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A rapid novel RP- HPLC stability indicating assay method development and validation of dipyridamole in dipyridamole extended release capsules

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ABSTRACT

A simple, rapid and sensitive RP-HPLC method was developed and validated for the quantification of Dipyridamole in bulk drug and tablet formulation. The separation was achieved on a Inertsil X-terra MS C 18 (100 x 4.6 mm, 3.5µm). The mobile phase containing a gradient mixture of mobile phase A (1.0 g Potassium dihydrogen phosphate buffer, pH adjusted to 7.0 with 5% sodium hydroxide solution and Methanol in the ratio of 40:60 v/v) and mobile phase B (1.0 g Potassium dihydrogen phosphate buffer, pH adjusted to 7.0 with 5% sodium hydroxide solution and Methanol in the ratio of 5:95 v/v). At a flow rate of 1.5 mL min^{-1} and detection was performed at 282 nm using photodiode array (PDA) detector. The drug was subjected to various ICH prescribed stress conditions including hydrolysis (neutral, acid and alkaline), oxidation, photolysis and thermal degradation. The drug in solution was found to degrade significantly in alkaline hydrolysis and when exposed to sunlight. The proposed method was validated with respect to specificity, linearity, accuracy, precision, stability, ruggedness and robustness as per ICH guideline. The peak purity achieved from PDA detector and satisfactory Separations between drug and its degradants established the specificity of the method. The developed method was found to be successively applied for the quality control of Dipyridamol in bulk drug and tablets as well as the stability studies.

KEYWORDS

Dipyridamole;
Bulk drug;
Forced degradation;
RP-HPLC;
Stability-indicating method tablets;
Validation.

INTRODUCTION

Dipyridamole is a classic platelet inhibitor, whose inhibitory effect is thought to be due to inhibition of the adenosine transporter leading to an increase in cAMP, an inhibitor of platelet aggregation[1,2]. chemically described as 2,2',2'',2'''-[(4,8-Dipiperidino pyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]-tetra ethanol. Its empirical formula is C24H40N8O4, which corresponds to a molecular weight of 504.63. (Figure 1). Its solid oral dosage form is available as capsule. Each modified release capsule contains Dipyridamole 200 mg. some studies have also suggested that Dipyridamole also possesses beneficial properties to vasculature such as inhibition of proliferation, antioxidant and anti-inflammatory properties[3,4]. The analysis of Dipyridamol has been determined by a few LC methods have been reported for determination of Dipyridamole in pharmaceutical

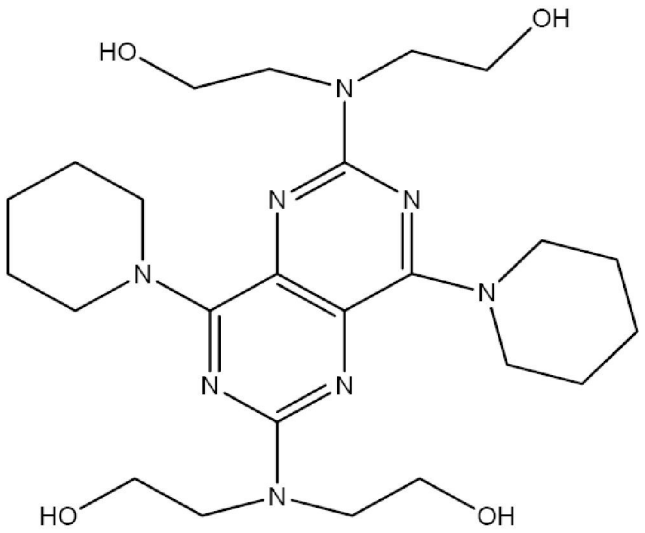

Figure 1 : Chemical structure of dipyridamole

preparation[5,6] and few method were reported for Dipyridamole and its degradation product[7]. However, several methods were reported for determination of Dipyridamole in combination with other drug[8-10]. Estimation of Dipyridamole, and its metabolites in human plasma by LC-MS and HPLC has been performed[11-14]. The present studies describe the development and validation of gradient reverse phase HPLC method. The development required investigating several factors including buffer ionic strength, mobile phase PH, Organic composition, and selection of the stationary phase. The validated method is sensitive, accurate, and reproducible for the determination of degradation products in dipyridamol[15,16].

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade of Dipyridamole drug substance and Drug product supplied as a gift sample by regional Pharmaceutical Company, Hyderabad, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. High purity water was prepared by using Milli-Q water purification system.

Instrumentation

HPLC assay was performed on a Liquid Chromatography waters HPLC PDA 2996 system used consists of Quaternary solvent manager, sample manager and waters Empower3 software was used to control the equipment and to calculate data and responses from the LC system. Cintex digital water bath was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India).

Preparation of stock and standard solutions

A standard stock solution of Dipyridamol (1.0 mg/mL) prepared by dissolving 60 mg of Dipyridamol reference standard in 100mL methanol. Required dilutions of stock solution are done to obtain working solution of standard with a concentration of 60µg/mL which is used for the Assay determination (Figure 2).

