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Development and Validation of RP-HPLC Method for Assay of Dipyridamole in Formulations

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Abstract:

A simple, sensitive and accurate gradient reverse phase high performance liquid chromatography method was developed for determination of dipyridamole in formulation. The effective separation was achieved on phenomenex-C18; 75 x 3.0 mm, 3µm. The mixture of buffer and methanol in the ratio 25: 75v/v used as a mobile phase-A. The buffer was prepared as 1.5 g of potassium dihydrogen phosphate in 1000 mL of water; adjust the pH to 7.2 with 5% potassium Hydroxide solutions. The mixture of buffer and methanol in the ratio of 10: 90 (v/v) used as mobile phase-B. The flow rate of the mobile phase was 1.5 mL/min and the total elution time was 8 minutes. The UV detection wavelength was carried at 282 nm and experiments were conducted at 45°C. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy and robustness as per the ICH guidelines.

Key Words: Dipyridamole, Method development, Validation and RP-HPLC

1. INTRODUCTION:

Dipyridamole is a medication that inhibits blood clot formation [1] when given chronically and causes blood vessel dilation when given at high doses over a short time. Dipyridamole is an odorless yellow crystalline powder, having a bitter taste. It is soluble in dilute acids, methanol and chloroform, and practically insoluble in water. Each Dipyridamole tablet USP, for oral administration, contains 25 mg, 50 mg, or 75 mg Dipyridamole, USP and contains the following inactive ingredients: colloidal silicon dioxide, hypromellose, lactose anhydrous, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, propylene glycol, stearic acid, sodium starch glycolate, and titanium dioxide.

Dipyridamole inhibits the uptake of adenosine into platelets, endothelial cells and erythrocytes in vitro and in vivo; the inhibition occurs in a dose-dependent manner at therapeutic concentrations (0.5 to 1.9 mcg/mL). This inhibition results in an increase in local concentrations of adenosine which acts on the platelet A2-receptor thereby stimulating platelet adenylate cyclase and increasing platelet cyclic-3',5'-adenosine monophosphate (cAMP) levels. Via this mechanism, platelet aggregation is inhibited in response to various stimuli such as platelet activating factor (PAF), collagen and adenosine diphosphate (ADP).

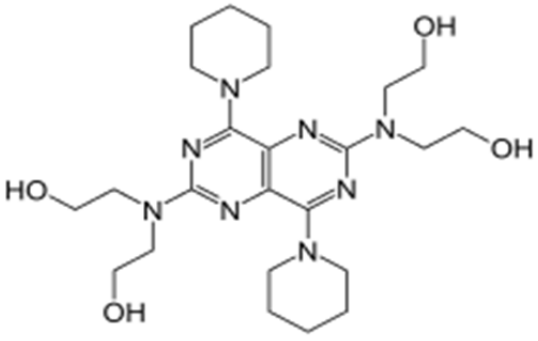

Figure-1: Chemical structure of dipyridamole

In the literature survey there were quite a few LC methods have been reported for determination of Dipyridamole in pharmaceutical preparation [2, 3] and few method were reported for Dipyridamole and its degradation product [4]. However, several method were reported for determination of Dipyridamole in combination with other drug [5-7]. Estimation of Dipyridamole, and its metabolites in human plasma by LC-MS and HPLC has been performed [8-10]. The developed LC method was validated with respect to specificity, LOD, LOQ, linearity, precision, accuracy and robustness. Force degradation studies were performed on the placebo and drug products to show the stability-indicating nature of the method. The present work describes a simple, gradient RP-HPLC method for the determination of dipyridamole as per ICH guidelines [11-13].

2. EXPERIMENTAL: MATERIALS AND REAGENTS:

2.1 INSTRUMENTATION AND SOFTWARE:

A high performance liquid chromatography system manufactured by Agilent which consist of VWD detector, Quaternary solvent manager, Sample manager, column heating compartment was used for assay determination of dipyridamole. HPLC instrument was controlled by Empower software. The phenomenex-C18; 75 x 3.0 mm, column with particle size of 3µm was used as stationary phase for chromatographic separation. Sartorius semi micro analytical balance was used for all weighing, Thermo pH meter was used for buffer pH adjustment, and Bandelin sonicator used to dissolve the standard, sample and were centrifuged by using Hermle centrifuge machine.

