Enliven Archive
Intensifying Thoughts

Research Article www.enlivenarchive.org Enliven: Bio analytical Techniques

RP-HPLC Method Development and Validation for Dipyridamole in both Bulk and Pharmaceutical Dosage Forms

Nalini Kanta Sahoo[1*], Dibyalochan Mohanty[2], Ch. Gurulatha[2], Vasudha Bakshi[2], and Akiful Haque[2]

[1]*Department of Pharmaceutical Analysis & Quality Assurance, MLR Institute of Pharmacy, Dundigal, Medchal, Telangana, 500043*

[2]*Department of Pharamaceutics, School of Pharmacy, Anurag Group of Institutions, Venkatpur, Ghatkesar, 500088*

***Corresponding author**: Nalini Kanta Sahoo, Department of Pharmaceutical Analysis & Quality Assurance, MLR Institute of Pharmacy, Dundigal, Medchal, Telangana, 500043, India, E-mail: sahoo.nalini@gmail.com

Received Date: 24th June 2019
Accepted Date: 20th July 2019
Published Date: 12th August 2019

Citation: Sahoo NK, Mohanty D, Gurulatha CH, Bakshi V, Haque A. RP-HPLC Method Development and Validation for Dipyridamole in Both Bulk and Pharmaceutical Dosage Form. Enliven: Bio analytical Techniques. 2019; 6(1): 001.

Copyright: @ 2019 Nalini Kanta Sahoo. This is an OpenAccess article published and distributed under the terms of the CreativeCommons Attribution License that permits unrestricted use, distribution andreproduction in any medium, provided the original author and source are credited.

Abstract

Ultra High performance liquid chromatography is a simple, sensitive and accurate gradient Reverse Phase Chromatography. It is a highly sophisticated instrument and was developed for the determination of Dipyridamole in pharmaceutical dosage form. The effective separation was achieved on phenomenex-C18; 4×250 mm, 5µm. The mixture of buffer and methanol which used as a mobile phase was taken in the ratio of 90:10 v/v. The buffer was prepared as 0.75 g of potassium dihydrogen orthophosphate in 500 ml of HPLC deionised water and the pH 7.2 buffer was adjusted with 2.5% of potassium hydroxide solution. The flow rate of the mobile phase was 1 ml/min and total elution time was 5 min. The UV detection wavelength was carried out at 291 nm and experiments were conducted at 40°C. The developed method was validated in terms of system linearity, precision, accuracy, repeatability, selectivity, and robustness, limit of detection and limit of quantification as per ICH guidelines. The results collectively demonstrated that the proposed method is selective.

Keywords: Dipyridamole; Validation; Method development; RP-HPLC

Introduction

Dipyridamole is a yellow crystalline odorless powder having a bitter taste. It is a platelet and phosphodiesterase inhibitor that blocks uptake and metabolism of adenosine by erythrocytes and vascular endothelial cells. When this drug is given chronically it causes blood vessel dilation at higher doses in a short span of time [1]. It is soluble in dilute acids and methanol, chloroform and practically insoluble in water 7.2 phosphate buffer, and slightly soluble in dilute acids having pH 3.3 or below. Dipyridamole likely inhibits the both adenosine deaminase and phosphodiesterase, preventing the degradation of cAMP, an inhibitor of platelet function. Secondly it inhibits the uptake of adenosine in platelets, endothelial cells and erythrocytes which increases the concentration of adenosine at the platelet vascular interface [2]. Dipyridamole, a non-nitrate coronary vasodilator that also inhibits platelet aggregation, is combined with other anticoagulant drugs, such as warfarin to prevent thrombosis in patients with valvular or vascular disorders. Dipyridamole is also used in myocardial perfusion imaging, as an anti platelet agent, and in combination with aspirin for stroke prophylaxis [3]. Each Dipyridamole tablet USP, for oral administration contains 25 mg, 50 mg and 75 mg of dipyridamole. It contains the inactive ingredients like colloidal silicon dioxide, lactose anhydrous, Chitosan, Carbopal, Microcrystalline cellulose, Polyethylene glycol, Titanium dioxide, Stearic acid, and Sodium starch glycolate [4] (Figure 1).

