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Full Length Research Paper

Rapid and simultaneous determination of aspirin and dipyridamole in pharmaceutical formulations by reversed-phase high performance liquid chromatography (RP-HPLC) method

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The combination of Dipyridamole and Aspirin and is widely used to reduce thrombosis in patients with thrombotic diseases. A rapid, simple, precise and cost effective and reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous determination of Aspirin and Dipyridamole in pharmaceutical formulations. Separation of both Aspirin and Dipyridamole was achieved within 5 min with required resolution, accuracy and precision thus enabling the utility of the method for routine analysis. Chromatographic separation was achieved on a waters symmetry C18 3.5 µm, 50 x 4.6 mm using a mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 75:25 at a flow rate of 1.0 ml per minute. The detection was made at 227 nm and the retention time of Aspirin and Dipyridamole were1.5 and 2.8 minutes respectively. The method was found linear over the range of 4 to 80 µg/ml for Dipyridamole and 0.5 to 10 µg/ml for Aspirin.

Key words: Aspirin, Dipyridamole, high performance liquid chromatography.

INTRODUCTION

Aspirin (ASP) is 2- (Acetyloxy) benzoic acid, and is cyclo oxygenase inhibitor which is best known as an anti-platelet drug (Patel et al., 2010) and is one of the major antithrombogenic agent widely used for the treatment and prevention of cerebro and cardiovascular conditions such as stroke (Purushotam et al., 2009). Dipyridamole is a platelet inhibitor chemically described as 2,2',2",2"'-[(4,8-Dipiperidinopyrimido [5,4-d]pyrimidine-2,6-diyl)dinitrilo]-tetraethanol. Dipyridamole is widely used as a coronary vasodilator in patients with high blood pressure, a prophylactic agent in patients with angina pectoris and an inhibitor of platelet aggregation in various thromboembotic conditions (Davood et al., 1999).

The combination of Dipyridamole and Aspirin and is widely used to reduce thrombosis in patients with thrombotic diseases. This antithrombotic action results from additive antiplatelet effects of both drugs. Aspirin inhibits platelet aggregation by irreversible inhibition of platelet cyclooxygenase and thus inhibiting the generation of Thromboxane A2. Dipyridamole inhibits the uptake of adenosine into platelets and endothelial cells, thus decreasing the adhesion of platelets to thrombogenic surfaces (Hassan et al., 2008).

Analytical methods based on high performance liquid chromatography (HPLC), HPTLC, LC-MS (Kachhadia et al., 2008; Vora et al., 2008; Wada et al., 2007; William et al., 1983; Rajput et al., 2008; Mishra et al., 2006) and other methods were reported earlier for the determination of Aspirin individually and in combination with other drugs.

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A few analytical procedures were also proposed for the determination of Dipyridamole in dosage forms in human plasma, serum, urine and feces (Zhang et al., 1997; Qin et al., 2010; Murillo Pulgarín et al., 1997).

Although the combinational use of Aspirin and Dipyridamole is continuously increasing, few methods were reported for the simultaneous determination of Aspirin and Dipyridamole using combination of liquid chromatographic and mass spectrometric detection (Wang et al., 2008), second-order derivative spectrophometry (Periasamy Umapathi, 1994) and by spectrofluorimetric method (Hassan et al., 2008). Simple and sensitive HPLC method for the estimation of Aspirin and Dipyridamole was seldom reported. The objective of the present work was to develop and validate simple, robust, sensitive, reproducible and cost effective analytical method for the simultaneous determination of Aspirin and Dipyridamole in pharmaceutical dosage forms. We describe herein a simple, sensitive and validated HPLC method utilizing isocratic mobile phase with short retention time for the simultaneous determination of these two components in pharmaceutical formulations. The developed method can be successfully applied to routine quality control and other analytical purposes.

MATERIALS AND METHODS

Chemicals and reagents

Aspirin and Dipyridamole working standards were procured from Cipla Labs, and the tested pharmaceutical formulations (Aspirin (25 mg) and Dipyridamole (200 mg) tablets) were procured from commercial pharmacy. Ortho phosphoric acid, acetonitrile, methanol and other reagents were of suitable analytical grade.

