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Development and Validation of Simple and Rapid UV Spectroscopic Method for estimation of Dipyridamole in Tablet Dosage Form

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Abstract:
Background*: A simple, specific, linear, precise, accurate and robust UV Spectroscopic method was developed for the determination of Dipyridamole in tablet dosage form. Solution was scanned over UV-visible range for its wavelength of maximum absorbance. The wavelength of maximum absorbance for Dipyridamole was found to be 295 nm. The method was validated as per ICH guidelines. Linearity range was observed in concentration of 5.2 – 15.5 µg/mL for Dipyridamole. The mean percentage recovery of Dipyridamole was found to be 100.04%. The correlation coefficient was close to 1. Developed method was found to be robust for the intended use. A simple, precise and cost-effective UV spectroscopic method is very beneficial for routine analysis in pharmaceutical industry*
Key Word*: Dipyridamole, UV Spectroscopy, Validation.*

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I. Introduction

Dipyridamole (2,6-bis-(diethanolamino)-4,8-dipiperidino-(5,4-d)-pyrimidine) displays antithrombotic and antiaggregatory activity. Refer figure (1) for the structural formula of Dipyridamole[1]. Dipyridamole is an antiplatelet drug. Dipyridamole is an odourless yellow crystalline powder, having a bitter taste. Dipyridamole exhibits a relatively short biological half-life of less than one hour. Therefore, extended release formulations of dipyridamole, which provide a continual administration of active ingredient over time, are preferred. Dipyridamole is soluble in acidic mediums with a pH below 4 and is practically insoluble in water. Therefore, dipyridamole is readily absorbed in the more acidic regions of the upper gastrointestinal tract, but remains insoluble in the more basic regions of the intestine. To obtain a constant level of dipyridamole in the blood, it is advantageous to formulate a dipyridamole dosage form that releases dipyridamole at a controlled rate and at a defined pH. Acidic components can be administered with dipyridamole to maintain a defined pH level throughout administration.[2]

Literature reveals different Assay methods like liquid chromatography-tandem mass spectrometry[3,6], high performance liquid chromatography-mass spectrometry[4], RP-HPLC[5], Phosphorimetry[7], TLC-Densitometric[8], HPLC[8,10], RP-UPLC[9] for estimation of Dipyridamole alone and with other drug substances. A successful attempt was made for quantitative determination of Dipyridamole by simple, rapid and easy to operate UV Spectroscopic method which is which is very beneficial to the pharmaceutical industry.

The aim of this study is to develop a simple, precise and accurate UV Spectroscopic method for the estimation of Dipyridamole in pharmaceutical dosage forms as per ICH guidelines [11].

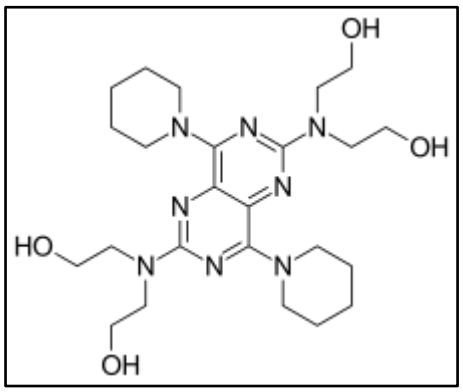

Figure 1 : Dipyridamole [1]

II. Material And Method Development

Instrumental:

The UV analysis was carried with UV 1800 (Shimadzu) UV Spectrophotometer. Quartz cell of 1.0 cm path length was used.

Reagents and chemicals

Dipyridamole was taken from commercial source and tablets were obtained from Medley Pharmaceutical Limited. All the chemicals used were AR grade.

Preparation of standard solution

25.88 mg of Dipyridamole working standard was weighed & transferred in to 50 ml volumetric flask. 25 mL of diluent was added and the solution was sonicated for 5 minutes. Volume was made up to the mark with diluent. Further 2 mL was diluted to 100 ml with diluent. Solution was mixed well and absorbance was measured. (Concentration: 10 ppm of Dipyridamole)

Preparation of sample solution

20 tablets were weighed and average weight was calculated. These tablets (Strength 25 mg per Tablet) were then crushed and transferred the crushed powder equivalent to the average weight into a 50 mL volumetric flask. 25 mL of diluent was added and sonicate for 15 minutes with intermittent shaking. Then solution was cooled and diluted up to the mark with diluent. Filter the solution.

