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Research Article

DEVELOPMENT AND VALIDATION OF A RAPID RP-UPLC METHOD FOR THE DETERMINATION OF ASPIRIN AND DIPYRIDAMOLE IN COMBINED CAPSULE FORMULATION

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ABSTRACT

A novel stability-indicating Ultra high-performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous estimation of Aspirin and Dipyridamole in the capsule dosage form.

Chromatographic separations were carried using Hypersil Gold C18, Column (1.9µm, 100 mm X 2.1 mm) with a mobile phase composition of triethylamine phosphate buffer (pH 2.5) and methanol in the ratio 50:50% (V/V) have been delivered at a flow rate of 0.5 mL min^{-1} and the detection was carried out using UV detector at wavelength 230 nm. The retention time for Aspirin and Dipyridamole were 0.83 and 1.62 minute respectively. The correlation coefficient values in linearity were found to be 0.9999 for both at concentration range 2.509-50.190 µg mL^{-1} and 20.093 - 401.860 µg mL^{-1} respectively. The recovery results were found in the range from 99.47- 101.08%. The results of study showed that the proposed RP-UPLC method is a simple, accurate, precise, rugged, specific, robust, Ultra fast and reproducible, which may be useful for the routine estimation of Aspirin and Dipyridamole in pharmaceutical dosage form.

Keywords: Aspirin, Dipyridamole, RP-UPLC, Simultaneous estimation.

INTRODUCTION

Combination therapy of Aspirin and Dipyridamole is used for the treatment of stroke[3], Aspirin (2-acetoxybenzoic acid) (figure 1) is a cyclooxygenase enzyme inhibitor, widely used for the treatment of strokes[9]. It may be used alone or in combination with other antiplatelet agents. It inhibits the production of thromboxane by inhibiting the cyclooxygenase enzymes. Dipyridamole [2,2', 2'', 2'''-(4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidine-2,6-diyl) bis(azanetriyl) tetraethanol] (figure 2). It acts by inhibition of platelet cAMP-phosphodiesterase by potentiation of adenosine inhibition of platelet function by blocking reuptake of vascular and blood cells, and subsequent degradation of adenosine. Aspirin and Dipyridamole has a half-life of 3.1-3.2 and 10 hour, 40 min respectively. Both are individually official in IP and USP.

The literature survey shows that several methods have been reported for the simultaneous estimation of aspirin and Dipyridamole individually and in combination in biological fluids and in pharmaceutical dosage form by UV Spectrophotometer, Ultra HPLC[1], HPLC and with tandem mass[2,4,5,6,7,8]. However there is no method available for the simultaneous estimation of Aspirin and Dipyridamole in the capsule dosage form by RP-UPLC. Therefore, an attempt was made to develop a new, rapid and sensitive method for the simultaneous estimation of Aspirin and Dipyridamole. The developed method was validated[13,14,15,16] as per ICH guidelines.

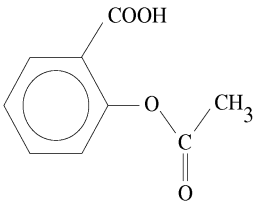


Fig. 1: Chemical structure of Aspirin

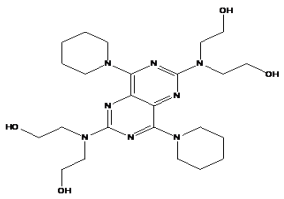


Fig. 2: Chemical structure of Dipyridamole

MATERIALS AND METHODS

Instrumentation

An Ultra High performance liquid chromatography (UPLC) system consisted of Waters Acquity with PDA detector and data-handling system Empower Pro and all pH measurements were performed on a pH meter (Metrohm, model 654 Herisau)

Reagents and chemicals

Aspirin and Dipyridamole were obtained as pure standards, samples (Capsules containing Aspirin and Dipyridamole in the ratio of 25mg; 200 mg respectively) from Wockhardt Research Center, Aurangabad, India. HPLC grade solvents, Methanol, Triethylamine, Orthophosphoric acid, Trifluoroacetic acid was from Merck-Specialties private Ltd., India. Water was deionised and further purified by means of a Milli-Q Plus water purification system, Millipore Ltd (U.S.A)

Chromatographic conditions and measurement procedure

The mobile phase consisted of a mixed and degassed solution of triethylamine phosphate buffer and methanol in the ratio of 50:50 (v/v). The buffer solution was prepared by adding 1 mL Triethyl amine in 1000 mL of water mixed and adjusted to pH 2.5 with orthophosphoric acid, filtered through 0.45µ or finer porosity

membrane filter. The peak separations were achieved on Hypersil Gold C18 (100 X 2.1 mm, 1.7µm) column. Maintained column oven temperature at 40^{0}C and detection with PDA detector at 230 nm, the injection volume was 1.0µl. flow rate of mobile phase was 0.5 mL min^{-1}.