Apparatus and chromatographic conditions

HPLC analysis was performed on waters HPLC system equipped with a 2696 separation module and 2996 Photo Diode Array Detector. Separations were carried on a waters symmetry C18 3.5 µm, 50 x 4.6 mm using isocratic elution. The flow rate was 1.0 ml min^{-1}. Detection was performed at 227 nm. Injection volume was 10 µl. Peak identities were confirmed by retention time comparison and the procedures and instrument operation was performed at room temperature.

Preparation of mobile phase

The mobile phase is composed of a mixture of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 75:25 (v/v)., filtered through a 0.45 µm nylon filter (Millipore, USA) and degassed by sonication prior to use.

Preparation of standard solution

The standard stock solution of Aspirin (0.25 mg/ml) and Dipyridamole (1 mg/ml) was prepared in methanol since both drugs are soluble in this solvent. The working standard solutions of Aspirin (5 µg/ml) and Dipyridamole (40 µg/ml) was prepared by diluting the stock solution in mobile phase solution.

Preparation of sample solution

Twenty tablets were weighed to get the average weight and the tablets were grounded and made into powdered form. From the powdered form amount of powder equivalent to 25 mg of Aspirin and 200 mg of Dipyridamole was transferred to a 500 ml volumetric flask and added 150 ml of methanol and kept on rotary shaker for 15 min at 200 RPM and added 200 ml of methanol and sonicated for 30 min with intermediate shaking. Finally, the volume was made up with methanol to obtain a solution containing 0.05 mg/ ml Aspirin and 0.4 mg/ ml Dipyridamole. An aliquot was then removed and centrifuged at 5000 rpm for 10 min and the centrifuged solution was filtered using 0.45 µm membrane filter paper. After filtration, the solutions were diluted with mobile phase to get the final concentration of Aspirin (5 µg/ml) and Dipyridamole (40 µg/ml).

RESULTS AND DISCUSSION

Method development

Drug quality control, stability, metabolism and pharmacokinetics studies including the toxicity studies necessitate the determination of drugs in pharmaceutical formulations and biological samples. Correspondingly efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminary optimized to develop a LC method for simultaneous determination of Dipyridamole and Aspirin with short run time (<5 min), and acceptable resolution (Rs > 2). The polarity of Dipyridamole and Aspirin differ greatly as Aspirin is more lipophilic than Dipyridamole. The sample retention increases with increased column length so a shorter column (50 x 4.6 mm) was selected to have a shortest possible runtime without compromising on the resolution. Separation of Aspirin and Dipyridamole were achieved on waters symmetry C18 3.5 µm, 50 x 4.6 mm column using isocratic flow. The column was chosen as both the analytes were separated with acceptable resolution and with in short run time. Isocratic elution was chosen as the required resolution was achieved and hence the complex gradient elution program was not used. Lower particle size (3.5 µm) column was chosen to increase the resolution between the Dipyridamole and Aspirin.

In order to identify a suitable organic modifier along with 0.1% ortho phosphoric acid, various compositions of acetonitrile and methanol were tested. Methanol produced a higher retention time for Dipyridamole and higher column pressures due to the high viscosity. Acetonitrile was found to display advantageous separations. Change of percentage of acetonitrile in the mobile phase provided great influence on retention time of the two drugs. When the acetonitrile content was lower than 20%, retention time of Dipyridamole increased rapidly and when the acetonitrile content was higher than 30%, resolution between Dipyridamole and Aspirin was