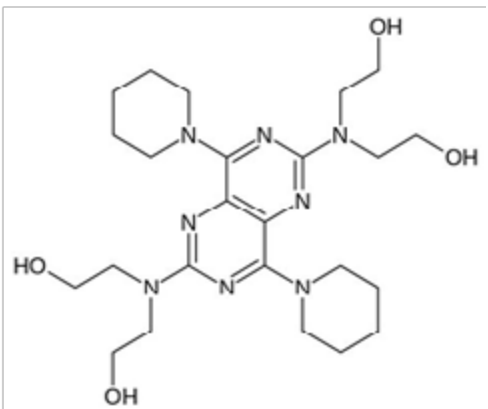


Figure 1: Structure of Dipyridamole

Liquid chromatography methods have been reported for the determination of Dipyridamole in pharmaceutical preparation and few methods were reported for the degradation products. The developed LC method was validated with respect to selectivity, LOQ, LOD, Linearity, Precision and Accuracy and Robustness. Forced degradation studies were performed on the placebo and drug products to show the stability indicating the nature of the method. The present work describes a simple, gradient RP-HPLC method for the determination of Dipyridamole as per ICH guidelines [5-7].

Experimental Materials and Reagents

Instrumentation and Software

A high performance liquid chromatography (HPLC) system manufactured by shimadzu - Japan which consists of PDA detector, quaternary solvent manager, sample manager column heating compartment was used for the assay of the dipyridamole. The HPLC instrument was controlled by empower software. LC solutions the phenomenon C18;4.6×250mm C18 column with a particle size of 5µm was used as a stationary phase for chromatographic separation. Sartorius semi micro analytical balance was used for buffer adjustment and ultra sonication was used to dissolve the standard and samples [8].

Chromatographic Conditions

Mobile phase: Mix 90% Methanol and 10% 7.2 phosphate buffer in the ratio of 90:10 (v/v).
Absorption maxima: 291 nm
Injection Volume: 10 to 60µl
Column: C18;4.6x250 mm C18 column with a particle size of 5µm was used as a stationary phase for chromatographic separation.
Flow rate: 1 ml/min
All the chemicals and reagents were of analytical reagent grade and distilled water as well as de-ionized HPLC grade water were utilised. Potassium di-hydrogen phosphate, methanol, orthophosphoric acid and potassium hydroxide solution were employed [9].

Methodology

Buffer Preparation

Dissolve 0.75g of Potassium dihydrogen phosphate in 500ml of HPLC grade water and adjust the pH to 7.2 with 5%potassium hydroxide solution filter through 0.45µ nylon 66 membrane filters and degas in ultra sonicater for 10 minutes.

Mobile Phase

Mix 90% Methanol and 10% 7.2 pH phosphate buffer in the ratio of 90:10 (v/v) respectively.

Preparation of Standard Solutions

Accurately weigh and transfer 10mg of Dipyridamole into 100 ml of volumetric flask and add about 90ml of methanol and 10ml of phosphate buffer and allow dissolving the drug completely by ultra sonication for 15minutes 1000µg/ml (STOCK 1).

Preparation of Sample Solutions

From STOCK1 solution measure accurately 1ml of solution and add 9ml of mobile phase and allow to dissolve completely by ultrasonicater for 15 min 100µg/ml (STOCK 2).

Preparation of Dilutions

From the STOCK 2 solution take 10µg/ml with micropipette and add 990µg/ml mobile phase simultaneously. Simultaneously prepare 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml and 60µl/ml.

Method Validation Parameters

The HPLC system suitability was conducted using standard preparation and evaluated by injecting the five replicates. Specificity parameter was performed by injecting diluents, placebo into the chromatographic system and evaluated by peak at the retention time. Linearity of the dipyridamole was found in the range of 99% of the specification .Calculate and record the area for each level and slope, intercept and correlation coefficient were calculated. The analytical method of the precision among the individual test results is performed when the sample is repeatedly to multiple sampling of homogeneous samples. The precision of the analytical method is usually expressed as the standard deviation (SD) and Relative standard deviation (RSD) of series of the precision was conducted using Dipyridamole and evaluated by making six replicate injections. The accuracy of the method by recovery of Dipyridamole sample solution at different concentration levels ranging from 10µg/ml to 60µg/ml respectively. The analytical method of robustness is a measure of its capacity to remain unaffected by small but deliberate variations in method parameter and provides an indication of its reliability during normal usage. The linearity was performed by different concentrations ranging from 10µg/ml to 60µg/ml with 1ml per minute flow rate at 291nm [10].