Preparation of solutions

Preparation of diluent-1

2 ml of Trifluoroacetic acid was added in 1000 ml of Water and mixed well.

Preparation of diluent-2

The suitable Buffer pH 2.5 and Methanol was mixed in the ratio 50: 50 (v/v).

Preparation of standard solution

Accurately weighed 25 mg of Aspirin working standard and 200 mg of Dipyridamole working standard was added into 100 ml volumetric flask then about 70 ml of diluent-1 added and sonicated to dissolve and diluted to volume with diluent-1 and mixed well. Transferred 5 ml of standard stock solution into 50 ml volumetric flask and diluted to volume with diluent-2 and mixed well. (To get a final concentration of 25 µg mL^{-1} and 200 µg $mL^{-1}$of Aspirin and Dipyridamole respectively) Filtered through 0.45 µm membrane filter.

Preparation of sample

Average net content of 10 capsules was determined. Accurately transferred quantitatively the whole content of 10 sample capsules carefully (Equivalent to 250 mg of Aspirin and 2000 mg of Dipyridamole) in 250 ml volumetric flask and added about 200 ml of diluent-1 and sonicated for about 30 minutes with intermittent shaking volume made with diluent-1 and mixed well. Further diluted 5ml of this solution into 50 ml volumetric flask and diluted the volume with diluent-2 and mixed well. Further diluted 5 ml of this solution into 20 ml with diluent-2 and mixed well. Filtered through 0.45 µm nylon membrane filter by discarding first few mL filtrates.

RESULTS AND DISCUSIONS

Selection of wavelength maximum

Aspirin showed two absorbance maxima at 226.3nm (λ-1) and 275.3nm (λ-2) where as Dipyridamole showed two absorbance maxima at 231.2nm (λ-1) and 283.9nm (λ-2). Simultaneous estimation of both Aspirin and Dipyridamole a common absorption point was selected as wavelength maxima at 230 nm (**Fig. 3**)

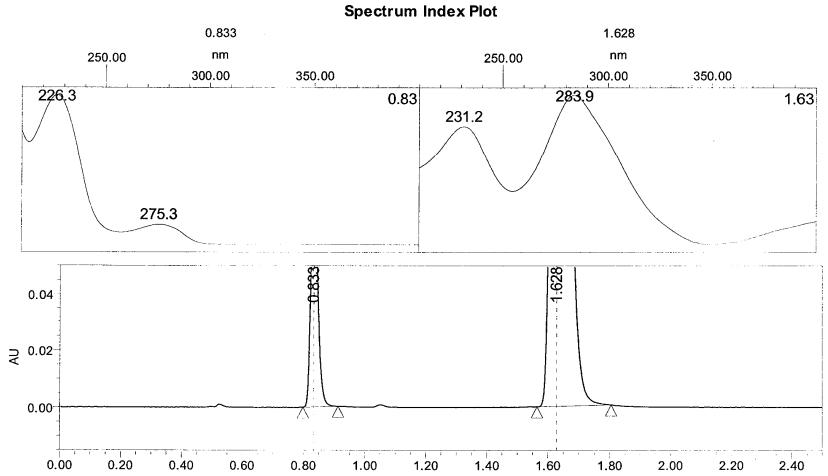
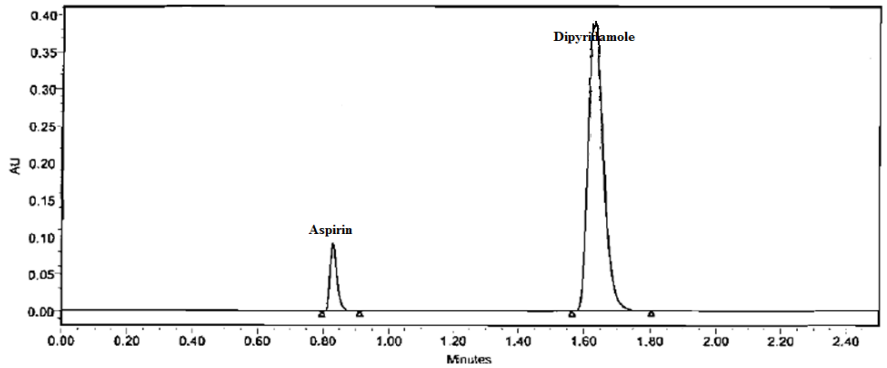