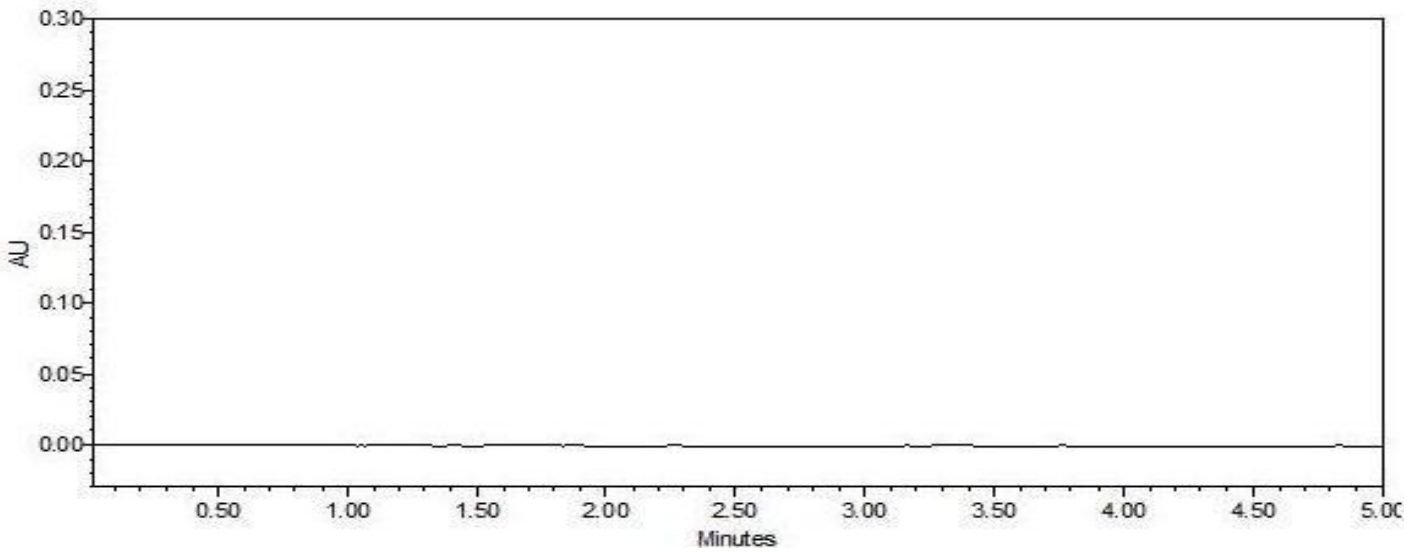

Figure 1. Chromatogram of placebo.

reduced. Finally the separation with acceptable resolution was achieved with the mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 75:25. Effects of the mobile phase pH on retention of the both drugs were investigated at pH values of 3, 4, 5, 6, and 7, respectively. It was found that the mobile phase pH has no effect on the retention of Aspirin and Dipyridamole. Operating wavelength of 227 nm was selected based on the absorbance maxima in the diluents. Flow rate of 1.0 ml per minute was optimized to yield the shorter retention time with required resolution and intensity. Lesser flow rates resulted in increased retention times and higher flow rates of more than 1.0 ml per minute not provided the required resolution and intensity.

Finally separation for simultaneous determination of Dipyridamole and Aspirin was carried out by isocratic elution using 25% acetonitrile with a flow rate of 1.0 ml per minute. With the above chromatographic conditions, retention time Aspirin and Dipyridamole were 1.5 and 2.8 min, respectively.

The above described method is suitable for routine pharmaceutical applications involving the analysis of Aspirin and Dipyridamole. The retention time of each analyte was very reproducible with relative standard deviations between 0.03 and 0.04% (n = 6) for Dipyridamole and Aspirin respectively. The peak area responses were also reproducible with relative standard deviations of 0.6 and 0.5% (n = 6) for Dipyridamole and Aspirin respectively.

Method validation

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

System suitability

In order to determine the adequate resolution and reproducibility of the proposed methodology, suitability parameters including retention time, resolution, tailing factor, %RSD of retention time and peak areas were investigated and the results were found within the acceptable specifications.