Results And Discussion

Optimization of Chromatographic Conditions

The development method of RP-HPLC includes selection of appropriate chromatographic conditions and factors like detection of wavelength and optimization of mobile phase. Preliminary development trails were performed with various columns of different types and dimensions from different manufacturer were tested for the peak shape and the number of theoretical plates for specification for concentrations. The wavelength of 291nm were selected which minimizes problems that may exhibit around the active ingredient attempting to quantify dipyridamole. Column with particle size of 3µm and C18 and dimensions of 4.6x250mm. The results reported are better than the existing method [8]. There was a significant improvement in the peak shape with good number of theoretical plates and tailing factor.

Method Validation

Suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of Dipyridamole to be performed. The suitability of the chromatographic system was demonstrated. The RSD from five replicate injection of diluted standard preparation was 0.1 % system suitability data was given in table: (Table 1).

S. no.	System suitability	Observed Value for Dipyridamole peak	Acceptance criteria
1	Tailing factor	0.75	NMT 2.0
2	Theoretical plates	9550	NLT 2500
3	RSD	0.7	NMT 2.0

Table 1: RSD from five replicate injection of diluted standard preparation was 0.1% system suitability data.

Selectivity

The selectivity of the method was tested analyzing the standard diluent solution by itself to show that no peaks interfere with analyte retention times during chromatographic separation. Standard diluent solution spiked with blank was analyzed. The specificity parameter was performed by injecting the standard and sample preparation into chromatographic system and record the retention times.

Linearity

Linearity of the analytical method was assessed considering six concentrations levels in the range from 10µg/ml to 60µg/ml concentrations are taken. Samples were analyzed six times per concentration; peaks areas were recorded, analyzed and results of the regression statistics are reported to demonstrate the linearity with Dipyridamole standard in the range of 10% to 60% of specification limit. The coefficient correlation of dipyridamole was 1.00 .the linearity was shown in the table. (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8) (Table 2).