Fig. 3: UV Spectrum of Aspirin and Dipyridamole peaks



	RT	Area	USP Plate Count	USP Tailing	USP Resolution	K Prime	Purity1 Angle	Purity1 Threshold
1	0.833	136140	7340	1.30		1.23	0.14	1.12
2	1.628	1230213	5994	1.54	12.60	1.72	0.02	1.02

Fig. 4: Representative chromatogram of Aspirin and Dipyridamole

Method development

During method development[12,17] and optimization of chromatographic separation of major three component peaks were critical, two active ingredients aspirin and dipyridamole and salicylic acid a main degradation impurity of aspirin. pH of buffer was tried as acetate buffer 4.4 to phosphate buffer 2.5 in various combinations of methanol. Each trial mixture of known components were injected and observed resolution and tailing factor of peaks. Addtion of triethyl amine in buffer showed improved peak symmetry and resolution. Aspirin degraded rapidly in water: methanol and 2% aqueous acetic acid: methanol solutions. Both Aspirin and dipyridamole were found to be soluble and stable in a mixture of 2% aqueous trifluoro acetic solution and methanol. Dipyridamole is highly sensitive to mobile phase composition. Finally mobile phase composition was optimized to 50:50 v/v as it was found that both peaks were well resolved ,Resolution 12.60, USP tailing 1.30 and 1.54, K prime value 1.23 and 1.72 for Aspirin and Dipyridamole respectively. The RT of Aspirin was found to be 0.83 min. and for Dipyridamole 1.62 min. chromatogram shown in Fig. 4

System suitability

System suitability parameters such as no. theoretical plates, peak tailing and K prime value were determined. The results obtained are shown in Table-1.

Method validation

Specificity

Specificity of analytical assay method carried out by analyzing blank, placebo and sample solution spiked with known impurities at 1 % level in triplicate with two injections of each**.** The % assay difference is < 2 .The results were tabulated in Table no.2

It is evident from the above data that all the impurities are well resolved from each other and all the peaks are pure. Hence the method is specific[13,14,15,16.]

Table 1: Results of system suitability

Serial No	Parameters	Aspirin	Dipyridamole
1	No. of theoretical plates	7340	5994
2	Tailing factor	1.30	1.54
3	K prime	1.23	1.72

Table 2: Results of specificity

Specificity	Mean % assay	Aspirin	Dipyridamole
Control sample, Method precision (n-6)		100.27	98.57
Sample spiked with impurities (n-3)		101.54	98.03
% difference w.r.t. method precision		1.27	0.55

Table 3: Data of forced degradation

Sample Condition	Component Name	%Assay (w.r.t.Untreated)	% Difference	PDA Peak Purity Purity angle	Purity Threshold
Untreated sample	Aspirin	100.27	-	0.191	1.071
	Dipyridamole	98.57	-	0.191	1.071
Acid treated	Aspirin	83.72	16.51	0.300	1.100
	Dipyridamole	85.70	13.06	0.020	1.020
Alkali treated	Aspirin	84.07	16.16	0.140	1.110
	Dipyridamole	85.88	16.16	0.140	1.110
Peroxide treated	Aspirin	79.63	20.58	0.140	1.110
	Dipyridamole	95.24	3.38	0.020	1.020
Thermal exposed	Aspirin	83.42	16.80	0.120	1.100
	Dipyridamole	85.34	13.42	0.020	1.020
Photolytic	Aspirin	83.76	16.47	0.130	1.100
degradation	Dipyridamole	86.07	12.68	0.020	1.020

Forced degradation

For analytical methods for determination of assay specificity shall also be demonstrated by performing force degradation study[19] of placebo and drug product under various stress conditions like Acid degradation, Alkali degradation, Oxidative degradation, Photolytic degradation and Thermal degradation. When exposed to moisture, aspirin hydrolyzes into salicylic and acetic acids. The Data for Forced degradation are tabulated in Table no.3

There was no interference of any peak at the retention time of analyte peaks from blank and placebo observed, Peak purity of all forced degradation treated samples were passed, from this study it has been concluded that the proposed method is Specific and stability indicating[18,19] for the estimation of Aspirin and Dipyridamole in the capsule dosage form.