Specificity

The specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products etc. Specificity was evaluated by preparing the analytical placebo and it was confirmed that the signal measured was caused only by the analytes. A solution of analytical placebo (containing all the tablet excipients except Dipyridamole and Aspirin was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients (placebo), standard solutions, and the commercial pharmaceutical preparations including Dipyridamole and Aspirin were analyzed by the developed method. The representative chromatograms as shown in Figures 1, 2 and 3 did not show any other peaks, which confirmed the specificity of the method. Peak purity of Aspirin and Dipyridamole were

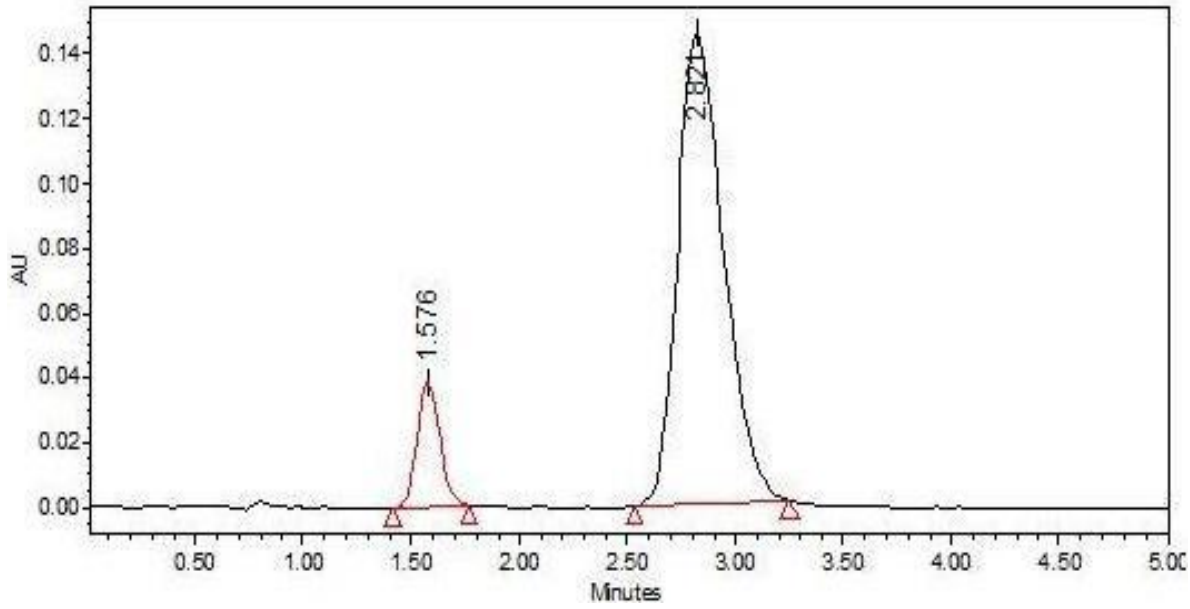

Figure 2. Chromatogram of standard.

