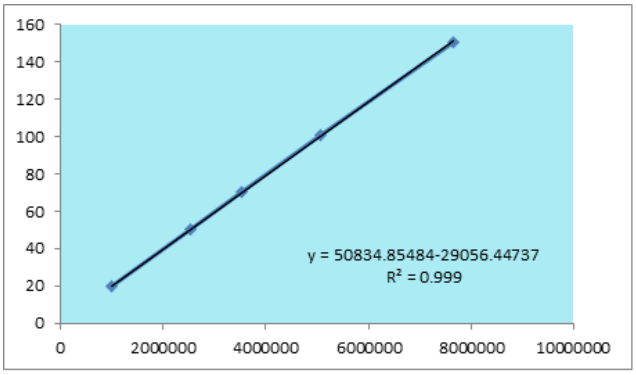

Figure.3. Linearity of detector response for Dipyridamole

4. CONCLUSION

An exertion has been made for a simple, rapid, accurate and precise method for the estimation of Dipyridamole in formulation by an isocratic RP – HPLC method.

With the optimized chromatographic conditions, the drugs were linear in the concentration range of 10 µg/ml to 30 of µg/ml Dipyridamole, methyl paraben and propyl paraben. The correlation co – efficient was found to be above 0.999 for all three drugs. The percentage purity was found to be 99.9 and 100.1 for Dipyridamole. The precision of the method was confirmed by repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies.

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