Linearity

The linearity of this method for assay determination was carried out by analyzing in the range from about 50 % to 150 % of test concentration. Peak responses of the components on Y-axis and the corresponding concentrations on X- axis were drawn and the correlation coefficient (r) estimated. The linearity study showed that the calibration curve for Aspirin and Dipyridamole was found to be linear with correlation coefficient (r^2) values 0.99998 and 0.99998 respectively. Linearity plot of Aspirin and Dipyridamole were shown in **Fig. 5** and **Fig .6** respectively.

Accuracy

The accuracy study of assay was performed with known amount of drug substance (API) was spiked in placebo at about 50 %, 100 % & 150 % of test concentration in triplicate at each level and was

injected each level in duplicate. Amount of drug recovered was quantified and % recovery was calculated from amount found and actual amount added. The accuracy study of this method for estimation of percent assay of Aspirin and Dipyridamole in capsule dosage was found to be in the range of 99.47 % - 101.08 % as shown in Table-4.

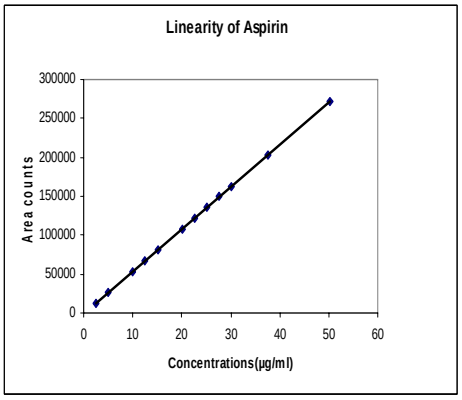


Fig. 5: Linearity plot of Aspirin

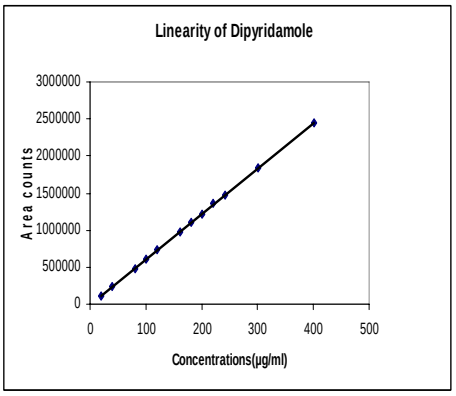


Fig. 6: Linearity plot of Dipyridamole

Table 4: Results of accuracy (recovery)

	Spiked level %, n=3)	Amount added (mg)	Amount recovered (mg)	Amount recovered (mg)
Aspirin	50	12.5	12.4	99.47
	100	25	24.9	99.60
	150	37.5	37.8	100.80
Dipyridamole	50	100	99.9	100
	100	200	199.5	99.75
	150	300	303.2	101.08

Precision

Reproducibility (Method precision) and intermediate precision

The method precision study showed that the results of percent assay in six different samples preparations of same sample were within limits (%RSD < 2) as shown in Table-5.

The Intermediate Precision study was performed within laboratory variation by different analysts, on different days, different instruments, and different column by using different standard and sample solution of the same sample as specified in method precision and the results were compared with method precision; The ruggedness study showed that it passes the limits (%RSD < 2) shown in Table-5.

Repeatability

Injecting six replicate injections of standard solution from the same vial performed the system precision study; the results found were within limits (%RSD < 2) shown in Table-6.