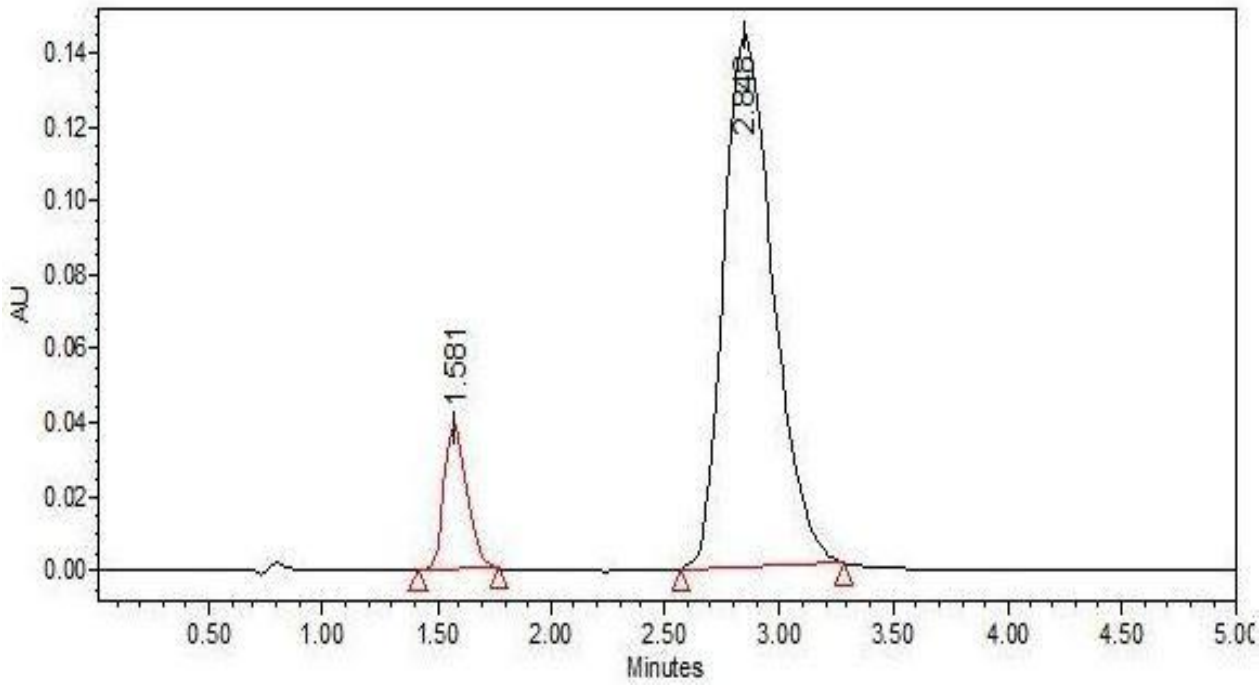

Figure 3. Chromatogram of sample.

also evaluated for confirming the purity of the individual peaks. The peak purity evaluation profiles indicate that there were no interference from blank components. The peak purity profiles of Aspirin and Dipyridamole were shown in Figures 4 and 5, respectively.

Linearity

The linearity of an analytical method is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in

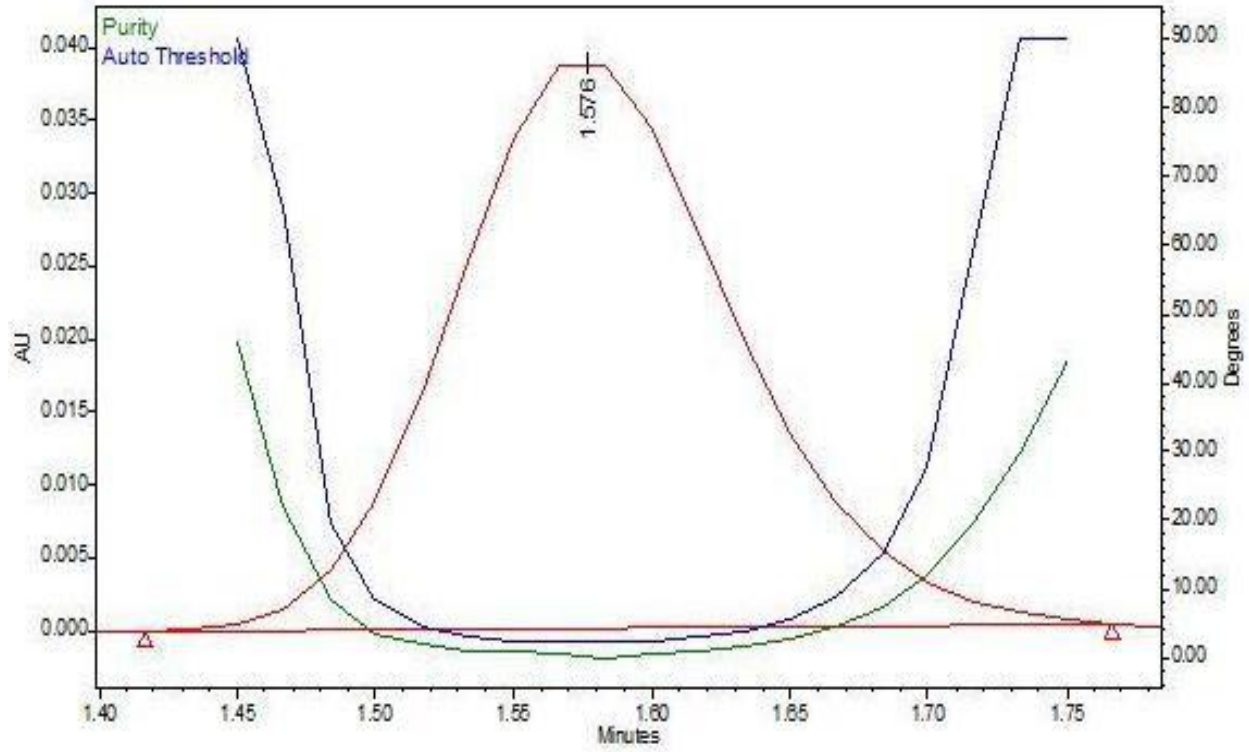

Figure 4. Peak purity plot of asprin.