2.2 CHEMICALS AND REAGENTS:

All the reagents were of analytical reagent grade unless stated otherwise. Distilled and de-ionized HPLC-grade water, HPLC grade methanol, potassium dihydrogen phosphate, potassium dihydrogen phosphate,

orthophosphoric acid and potassium Hydroxide solutions was purchased from Merck, Mumbai.

Buffer preparation:

Dissolve 1.5 g of potassium dihydrogen phosphate in 1000 ml of water; adjust the pH to 7.2 with 5% potassium Hydroxide solutions. Filter through 0.45µ Nylon 66 membrane filter and degas in sonicator for 10 minutes.

Mobile phase A:

Mix Buffer and methanol in the ratio of 25:75 (v/v) respectively.

Mobile phase B:

Mix Buffer and methanol in the ratio of 10:90 (v/v) respectively.

Preparation of diluents:

Potassium dihydrogen phosphate buffer with pH to 3.0 and methanol in the ratio of 65:35 (v/v) used as diluent for preparation of all solutions.

2.3 PREPARATION OF STANDARD SOLUTIONS:

Accurately weigh and transfer 60 mg of dipyridamole working standard, accurately weighed in to a 100 mL volumetric flask, add about 70 mL of methanol and sonicate to dissolve the material completely. Dilute to volume with methanol and mix. Pipette 5.0 ml of the resultant solution into a 50 ml volumetric flask, dilute to volume with diluent and mix.

2.4 PREPARATION OF SAMPLE SOLUTIONS:

Weighed and transferred dipyridamole pellets equivalent to 200 mg of dipyridamole into a 100 mL volumetric flask, add about 70 mL of methanol and sonicate for 20 minutes with intermediate shaking or till pellets dissolves completely. Dilute to volume with methanol. Centrifuge the resultant solution at 3500 RPM for 15 minutes. Pipette 3 mL of the supernatant solution into 100 mL volumetric flask, dilute to volume with diluent and mix well.

3. METHOD VALIDATION PARAMETERS:

The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with dipyridamole in the range of 50 to 150% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using dipyridamole and evaluated by making six replicate injections. The accuracy of the method by recoveries of dipyridamole sample solutions at different concentration levels ranging from 50 to 150%.The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

4. RESULTS AND DISCUSSION:

4.1 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 282 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify dipyridamole. Preliminary development trials were performed with various columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to phenomenex-C18; 75 x 3.0 mm, column with particle size of 3µm, there was significant improvement in the peak shapes with 1.0 tailing factor and got good number of theoretical plates (9450).

5. METHOD VALIDATION:

5.1 SYSTEM SUITABILITY:

The RSD from five replicate injections of diluted standard preparation was 0.1 %. System suitability data is given in Table-1

Table-1: System suitability results of dipyridamole

System suitability	Observed value for Dipyridamole peak	Acceptance criteria
Tailing factor	1.0	NMT 2.0
Theoretical Plates	9450	NLT 2500
RSD	0.1	NMT 2.0

5.2 SELECTIVITY:

Performed the specificity parameter of the method by injecting diluent, standard preparation and sample preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of lacosamide. The placebo, standard and sample chromatograms shown in the Figures 2 & 3.