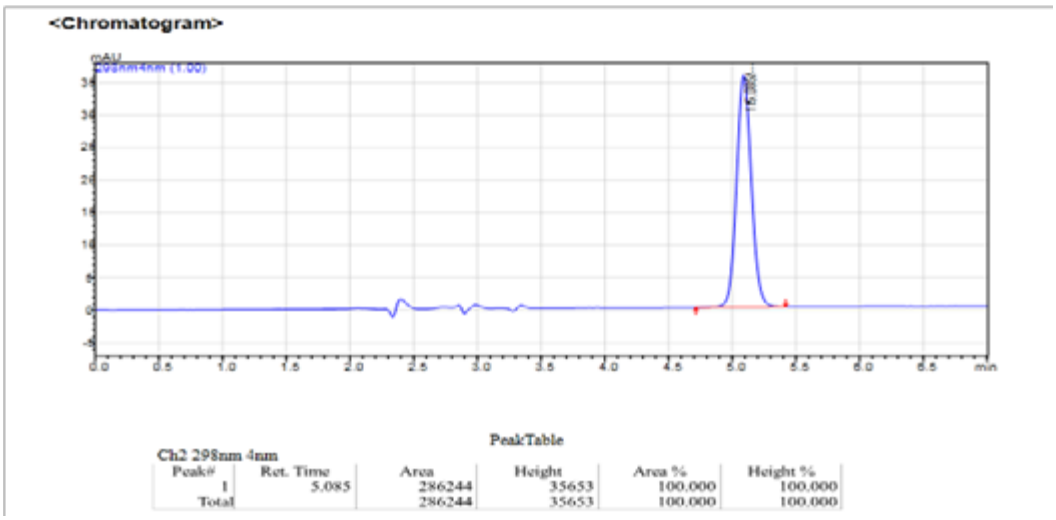


Figure 2: Chromatogram 10µg/ml

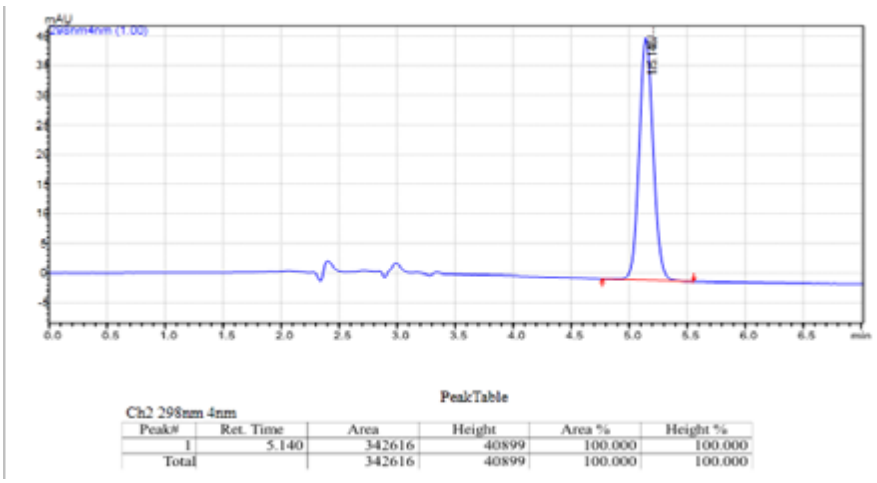
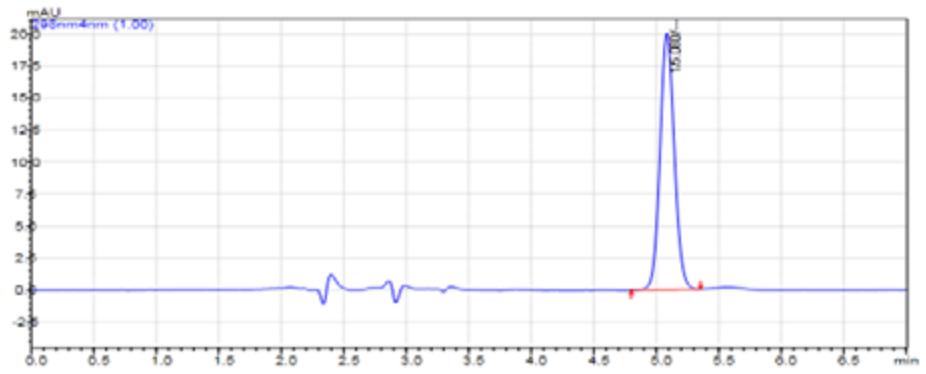


Figure 3: Chromatogram 20µg/ml

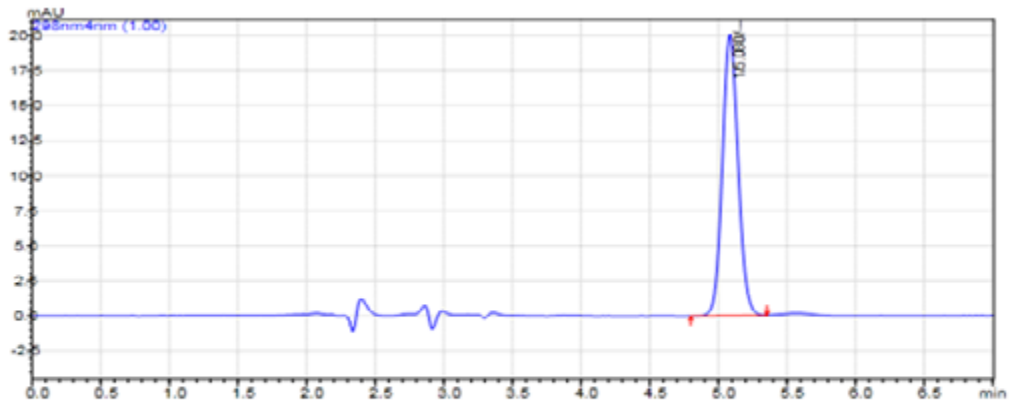


PeakTable

Ch2 298nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.080	159378	20060	100.000	100.000
Total		159378	20060	100.000	100.000