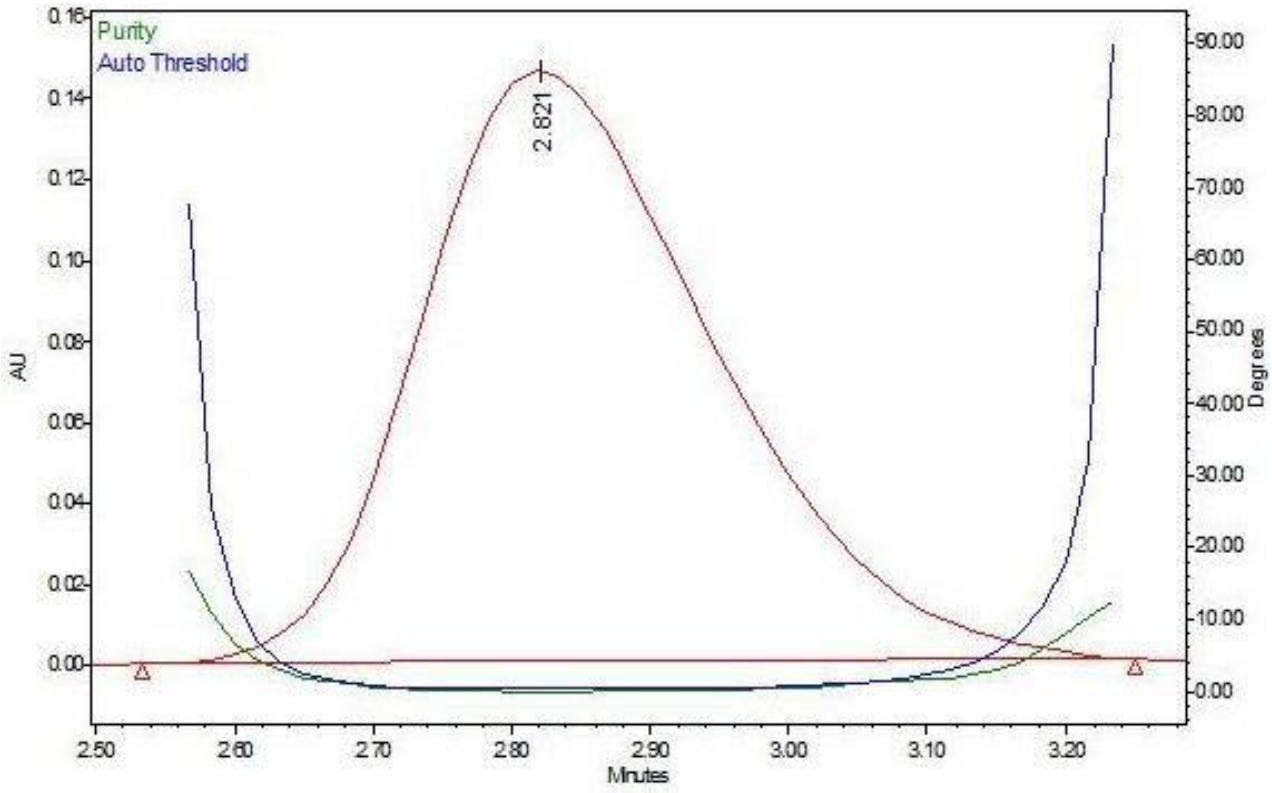

Figure 5. Peak purity plot of dipyridamole.