Further diluted 2.0 mL of the above solution to 100 mL with diluent. Then solution was mixed well and absorbance was measured. (Concentration:10 ppm of Dipyridamole).

Procedure

Absorbance of reference & test solution at wavelength 295 nm was measured after doing blank correction with diluent and % RSD (Relative Standard Deviation) was calculated.

Method development

The reference solution was scanned with medium scanning speed for a whole range of UV/VIS Spectrophotometer, the ranging from 800-200 nm with a diluent as a blank. After acquiring the spectrum, λmax was identified. The above method was repeated thrice.

III. Material Validation

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH 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. They were tested using the optimized chromatographic conditions and instruments. [11,12]

Specificity

Spectral purities of Dipyridamole were evaluated for the interference of the blank and placebo, as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks. Maxima /spectral pattern of test solution should match with that of reference solution.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of for Dipyridamole and Dipyridamole was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations such as 5.18, 7.76, 10.35, 12.94 & 15.53 µg/mL for Dipyridamole were prepared and analyzed as per table (1) Correlation coefficient & %Y-axis intercept were calculated. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Table 1: Linearity Concentration Levels of Dipyridamole

% level	Volume of stock solution	Diluted to (mL)	Final concentration in ppm
50%	1.0 ml	100	5.18
75%	1.5 ml	100	7.76
100%	2.0 ml	100	10.35
125%	2.5 ml	100	12.94
150%	3.0 ml	100	15.53

Accuracy

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed concentration of the pre-analyzed Tablet sample. Percent recovery was calculated by comparing the area with pre-analyzed sample. Three different solutions of Dipyridamole were prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration (5, 10, 15 µg/mL). and the percentage mean and individual recovery was calculated.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by carrying out six independent assays of Dipyridamole at 10 µg/ml concentration. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

Intermediate precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day was taken from the analysis of "Repeatability". The second set of experiments was performed by a different analyst or on different instrument.The standard deviation, relative standard deviation and mean value difference was calculated from the results obtained on each day.

Robustness

The robustness of an analytical procedure 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. It was observed that the variations like sonication time & change in wavelength etc.

IV. Result & Discussion

The proposed method for determination of Dipyridamole showed molar absorptivity of 2.76 x 10^4 L/mol.cm. Linear regression of absorbance on concentration gave the equation y = 0.06x -3.4 with a correlation coefficient (r) of 0.9996. The optical characteristics such as Beer's law limit and Sandell's sensitivity were calculated and are summarized in Table 1

Table 1. Optical characteristics of Dipyridamole.

Parameter	**Results**
λmax	295 nm
Beer's law limit	5.18 – 15.53 µg/m
Molar absorptivity	2.7×10^{-8}
Sandell's sensitivity (µg cm-2 / 0.001 absorbance unit)	0.017

Regression equation (Y = a + bC)	Y=-3.4 + 0.06b
Slope (b)	0.06
Intercept (a)	-3.4
Correlation coefficient (R)	0.9996
% Range of error (Confidence limits) 0.05 Level 0.01 Level	 0.0016 0.0027

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated 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. Dipyridamole showed maximum absorbance at 295 nm.

Specificity:

By comparing the spectra of blank solution, placebo solution, reference solution & test solution it was observed that no interference was observed from blank & placebo solution. Maxima /spectral pattern of test solution was matching with that of reference solution. Refer figures (2), (3), (4) an (5) for UV scans of blank, reference solution, test solution and placebo solution respectively.