Preparation of sample solutions

Open 20 capsules and take only Dipyridamole pellets in a poly bag and mix well. A quantity of pellets equivalent to 200 mg of Dipyridamol was transferred to a 100 mL volumetric flask, 70 mL methanol was added. The mixture was then sonicated for 20 minute and diluted to volume to give a solution containing 60 µg/mL of Dipyridamol. The above solution was centrifuged at 4000rpm for 10 minutes in order to eliminate insoluble excipients and filtered through a 0.45 µm pore

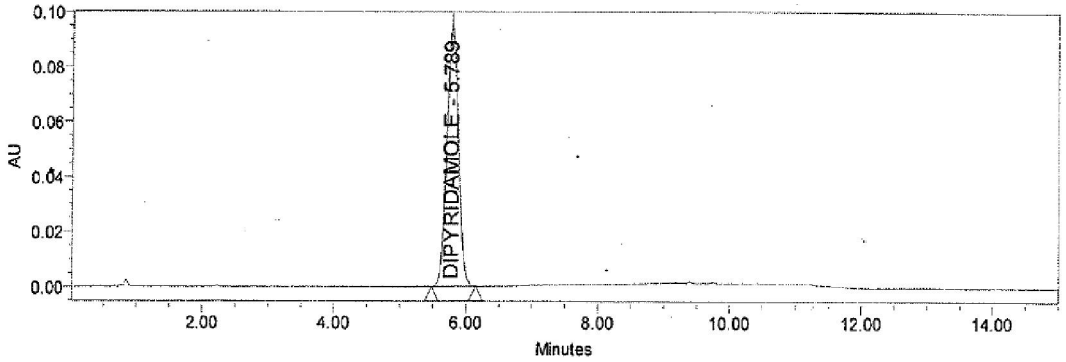

Figure 2 : Chromatogram of standard sample

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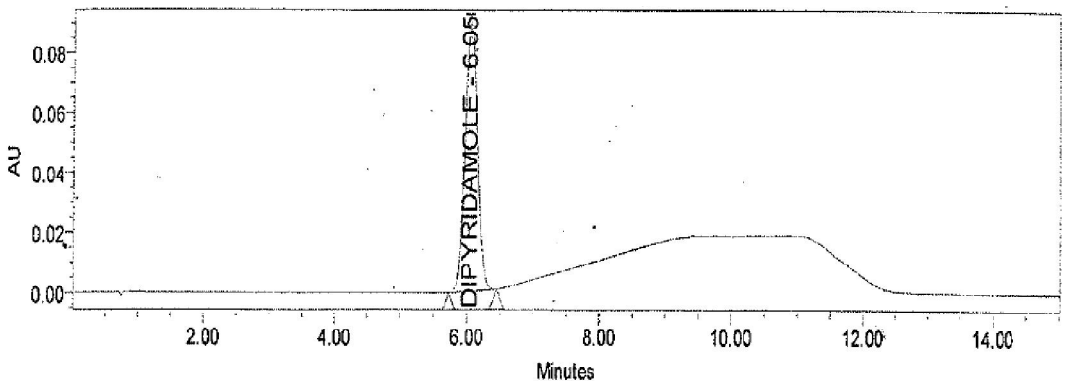

Figure 3 : Chromatogram of sample

size Nylon 66 membrane filter and inject in HPLC system as per chromatographic conditions mentioned (Figure 3).

Chromatographic conditions

The method was developed using X-terra MS C 18 (100 x 4.6 mm, 3.5µm) column with mobile phase containing a gradient mixture of mobile phase A (1.0 g Potassium dihydrogen phosphate buffer, pH adjusted to 7.0 with 5% sodium hydroxide solution and Methonol in the ratio of 40:60 v/v) and mobile phase B (1.0 g Potassium dihydrogen phosphate buffer, pH adjusted to 7.0 with 5% sodium hydroxide solution and Methanol in the ratio of 5:95 v/v). The mobile phases were filtered through nylon 0.45µm membrane filters and degassed in sonicator. The flow rate of the mobile phase was 1.5 mL/min, with a gradient programme of Time / % mobile phase –B 0.001/00, 5/00, 8/100, 10/100, 11/00, 15/00. The column temperature was maintained at 45° C and the eluted compounds were monitored at the wavelength of 282 nm. The injection volume was 10µl.

SPECIFICITY

Placebo interference

A study to establish the interference of placebo was conducted. Assay test was performed on Placebo equivalent to the amount present in the test preparation in duplicate as per test method and injected into HPLC. Chromatograms of placebo solutions showed no peaks at the retention time of Dipyridamole. This indicates that the excipient used in the formulation does not interfere in the estimation of Dipyridamole in the assay test method (Figure 4).

Impurity interference

A study to establish the interference of known impurities was conducted. Assay was performed by spiking known impurities at the level of 0.3% to the test concentration and injected as per test method. All the known impurities are well separated from the Dipyridamole peak. This indicates that the known impurities are not interfering in the estimation of Dipyridamole in the assay test method.