Figure 4: Chromatogram 30µg/ml

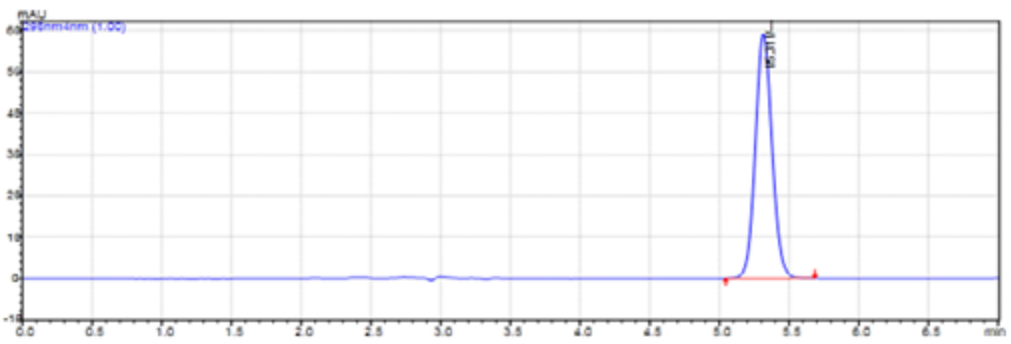


PeakTable

Ch2 298nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.080	159378	20060	100.000	100.000
Total		159378	20060	100.000	100.000

Figure 5: Chromatogram 40 µg/ml

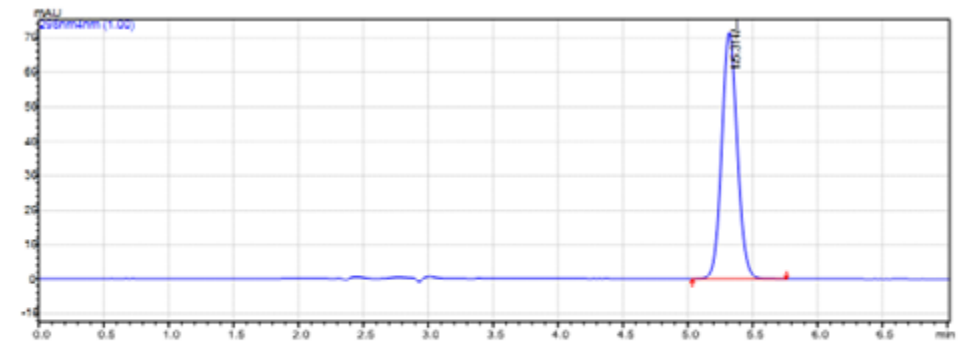


PeakTable

Ch2 298nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.311	493292	59275	100.000	100.000
Total		493292	59275	100.000	100.000

Figure 6: Chromatogram 50µg/ml



PeakTable

Ch2 298nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.314	598512	71632	100.000	100.000
Total		598512	71632	100.000	100.000

Figure 7: Chromatogram 60µg/ml

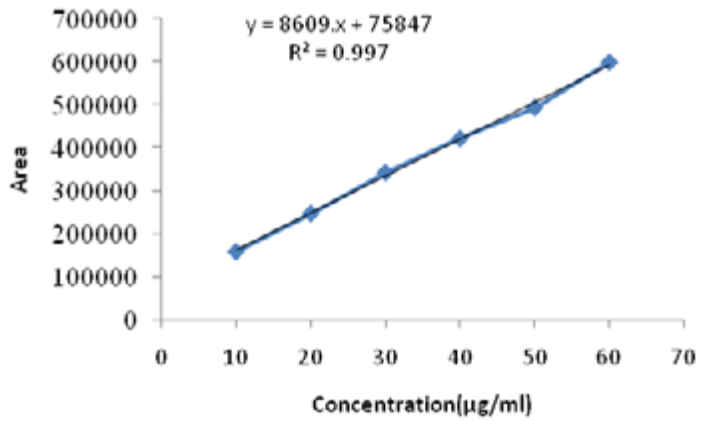


Figure 8: Linearity curve of Dipyridamole

Table 2: Linearity results for Dipyridamole

Linearity	Concentration of dipyridamole in µg/ml (ppm)	Dipyridamole peak area
0	0	0
10	159369.19	159378
20	247211.19	247220
30	342607.19	342616
40	42206.19	422070
50	493283.19	493292
60	598503.19	598512
	Correlation coefficient(R)	0.997
	Slope(m)-(uV*sec/ppm)	8609
	Intercept	75847

Precision

The test methods was validated by assessing six samples prepared on Dipyridamole and calculate relative standard deviation of area .The precision results are given: (Table 3).