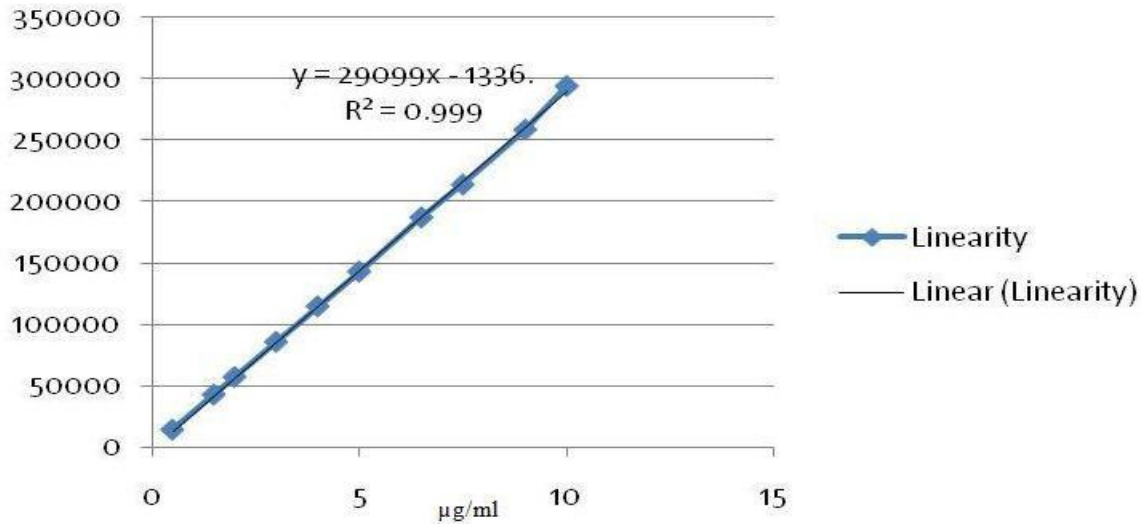

Figure 6. Linearity graph of aspirin.

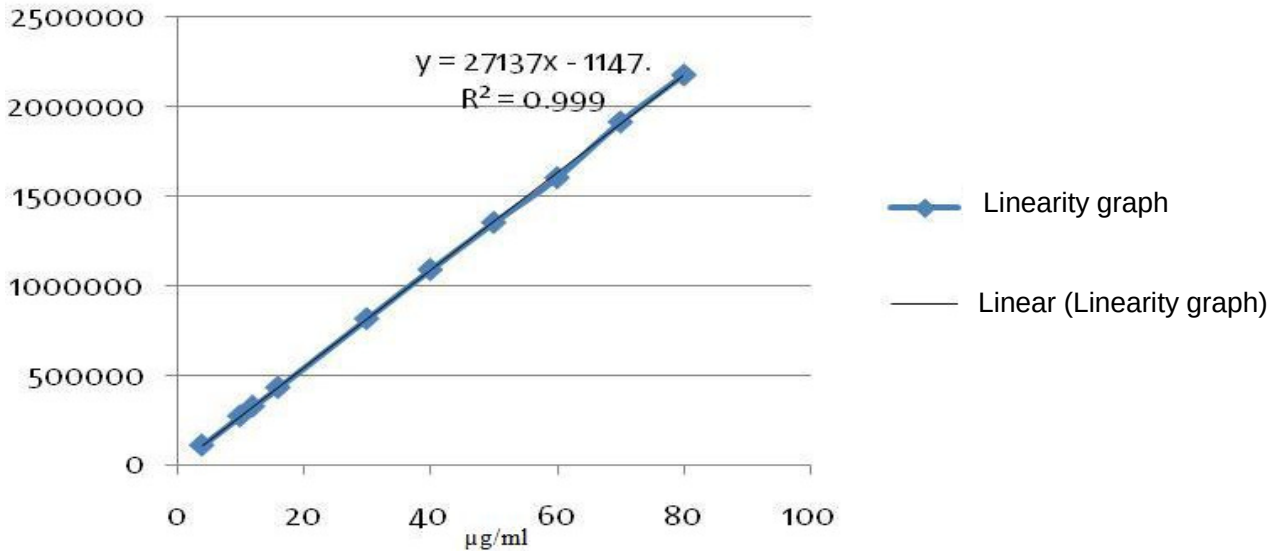

Figure 7. Linearity graph of dipyridamole.