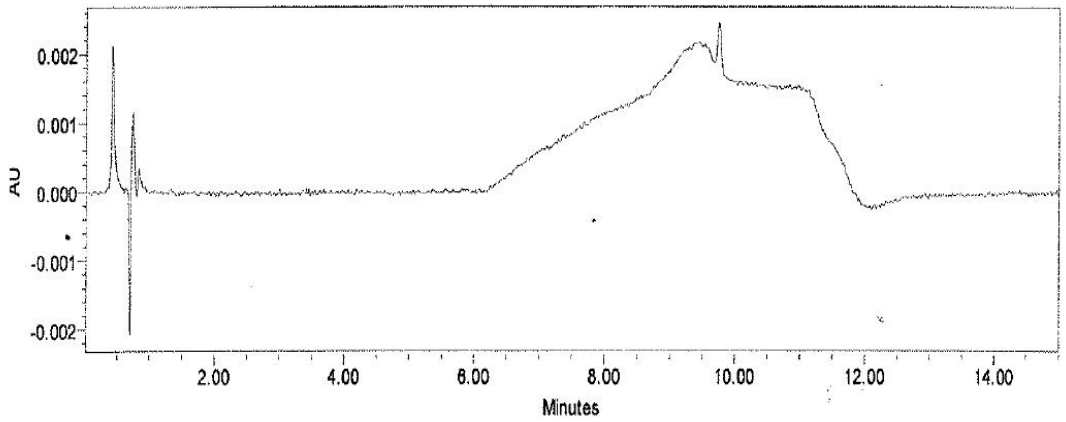

Figure 4 : Chromatogram of placebo sample

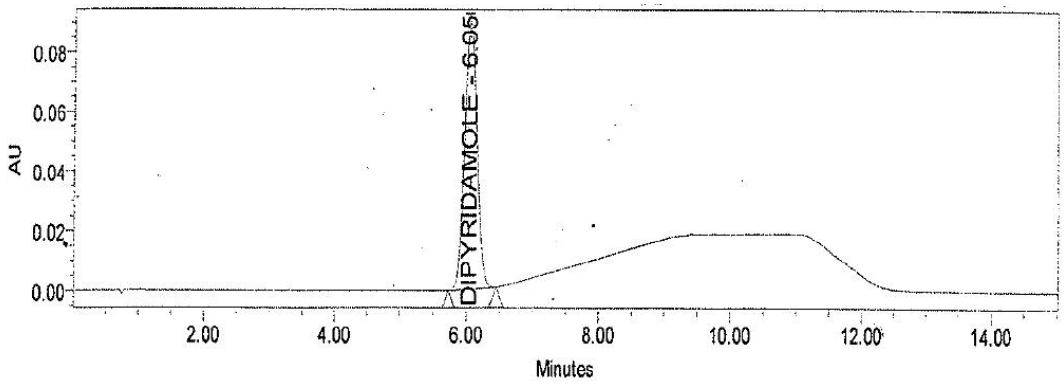

Figure 5 : Chromatogram for as such sample

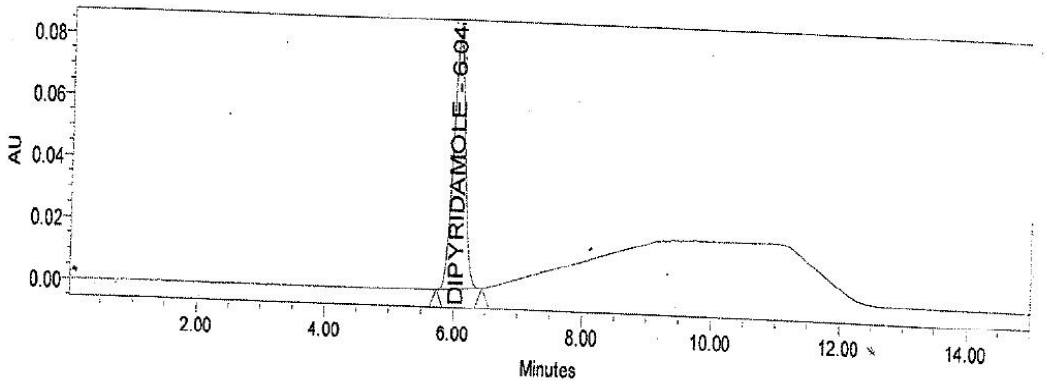

Figure 6 : Chromatogram of acid stress sample