Table 3: Precision results of Dipyridamole

S .No	Sample number	% Assay of Dipyridamole
1	10	99.4457357
2	20	99.481596
3	30	97.4309431
4	40	97.4639841
5	50	99
6	60	99
	Average	98.96
	SD	0.94253512
	%RSD	0.95

Repeatability

It was determined by preparing six replicates of same concentration (30µg/ml in HPLC method respectively) of sample and their absorbance/area measured.

Reproducibility

Intraday precision study was carried out by preparing drug solution of same concentration (30µg/ml in HPLC method respectively) and analyzing it at three different times in a day. The same procedure was followed for three different days to determine intraday precision. The results were indicated as % RSD.

Accuracy

According to ICH recommendations, to assess accuracy of the method for drugs formulation, studies are frequently performed by the addition of known amounts of drugs to the placebo, working at three concentration levels into the linear range of detection of the analyte. Thus, accuracy of the method was assessed using the average recovery values of the lowest, intermediate and upper concentration levels of calibration curve covering the linear range of analytes. The study of accuracy was found that the mean % of recovery was more than 100 % and less than 120 % at each level 80% 100% 120% of concentrated levels. Hence method is accurate [11].

The accurate results are given: (Table 4, Table 5).

Sample number	Spike level	% assay of Dipyridamole
1	80	98.4%
2	100	97.7%
3	120	99.8%

Table 4: Results for accuracy

Table 5: Precision results

Method Precision	RP – HPLC (%RSD)
Repeatability	0.337
Intra Day 1. 2. 3.	 0.337 0.361 0.962
Inter Day 1. 2. 3.	 0.361 0.594 0.643

Limit of Detection and Quantification

To check the Robustness of proposed methods analysis was carried out at two different temperatures, room temperature and at 39°C. This method was studied by injecting the system suitability solution at a change in the pH 6.8 of buffer solution flow rate was 0.08ml/minute and column temperature is 39°C. The results obtained were given in the table [9] (Table 6).

Table 6: Robustness results

Condition	%RSD(NMT:2.0)	Theoretical plates(NLT:2500)	Tailing factor(NMT:2.0)
Normal condition	0.1	9445	1.0
Change in buffer pH 6.8	0.12	13697	1.01
Change in buffer pH 7.2	0.14	18283	1.01
Column temp 40°C	0.17	18592	1.03
Column temp 39°C	0.22	6221	1.04
Flow rate 0.08ml /minute	0.10	22780	1.05
Organic modifier-10%	0.15	9775	0.99
Organic modifier+ 10%	0.07	10743	0.98

Limit of Detection and Quantification

The limit of detection (LOD) is defined as the analysis of samples with known concentrations of analyte and by establishing through visual evaluation the minimum level at which the analyte could be reliably detected. Where as the limit of quantification (LOQ) is the lowest concentration of an analyte that can be determined with the acceptable precision and accuracy in a sample under the stated operational conditions. LOD and LOQ were calculated to the formulae given in the ICH guidelines [13] (Table 7).

Table 7: Results for LOD and LOQ

S.No	Parameters	Mean of Calibration	SD	LD	LQ
1.	Slope	8609	0.94253512	61.8099314	187.302822
2.	Intercept	75847			
3.	R^2	0.998			

Conclusion

A simple gradient HPLC method has been developed and validated for the determination of Dypiridamole. The developed method has been found to accuracy, precision, linearity, robustness, and stability indicating method. 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.

Acknowledgement

I would like to express our special thanks to department of pharmaceutical Analysis of CVSR School of pharmacy, Anurag group of institutions, for the necessary facilities for carrying out the project.