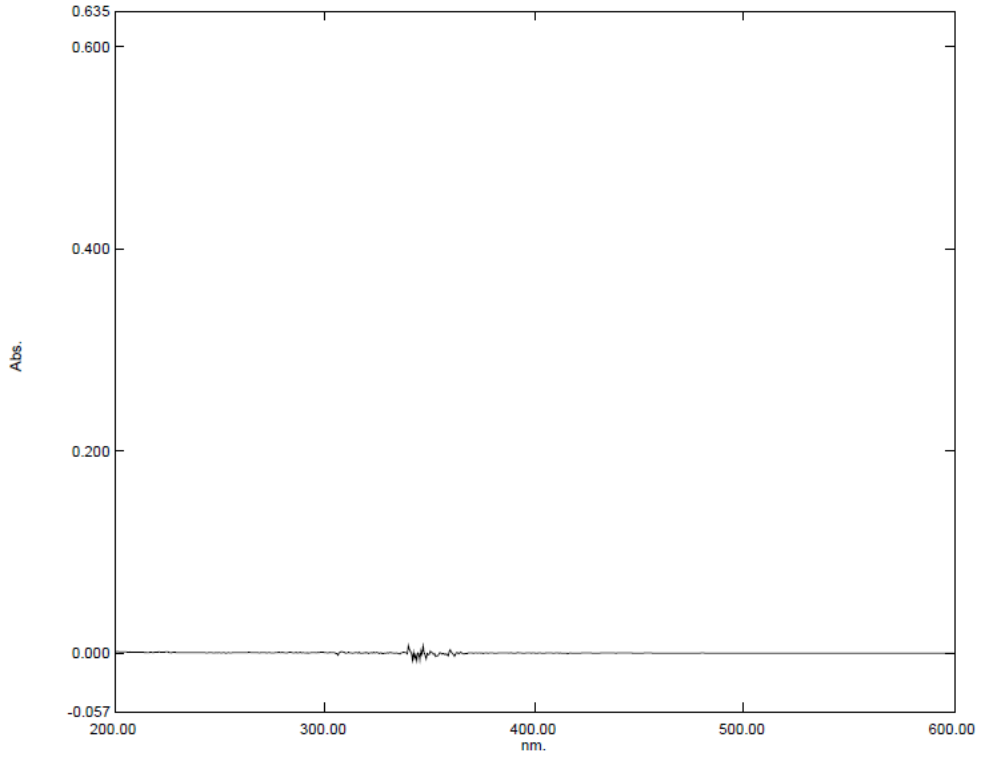

Figure 2: Scan of blank solution

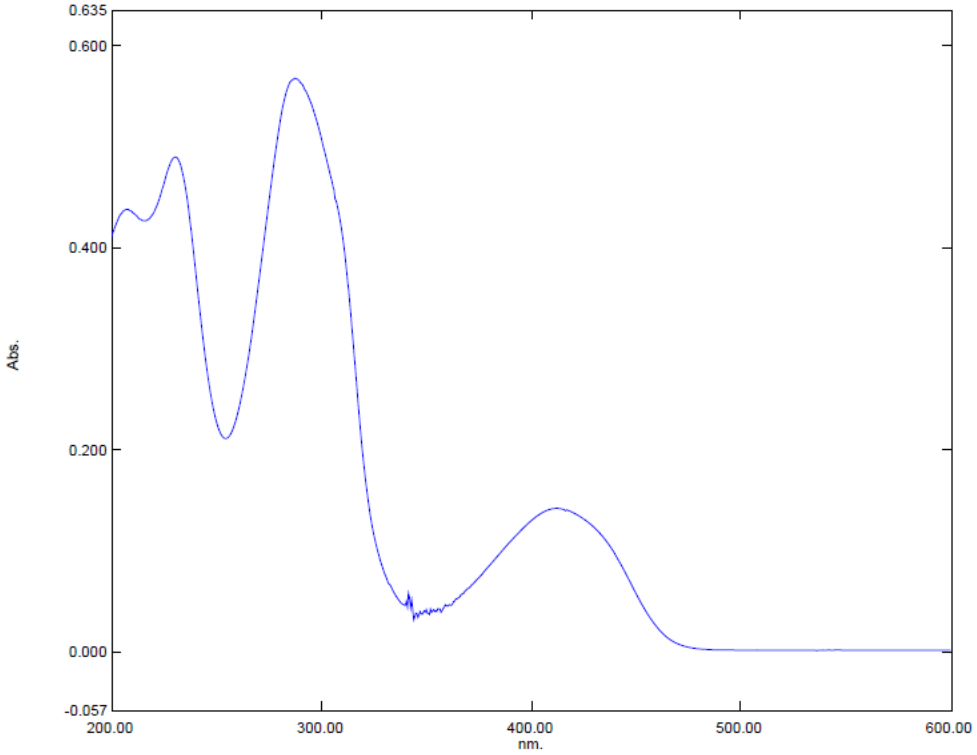

Figure 3: Scan of standard solution

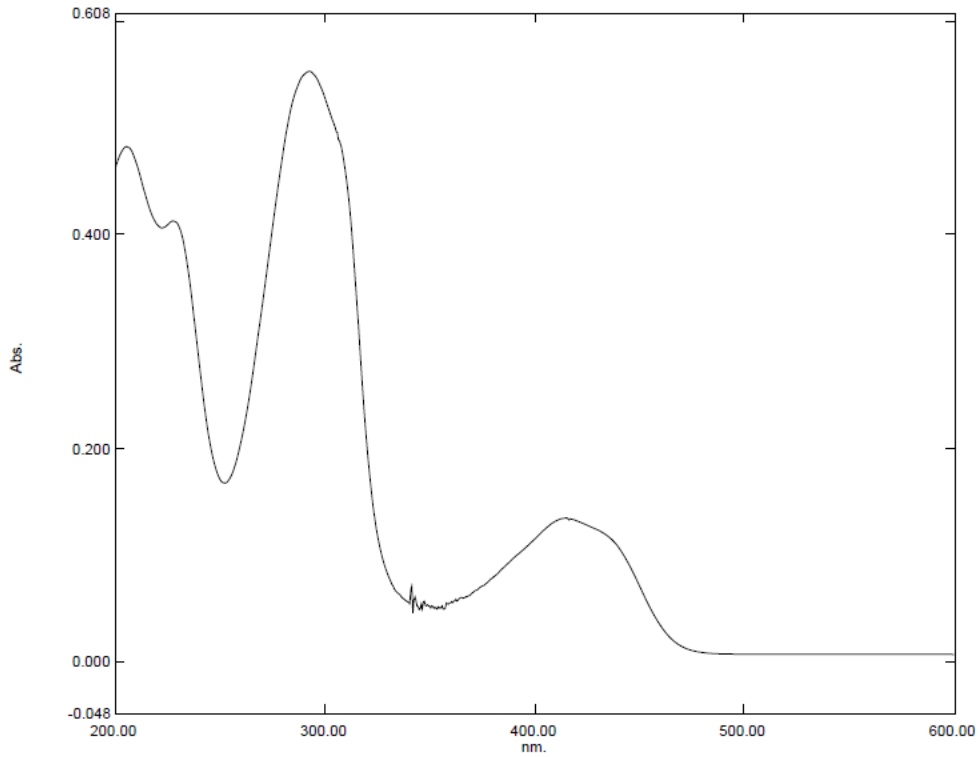

Figure 4: Scan of sample solution

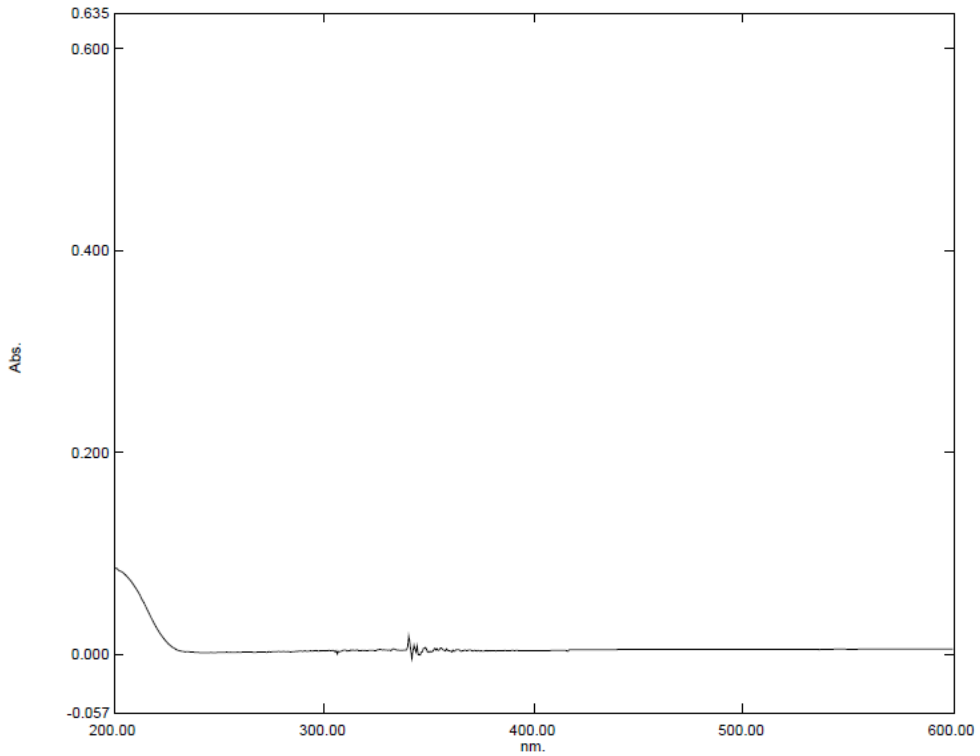

Figure 5: Scan of placebo solution

Linearity

Five concentrations such as 5.18, 7.76, 10.35, 12.94 & 15.53 µg/mL for Dipyridamole were prepared and the linearity graph was plotted using absorbance verses concentration as shown in Figure (6). Graph of Residuals against concentration was also plotted as per shown in Figure (7). A linear relationship was obtained in the range of 50 to 150% (5.18 – 15.53 µg/mL for Dipyridamole as Correlation coefficient R and %Y – axis intercept was within the acceptance criteria (refer table 2).