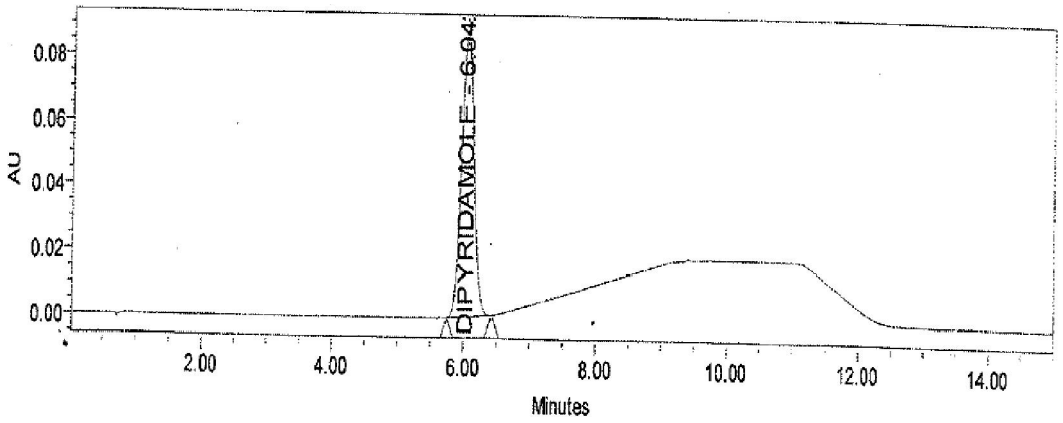

Figure 7 : Chromatogram of base stress sample

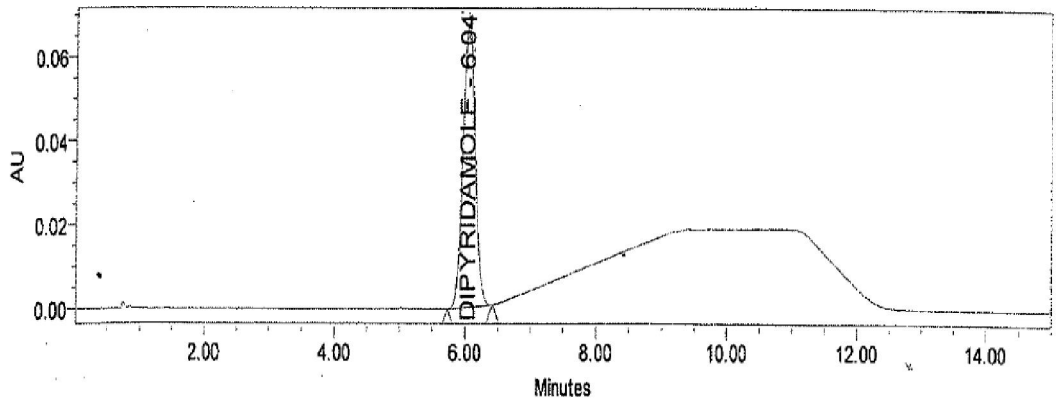

Figure 8 : Chromatogram of oxidation stress sample

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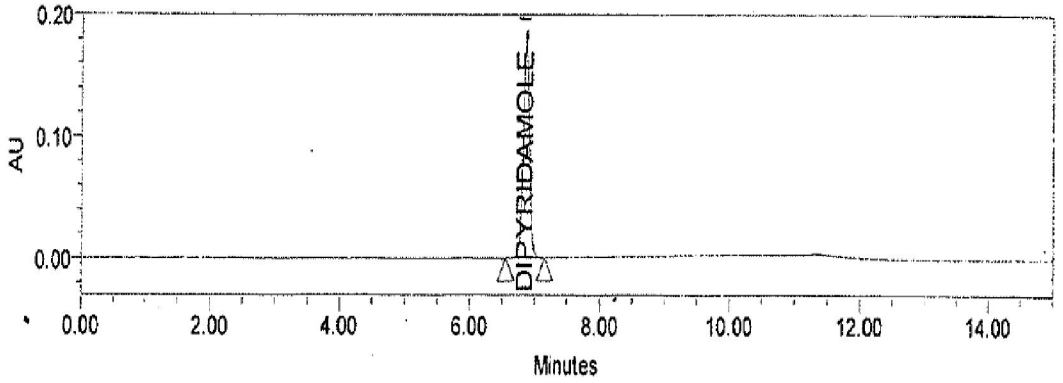