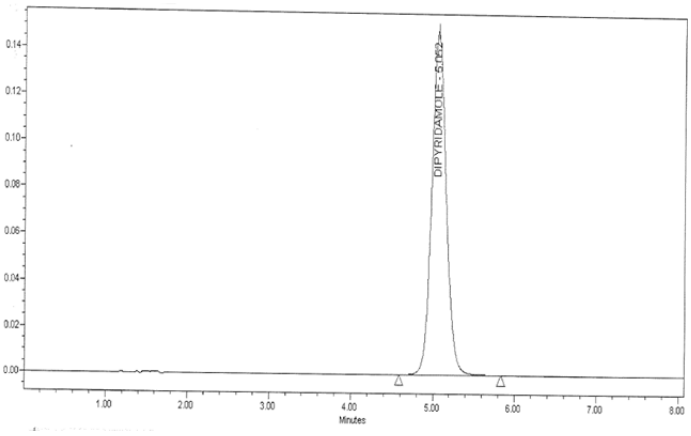

Fig-3: Chromatogram of Standard

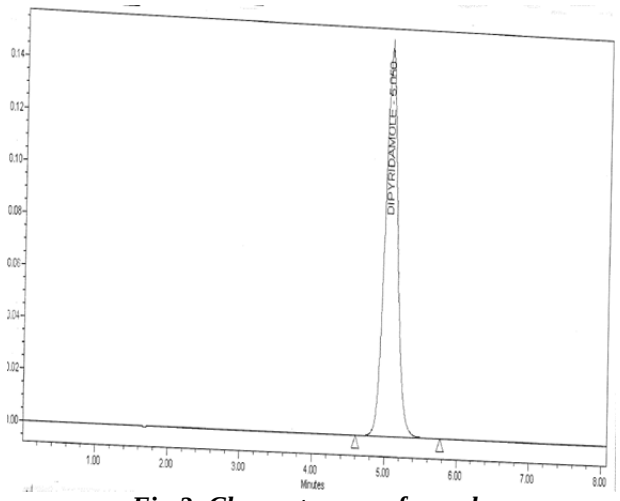

Fig-3: Chromatogram of sample

5.3 LINEARITY:

To demonstrate the linearity with dipyridamole standard in the range of 50 to 150% of specification limit**.** Correlation coefficient of dipyridamole was 1.00.The linearity results shown in the below Table -2. Linearity curve of dipyridamole shown in the Figure-4

Table-2: Linearity results of dipyridamole

Linearity level	Concentration of dipyridamole in µg/ml (ppm)	Dipyridamole peak Area
0	0	0
50%	30.08	2114912
75%	45.12	3204093
100%	60.16	4265933
125%	75.20	5284663
150%	90.24	6261908
Correlation coefficient (R)		0.999903
Slope (m)-(µV*Sec/ppm)		20156.76
Intercept (C)		5862.8

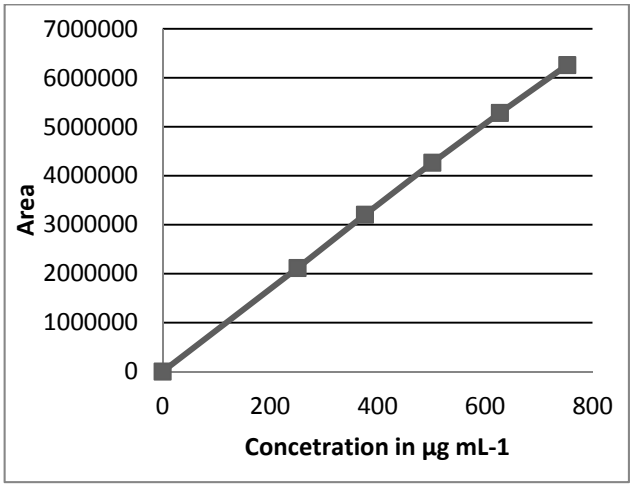

Figure-4 Linearity curve of dipyridamole

5.4 PRECISION:

The precision of test method was validated by assaying six samples prepared on dipyridamole and calculate relative standard deviation of area results. The precision results are given Table-3.

Table-3: Precision results of dipyridamole

Sample No.	% Assay of Dipyridamole
01	100.2
02	99.1
03	99.8
04	99.5
05	100.6
06	100.1
Average	99.9
SD	0.53
%RSD	0.54

5.5 ACCURACY:

Accuracy study found that the mean % of recovery was more than 98.0% and less than 102.0% at each level 50 to 150% of concentration levels, hence method is accurate. The accuracy results are given Table-4.

Table-4: Accuracy results

Sample No.	Spike level	% Assay of Dipyridamole
01	50	100.4
02	75	100.1
03	100	100.0
04	125	100.4
05	150	100.0

5.6 ROBUSTNESS

The method robustness was studied by injecting the system suitability solution at change in the pH of buffer solution, organic modifier, flow rate, and column temperature. The results were obtained as shown in the below Table-5.

Table-5: Robustness results

Condition	% RSD (NMT: 2.0)	Theoretical Plates (NLT: 2500)	Tailing factor (NMT: 2.0)
Normal Condition	0.1	9450	1.0
Change in buffer pH 6.8	0.13	13697	1.01
Change in buffer pH 7.2	0.16	17283	1.01
Column temperature 40°C	0.18	19592	1.02
Column temperature 50°C	0.23	6221	1.05
Flow rate 1.3 mL/min	0.10	21720	1.06
Flow rate 1.7 mL/min	0.44	4103	1.04
Organic modifier - 10%	0.15	9775	0.99
Organic modifier +10%	0.07	10743	0.97

6. CONCLUSION:

A simple gradient HPLC method has been developed and validated for the determination of dipyridamole. The developed method has been found to selective, sensitive, precise, robust and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of dipyridamole and also for the analysis of stability samples.

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