References

1. Brown DG, Wilkerson EG, love W. A review of traditional and noval oral anticoagulant and anti platate therapy for dermitalogist and dermatologics surgeons. J Am Acad Dermatol. 2015 Mar; 72(3): 524-534. PubMed PMID: 25486915
   https://www.ncbi.nlm.nih.gov/pubmed/25486915
2. Bridle JH, Brimble MT. A Stability indicating methods for Dipyridamole. Development and Industrial Pharmacy. 1993 Jan 1; 19(3): 371-381.
   https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=A+Stability+indicating+methods+for+Dipyridamole&btnG=
3. Zoesta AR, Watsona JE, Hunga CT, Wimolrukb S. A Rapid Isocratic HPLC Assay for Dipyridamole using a Microbore Column Technique. Journal of Liquid Chromatography. 1991 Jun 1; 14(10): 1967-1975
   https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=A+Rapid+Isocratic+HPLC+Assay+for+Dipyridamole+using+a+Microbore+Column+Technique+&btnG=.
4. Pharmacopoeia I, Government of India, Ministry of Health and Family Welfare. Delhi: Controller of Publications. 1996; 2: A117-124.
   https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=+Indian+Pharmacopoeia%2C+Vol.+II%2C+Govt.+of+India%2C+Ministry+of+Health+and+Family+Welfare.+New+Delhi%3B+Published+by+The+Controller+of+Publications%3B+1996.&btnG=

5. Guideline IH. Stability Testing of New Drug Substance and Product. Q1A (R2). In Proceedings of the International Conference on Harmonization, 2003 Feb; 4:1-24.
https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=ICH+Stability+Testing+of+New+Drug+Substance+and+Product+Q1A+%28R2%29+International+Conference+on+Harmonization+%2C+2003.&btnG=
6. Guideline IH. ICH Validation of Analytical Procedures Text Methodology Q2 (R1). In Proceedings of the International Conference on Hormanization, Geneva, Switzerland 2005 Nov 10; p.11-12.
https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Validation+of+Analytical+Procedures+Text+Methodology+Q2+%28R1%29+&btnG=
7. Validation of Analytical Procedures: Methodology (Q2B) ICH Harmonized Tripatte Guidelines, Geneva.
8. Rao AS, Rao MK, Dadichand AS, Rao AP, Balaswami B. Development and Validation of RP-HPLC Method for Assay of Dipyridamole in Formulations. Journal of Pharmaceutical Sciences and Research. 2016 May 1; 8(5): 256.
https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Development+and+Validation+of+RP-HPLC+Method+for+Assay+of+Dipyridamole+in+Formulations%2C+Journal+of+Pharmaceutical+Sciences+and+Research&btnG=
9. Jain S, Diwan A, Sardana S. Development and Validation of UV Spectroscopy Method RP-HPLC Method for Estimation of Imiquimol, International Journal of Pharmaceutical Sciences Review and Research 2015; 35(2): 16-21.
https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Development+and+Validation+of+UV+Spectroscopy+Method+RP-HPLC+Method+for+Estimation+of+Imiquimol&btnG=
10. Rajput AP, Manohar C, Sonasis. Development and Validation of A Rapid RP – HPLC method for Determination of Aspirin and Dipyridamole in Combined Capsule Formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(2): 156-160.
https://pdfs.semanticscholar.org/b16a/072d2b33f406136530cdc890c6d48521b6ad.pdf
11. ICH, ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology, Int. Conf. Harmon. 1994 (2005)17.
12. Usmangani K. Chhalotiya, Kashyap K. Bhatt, Dimal A. Shah, Sunil L. Baldania. Development and Validation of A Stability indicating RP – HPLC Method for Duloxitine Hydrochloride in its Bulk and Tablet dosage forms, Sci Pharm. 2010 Oct-Dec; 78(4): 857-868.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3007604/

Submit your manuscript at
http://enlivenarchive.org/submit-manuscript.php

New initiative of Enliven Archive

Apart from providing HTML, PDF versions; we also provide **video version** and deposit the videos in about 15 freely accessible social network sites that promote videos which in turn will aid in rapid circulation of articles published with us.