Figure 9 : Chromatogram of sunlight stress sample

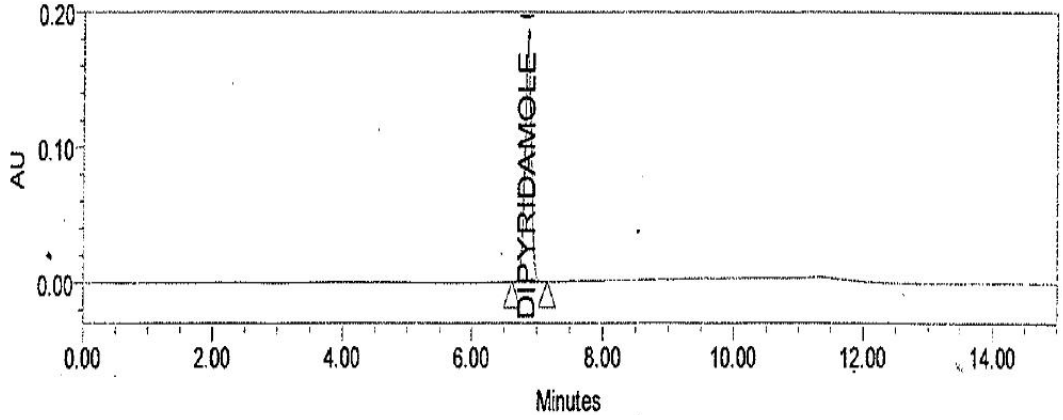

Figure 10 : Chromatogram of UV light stress sample

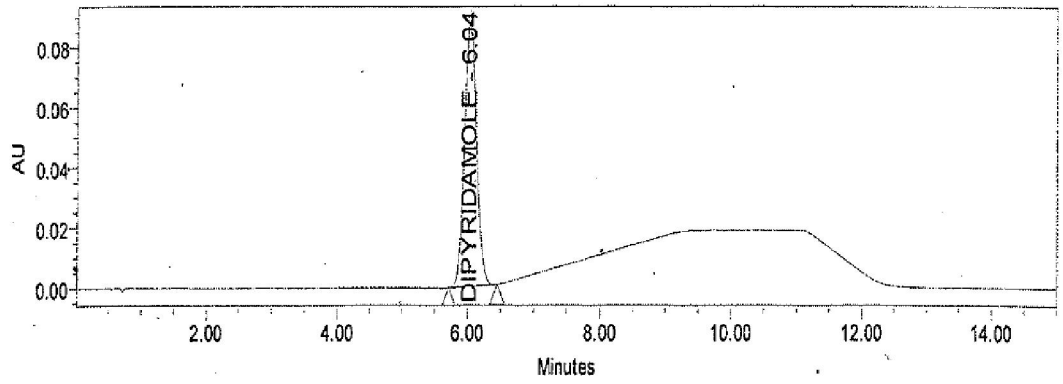

Figure 11 : Chromatogram of heat stress sample

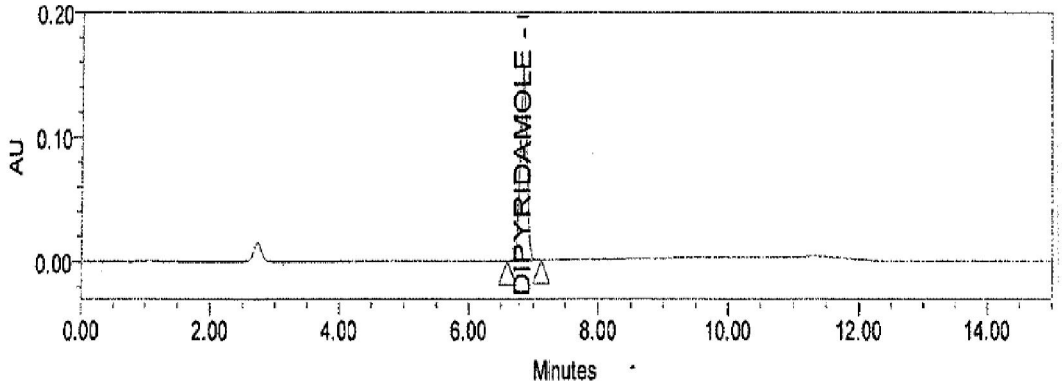

Figure 12 : Chromatogram of humidity stress sample

Forced degradation studies

A study to establish the interference of degradation product was conducted. Forced degradation was performed on Dipyridamole extended release pellets and on placebo individually. Degradation was conducted by sample as such(Figure 5), 1N Acid hydrolysis refluxed at 60^0c (Figure 6), 5N Base Hydrolysis refluxed at 60^0cn (Figure 7), 3% Peroxide oxidation refluxed at 60^0c(Figure 8), degradation by sun light(Figure 9) and UV radiations(Figure 10),degradation by heat 105^0c for 5 h(Figure 11), humidity and water(Figure 12,13) conditions. All the samples were evaluated for peak purity using PDA detector (Waters Empower software 3) and are found to be pure. Dipyridamole peak did not have any flag in the purity results table. This indicates that all the degradation products are well separated from the Dipyridamole peak and the test method is stability indicating. The results are summarized in TABLE 1.

RESULTS AND DISCUSSION

The Present research work is to develop a stability indicating method for the estimation of the Dipyridamole in Dipyridamole extended release pharmaceutical dosage forms. The mobile phase was optimized by examining the effect of pH, The optimal buffer pH was determined to be 7.0. When a lower pH was used its impacting the Dipyridamole, The optimal ionic strength was determined to be 50 mM. Potassium dihydrogen phosphate and organic modifier as methanol. And several stationary phase columns were evaluated in optimizing the proper peak shape and better selectivity; a Waters µ Bondapak (3.9 x 300, 10 µm; Waters Associates, Milford, MA), Alltech Alpha bond C18 (3.9 x300, 10 µm, Alltech Assocaites, Inc., Deerfield, IL), Zorbax SB-18 (4.6 x 250 mm, 5 µm; Mac-Mod Analytical,Inc., Chadds Ford, PA), YMC Basic (4.6 x 150 mm,5 µm; YMC, Inc., Wilmington, NC) and X-