the sample. Linearity of detector response for Aspirin/Dipyridamole was established by analyzing serial dilutions of a stock solution of the working standard. Ten concentrations ranging from 10 to 200% of the test concentrations were prepared and analyzed. The final concentration of each solution in µg/ml was plotted against peak area response. The method was found linear over the range of 4 to 80 µg/ml for Dipyridamole and 0.5 to 10 µg/ml for Aspirin. Correlation coefficient (R) was found to be greater than 0.999 for both Aspirin and Dipyridamole. The linear graphs of Aspirin and Dipyridamole were shown in Figures 6 and 7, respectively.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Six replicate samples were prepared and analyzed as per the sample preparation procedure and the assay values were calculated.

The precision (n = 6) of the assay values for Aspirin and Dipyridamole was found to be 0.5 and 0.4% for Aspirin and Dipyridamole, respectively. The precision values are shown in Table 1.

Table 1. Precision of aspirin and dipyridamole.

Sample No.	Assay	
	Aspirin	Dipyridamole
1	10.2	80.2
2	10.1	80.1
3	10.3	80.8
4	10.2	79.7
5	10.3	80.5
6	10.1	80.6
Mean ($\overline{X}$)	10.20	80.32
S.D.	0.089	0.397
%RSD	0.9	0.5

Table 2. Recovery for aspirin and dipyridamole.

Dipyridamole			Aspirin		
Sample No.	**Spike level**	**Amount recovered (mg)**	**Sample No.**	**Spike level**	**Amount recovered (mg)**
1	50% (40 mg)	40.23	1	50% (5 mg)	4.87
2		38.75	2		5.29
3		40.32	3		5.21
4		39.35	4		5.17
5		40.15	5		4.94
6		41.05	6		5.03
Mean recovery		39.975	Mean recovery		5.085
Mean % recovery		99.9	Mean % recovery		101.7
1	100% (80 mg)	78.67	1	100% (10 mg)	10.36
2		79.45	2		9.79
3		82.67	3		9.48
4		80.32	4		9.75
5		79.24	5		10.47
6		83.24	6		10.14
Mean Recovery		80.598	Mean Recovery		9.998
Mean % Recovery		100.7	Mean % Recovery		100.0
1	150% (120 mg)	123.56	1	150% (20 mg)	19.42
2		126.38	2		20.35
3		116.29	3		19.61
4		118.89	4		20.87
5		124.67	5		20.94
6		117.98	6		19.13
Mean recovery		121.295	Mean recovery		20.053
Mean % recovery		101.1	Mean % recovery		100.3

Recovery

Recovery studies for dipyridamole and aspirin were performed at 50, 10%, and 150% of the highest concentration of the linearity range (80 µg/ml for dipyridamole 10 µg/ml for Aspirin) by spiking placebo blend with the drug substance. Six replicates each were spiked and analyzed after extraction. The amount spiked, amount recovered and mean percent recovery were calculated and reported in Table 2.

Range

The range of an analytical procedure is the interval

Table 3. Range for aspirin and dipyridamole.

Parameter	Acceptance criteria	Result	
		Dipyridamole	Aspirin
Linearity	R ≥ 0.999	0.9999	0.9998
Precision	%RSD of 6 replicates NMT 2.0%	0.9%	0.5%
Accuracy	Recovery 97.0 to 103.0%	99.9-101.1%	100.3 to 101.7

between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The results are shown in Table 3.

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. The variations like flow rate of mobile phase, column temperature and ratio of organic content in the mobile phase etc does not have any significant effect on the method performance.

Conclusions

A simple, rapid, reproducible and cost effective RP-HPLC method was developed for the simultaneous determination of Aspirin and Dipyridamole in pharmaceutical formulations by isocratic mode elution. The analytical conditions and the solvent system developed provided good resolution for Aspirin and Dipyridamole within a short run time. The HPLC method was validated and demonstrated good linearity, precision, accuracy and specificity. Thus, the developed HPLC method can be utilized for routine analysis during the analysis of Aspirin and/or Dipyridamole.

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