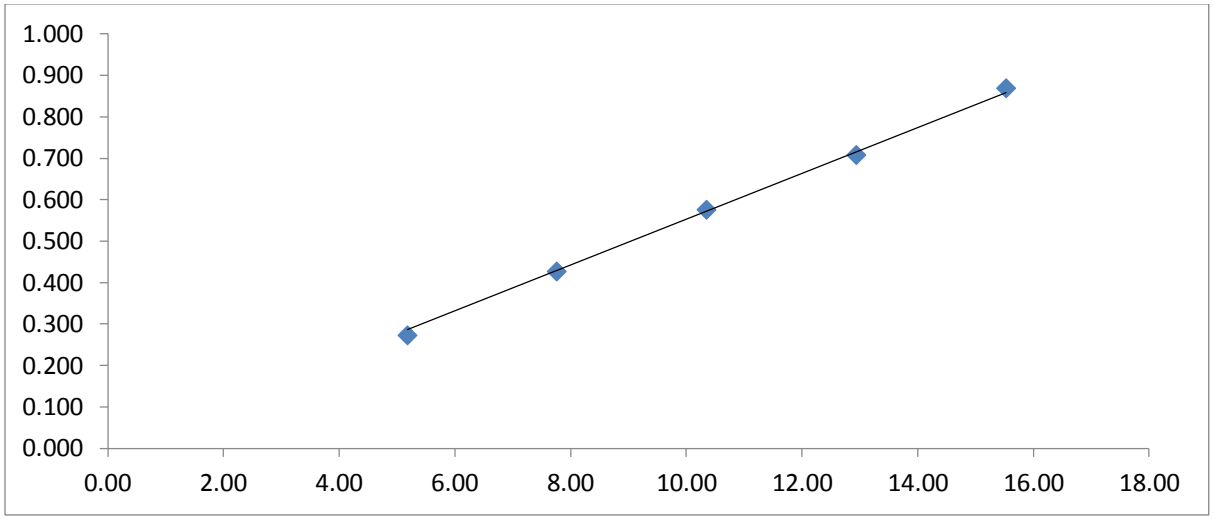

Figure 6: Linearity plot for Dipyridamole

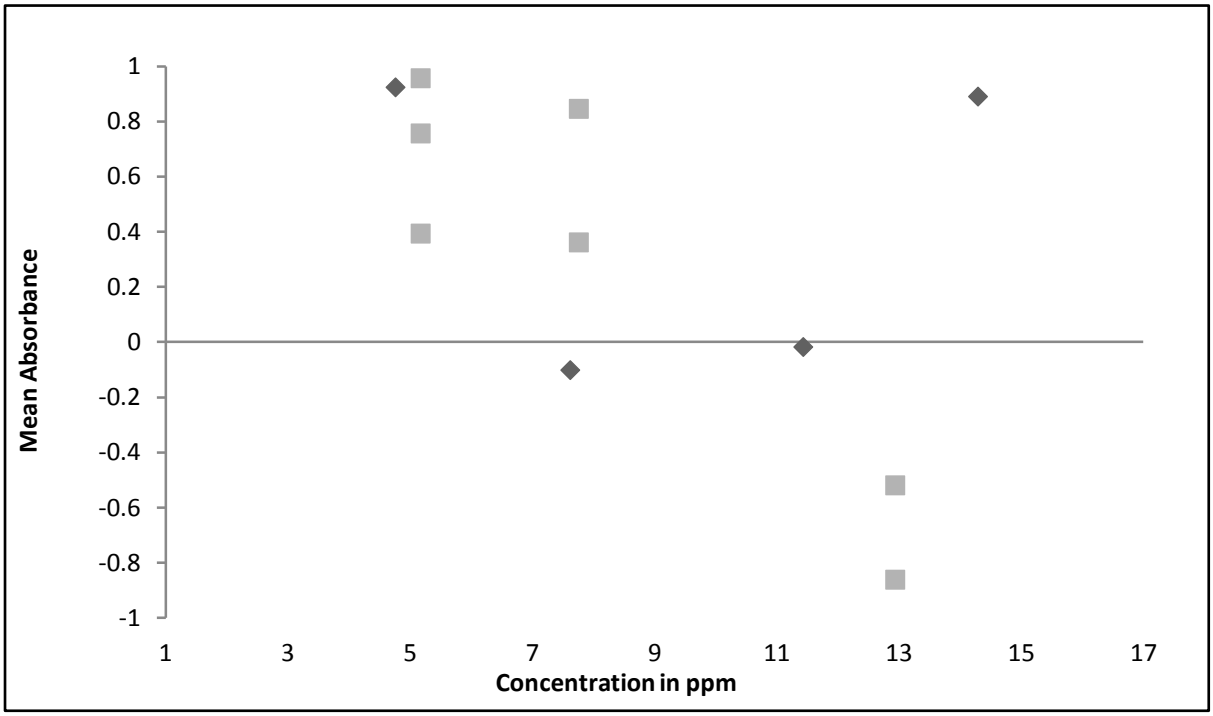

Figure 7: Plot of Residuals against concentration for Dipyridam

Table 2: Observation table for linearity of Dipyridamole

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	0.99961	$\geq$ 0.999
%Y – axis intercept	-3.4	$\leq \pm$ 5 %
Slope of regression line	0.06	To be reported
Residual sum of squares	0.0001678	To be reported

The method was considered to be linear in the range on 5.18 – 15.53 µg/mL for Dipyridamole as Correlation coefficient & %Y-axis intercept should be within the limit.

Accuracy

The percentage recovery of Dipyridamole was tabulated in table (3). The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table 3: Recovery at Different Concentration Levels

Accuracy level	% recovery of Dipyridamole
50%	100.39
	99.84
	99.99
100%	99.57
	99.98
	99.67
150%	99.56
	101.01
	100.32

Means recovery	**100.04**
Minimum recovery	**99.56**
Maximum recovery	**101.02**

Precision

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer table (4).

Table 4: Method Precision

Sample No.	% Assay of Dipyridamole
Sample 01	101.0
Sample 02	100.6
Sample 03	100.6
Sample 04	101.0
Sample 05	101.0
Sample 06	101.5
Mean	**101.0**
STD Dev	**0.33**
% RSD	**0.33**

Intermediate Precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer table (5) for % Assay of Dipyridamole and table (6) for comparison of two independent repeatability