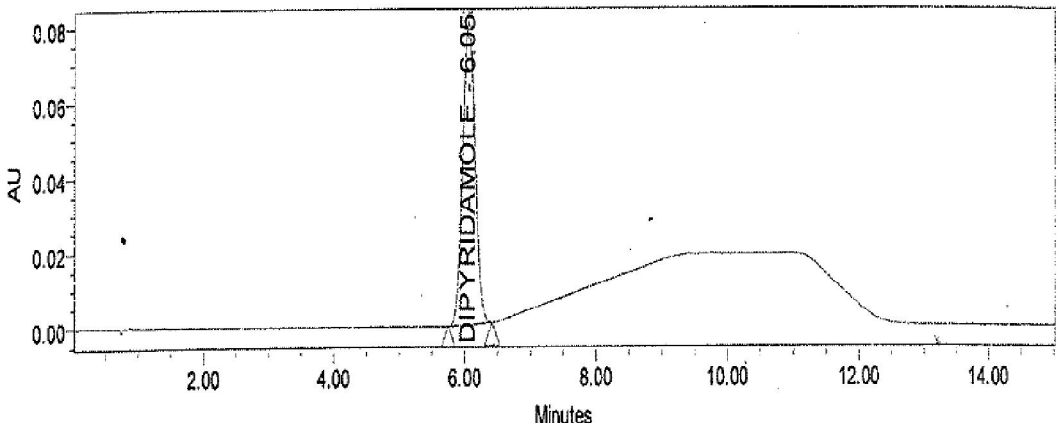

Figure 13 : Chromatogram of water stress sample

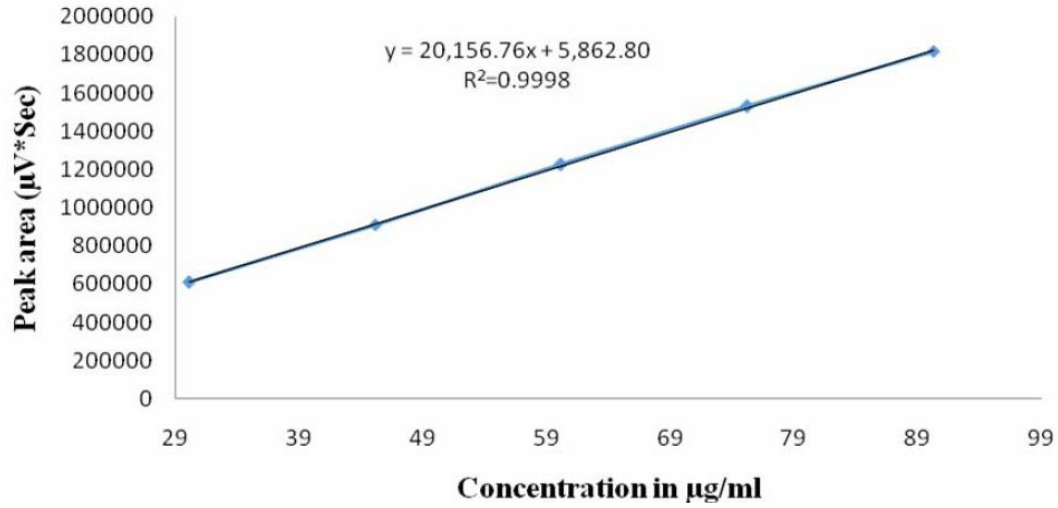

Figure 14 : Linearity plot

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TABLE 1 : Results of forced degradation

S.No	Stress Condition	Purity Angle	Purity Threshold	% Assay	Purity Flag (Yes/No)	% Degradation	% Impurity
1.	Sample as such	0.335	0.910	99.8	NO	NA	Nill
2.	1N HCl refluxed for 2hrs at 60°C	0.353	0.963	92.5	NO	7.3	7.3
3.	5N NaOH refluxed for 12hrs at 60°C	0.281	0.764	97.8	NO	2.0	2.0
4.	3% peroxide refluxed for 2hrs at 60°C	0.346	1.223	76.0	NO	23.8	23.8
5.	Sunlight for about 1.2 million lux hours	0.100	0.544	99.7	NO	0.1	0.1
6.	UV light for about 200 watt hours per square meter	0.098	0.539	99.2	NO	0.6	0.6
7.	Heated at 105°C for 5hrs	0.360	0.932	99.9	NO	0.1	Nill
8.	Humidity at 90% RH for 7days at 25°C	0.092	0.595	82.2	NO	17.6	17.6
9.	Refluxed with water for 30 minutes at 60°C	0.332	0.972	90.2	NO	9.6	9.6

TABLE 2 : System suitability and system precision

System suitability	Observed value	Acceptance criteria
Tailing factor for Dipyridamole peak area	0.97	NMT 2.0
Theoretical Plates	10360	NLT 2000
Injection Number	Dipyridamole peak area	Acceptance criteria
1	1218369	
2	1219911	
3	1219839	
4	1220308	The % Relative Standard Deviation of peak areas
5	1220917	Of Dipyridamole should be not more than 2.0
Mean	1219869	
Std.Dev.	941.23	
%RSD	0.08	

Terra MS C 18 (4.6x100 mm, 3.5 µm). After several logical trials the finalized chromatographic condition as follows, Mobile Phase compositions of mobile phase A(Mix Buffer solution and Methanol in the ratio of 40:60 v/v), Mobile phase B(Mix Buffer solution and Methanol in the ratio of 5:95 v/v) was selected for its enhanced resolution. The Waters, Intersil X-Terra MS C 18 (4.6x100 mm, 3.5 µm) column provided the best resolution with the shortest chromatographic run time and, consequently, was selected as the column of choice. A typical chromatogram illustrating the degradation profile of Dipyridamole Injection obtained from a 10 µL injection is provided. The overall chromatographic run time was 15 minutes.

Method validation

The above method was validated according to ICH and USP guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method

System suitability

The Relative standard deviation for Dipyridamole peak areas from five replicate standard injections should be not more than 2.0%. Tailing factor for Dipyridamole peak should be not more than 2.0. Theoretical plates for Dipyridamole peak should be not less than 2000.

METHOD VALIDATION SUMMARY

System precision

The Standard solution of assay was prepared by using Dipyridamole working standard as per test method and injected five times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits. The % RSD for peak areas from five replicate injections of Dipyridamole was found to be 0.08. The Tailing factor for Dipyridamole peak was found to be 0.97 and theoretical plate count was found to be 10360. The results are summarized inTABLE 2.

TABLE 3 : Precision results of dipyridamole

Sample No.	% Assay of Dipyridamole
01	99.8
02	100.2
03	99.8
04	99.8
05	100.1
06	99.8
Mean	99.9
Std.Dev.	0.1835
%RSD	0.2

TABLE 4 : Linearity

Linearity Level	Concentration in µg/ml (ppm)	Dipyridamole peak Area (µV*Sec)
50%	30.08	610033
75%	45.12	911357
100%	60.16	1225074
125%	75.20	1529008
150%	90.24	1816996
Correlation coefficient (R)		0.999903
Slope (m)-(µV*Sec/ppm)		20156.76
Intercept (C)		5862.8
Bias at 100% response		0.48

Precision

Six replicates (n=6) of sample solutions (60 µg mL^{-1}) were analyzed in the same day to determine method precision. The average % label amount was 99.9% with associated %R.S.D. values of 0.2. in TABLE 3

Linearity and range

A study to establish the linearity of detector response of Dipyridamole was conducted. Linearity of detector response of Dipyridamole was conducted from 50% level to 150% of assay concentration(TABLE 4). Plotted a linearity graph of Concentration in ppm versus peak area of the Dipyridamole at each level and the correlation coefficient was found to be 0.999903. These results show there was an excellent correlation between the peak area and concentration for the drug (Figure 14).

Accuracy

A study of Accuracy was conducted. Assay test was performed by analyzing six samples each at 50% and 150% levels and three samples each at 75%, 100% and 125% levels. Performed the accuracy by weighing the capsules content proportionately for each level to

TABLE 5 : Accuracy

Sample No.	Spike level	µg/ml added	µg/ml found	Individual % recovery	Mean %recovery
1.	50%	99.78	100.03	100.3	100.4
2.		100.05	100.16	100.1	
3.		100.07	100.25	100.2	
4.		100.04	100.54	100.5	
5.		99.96	100.75	100.8	
6.		99.96	100.64	100.7	
1.	75%	150.03	150.20	100.1	100.1
2.		150.28	150.16	99.9	
3.		149.86	150.18	100.2	
1.	100%	199.42	199.11	99.8	100.0
2.		199.15	199.62	100.2	
3.		199.64	199.32	99.8	
1.	125%	250.44	251.85	100.6	100.4
2.		250.82	251.24	100.2	
3.		250.65	251.59	100.4	
1.	150%	300.14	299.24	99.7	100.0
2.		299.84	299.73	100.0	
3.		300.44	299.87	99.8	
4.		299.91	300.05	100.0	
5.		300.37	300.69	100.1	
6.		300.16	300.61	100.2	

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TABLE 6 : Analyst to analyst, system to system, column to column and day to day variability

System suitability	Observed value		Acceptance criteria
	Analyst-1	Analyst-2	
Tailing factor	0.97	1.0	NMT 2.0
Theoretical Plates	10360	17821	NLT 2000
% RSD of replicate injections of standard preparation	0.08	0.1	NMT 2.0

Sample No	% Assay of Dipyridamole	
	Analyst-1	Analyst-2
01	99.8	99.7
02	100.2	100.3
03	99.8	100.0
04	99.8	99.4
05	100.1	100.1
06	99.8	100.1
Average	99.9	99.9
SD	0.1835	0.33
%RSD	0.2	0.33