Table 5: Intermediate Precision

Sample No.	% Assay of Dipyridamole
Sample 01	100.4
Sample 02	100.2
Sample 03	100.6
Sample 04	100.8
Sample 05	101.4
Sample 06	101.1
Mean	**100.8**
STD Dev	**0.44**
% RSD	**0.44**

Table 6: Comparison of two independent repeatability

Parameter	1st day Repeatability	2nd day Repeatability
Number of determinations	6	6
Mean (%) assay	101.0	100.8
RSD (%)	0.33	0.44
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**0.2**	

Robustness

Method was found to be robust as system suitability criteria was achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method

performance, which demonstrated that the developed UV method was robust. The results were shown in Table (7).

Table 7: Robustness Result For Dipyridamole

Parameter	System suitability	% Assay
	% RSD	
As per method		
--	0.33	101.0
Sonication time		
18 minutes	0.13	101.2
22 minutes	0.38	100.5
Wavelength change		
293 nm	0.23	101.3
297 nm	0.39	100.4

V. Conclusion

In this present work a new, selective, linear, precise, accurate and robust UV method was developed and validated for the estimation of Dipyridamole in pharmaceutical tablet dosage form in accordance with the ICH guidelines. The current work is worthwhile as developed UV spectroscopic method is selective, simple and rapid which can be very beneficial for the routine analysis of Dipyridamole in pharmaceutical tablet dosage form.

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