TABLE 7 : Bench top stability of standard and test preparations

Time in days	Similarity factor for Standard preparation	% Assay of Dipyridamole		Difference	
		Test-1	Test-2	Test-1	Test-2
Initial	NA	99.8	100.2	NA	NA
1	1.00	100.1	99.9	0.3	0.3
2	0.99	99.2	99.5	0.6	0.7
5	0.99	98.7	99.8	1.1	0.4

TABLE 8 : Bench top stability of mobile phase

System Suitability Parameters	Mobile phase stability				Acceptance Criteria
	Initial	1-day	2-day	5-day	
Tailing factor for Dipyridamole peak	0.97	1.00	0.97	1.01	NMT 2.0
Theoretical plates for Dipyridamole peak	10360	8362	8961	6912	NLT 2000
%RSD of five replicate injections of standard preparation	0.08	0.32	0.12	0.19	NMT 2.0

Time in days	% Assay of Dipyridamole		Difference	
	Test-1	Test-2	Test-1	Test-2
Initial	99.8	100.2	NA	NA
1	100.1	99.9	0.3	0.3
2	99.2	99.5	0.6	0.7
5	98.7	99.8	1.1	0.4

get the concentration of Dipyridamole equivalent to 50%, 75%, 100%, 125% and 150% of the amount of Dipyridamole present in the capsule assay test preparation as per the test method. The mean % recovery of Dipyridamole at all the levels and %RSD of individual % assay Dipyridamole at higher and lower levels was found to be within the limits (TABLE 5).

Ruggedness

Ruggedness of analytical method is degree of reproducibility of test result obtained by the analysis of the sample under verity of condition such as 1). System To System, Analyst To Analyst, Column To Column and Day To Day Variability (TABLE 6), 2). Bench Top

TABLE 9 : Effect of variation in flow rate

System suitability Parameters	Flow rate ml/min.			Acceptance criteria
	1.3	1.5 (initial)	1.7	
The tailing factor for Dipyridamole peak	1.05	0.97	1.07	NMT 2.0
Theoretical plates for Dipyridamole peak	22720	10360	3903	NLT 2000
% RSD of the Five replicate injections of Dipyridamole standard preparation	0.10	0.08	0.46	NMT 2.0

TABLE 10 : Effect of variation in pH of mobile phase buffer

System suitability Parameters	pH of Buffer			Acceptance criteria
	pH 6.8	pH 7.0 (Initial)	pH 7.2	
The tailing factor for Dipyridamole peak	1.00	0.97	1.03	NMT 2.0
Theoretical plates for Dipyridamole peak	13697	10360	21283	NLT 2000
% RSD of the Five replicate injections of Dipyridamole standard preparation	0.14	0.08	0.13	NMT 2.0

TABLE 11 : Effect of variation in column temperature

System suitability Parameters	Column temperature			Acceptance criteria
	40°C	45°C	50°C	
The tailing factor for Dipyridamole peak	1.01	0.97	1.08	NMT 2.0
Theoretical plates for Dipyridamole peak	22592	10360	5221	NLT 2000
% RSD of the Five replicate injections of Dipyridamole standard preparation	0.18	0.08	0.24	NMT 2.0

TABLE 12 : Effect of variation of methanol in mobile phase A

System suitability Parameters	Methanol in Mobile Phase A			Acceptance criteria
	90%	100%	110%	
The tailing factor for Dipyridamole peak	1.09	0.97	1.00	NMT 2.0
Theoretical plates for Dipyridamole peak	3940	10360	16504	NLT 2000
% RSD of the Five replicate injections of Dipyridamole standard preparation	0.15	0.08	0.07	NMT 2.0

TABLE 13 : Effect of variation of methanol in mobile phase B

System suitability Parameters	Methanol in Mobile Phase B			Acceptance criteria
	90%	100%	110%	
The tailing factor for Dipyridamole peak	0.97	0.97	0.98	NMT 2.0
Theoretical plates for Dipyridamole peak	9763	10360	10613	NLT 2000
% RSD of the Five replicate injections of Dipyridamole standard preparation	0.11	0.08	0.05	NMT 2.0

Stability Of Standard And Test Preparations (TABLE 7) and 3). Bench top Stability Mobile Phase (TABLE 8). The difference in % assay of test preparation from Initial to 5 days was found to be within the acceptance criteria.

Robustness

The Robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but liberate variation in method parameter and provides an indication of its reliability during normal usage and done by changing (TABLE 9, 10, 11, 12, 13),

1. Influence of variation in flow rates.
2. Influence of variation in mobile phases.
3. Influence of variation in column temperature.
4. Influence of variation in mobile phase A.
5. Influence of variation in mobile phase B.

The results are summarized as follows

CONCLUSION

A simple, fast and reliable HPLC method for the quantitative analysis of Dipyridamole in Dipyridamole

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Extended release capsules was developed and validated. The proposed method presented a good performance and demonstrated to be precise, accurate, sensitive and specific, eliminating the interferences from the degradents. Method can be used in routine and stability studies during pharmaceutical analysis.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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