Table 5: Reproducibility (n-6), %Assay of six different samples of same batch

Method Precision (Set-1)		**Inter analyst (Set-2)**		**Ruggedness**		
Aspirin	**Dipyridamole**	**Aspirin**	**Dipyridamole**	**Over all, Set-1 and Set-2**		
102.11	98.15	100.47	100.41	**Aspirin**	**Mean**	99.98
100.33	98.07	100.86	100.81		**SD**	1.653
99.48	97.65	100.86	100.81		**% RSD**	1.65
98.99	99.23	100.47	100.41	**Dipyridamole**	**Mean**	98.97
100.41	98.72	98.94	100.81		**SD**	1.817
100.31	98.57	98.68	100.41		**% RSD**	1.84

Table 6: Results of precision

Sr. No.	**Validation parameter**	**% Mean Area***		**S.D.**		**% RSD**	
		Aspirin	**Dipyridamole**	**Aspirin**	**Dipyridamole**	**Aspirin**	**Dipyridamole**
1.	Repeatability	136202	1231611	293	1515.2	0.21	0.12
2.	Intermediate precision	136241	1208183	1445.6	9553.2	1.06	0.79
3.	Intermediate precision (by different analyst)	135743	1207803	1117.4	2685.5	0.82	0.22

*Mean of three different samples of same batch injected in duplicate

Robustness

As per ICH guidelines small but deliberate changes have been made in parameters. The Robustness study for proposed analytical method for the determination of assay was performed and checked by preparing sample solutions in triplicate as per test method and was injected in duplicate by varying the organic phase/least component composition by ± 2 % (absolute) or 10 % relative which is lower, pH of the mobile phase by ± 0.1 unit, column oven temperature by ± 5 %, flow rate by ± 10 % and wavelength of the detector by ± 5 nm, the results showed that the percent assay of Aspirin and Dipyridamole was not more than 2% as compared to method precision results, hence the developed method was found to be robust.

Stability of sample solution

The sample solution was stable up to 37 hr. 48 min. at 5^0C temperature and did not show any appreciable change in sample area.

CONCLUSION

This intended study can be concluded as: the proposed method is economical, simple, ultra fast, sensitive and reliable and is found to be more accurate, precise, specific, stability indicating, rugged and robust hence it can be employed for routine estimation of capsules containing Aspirin and Dipyridamole.

Conventional reported HPLC methods may be replaced by the proposed UPLC method because of it's superiority in cost effectiveness, Savings of analysis time per sample and better detection. For faster samples testing routinely in QC lab the validated method may be used.

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REFERENCES

1. D. N. Vora; A. A. Kadav, "Validated Ultra HPLC Method for the Simultaneous Determination of Atorvastatin, Aspirin, and their Degradation Products in Capsules," Journal of Liquid Chromatography & Related Technologies, Volume 31, Issue 18 January 2008 , pages 2821 - 2837.
2. Na Wang, Fengguo Xu, Zunjian Zhang, Cheng Yang, Xiuhong Sun, Jinheng Li, "Simultaneous determination of dipyridamole and salicylic acid in human plasma by high performance liquid chromatography-mass spectrometry," Journal of Biomedical Chromatography, Volume 22 Issue 2, 12 Sep 2007, Pages 149 – 156.
3. Weale, A. R., Cooper, D. G., Hentschel, D. M., Dixon, B. S., Feldman, H. I. "Effect of Dipyridamole plus Aspirin on Hemodialysis Graft Patency." The New England Journal of Medicine, Volume 360:2191-2201, May 21, 2009, page no.720-721.
4. Ting Qin, Feng Qin, Ning Li, Shan Lu, Wei Liu, Famei Li, "Quantitative determination of dipyridamole in human plasma by high-performance liquid chromatography-tandem mass spectrometry and its application to a pharmacokinetic study," Journal of Biomedical Chromatography, 5 May 2009, page no. 1283.
5. Maria J. Nozal, Jose L. Bernal, Laura Toribio, Juan J. Jimenez, Maria T. Martin, " Validation of the removal of acetylsalicylic acid, Recovery and determination of residues on various surfaces by high performance liquid chromatographic," Journal of Chromatography A, 870 (2000) pages 69 - 75.
6. M. Gandhimathi, T.K. Ravi, Annie Abraham, Renu Thomas, "Simultaneous determination of aspirin and isosorbide 5-mononitrate in formulation by reversed phase high pressure liquid chromatography, "Journal of Pharmaceutical and Biomedical Analysis", 32 (2003) pages 1145 -/1148.
7. Emmanuelle Belougne-Malfatti, Omar Aguejouf, "Combination of Two Doses of Acetyl Salicylic Acid: Experimental Study of Arterial Thrombosis", Thrombosis Research 90 (1998) 215–221, Laboratoire d'He´ matologie, Faculte´ de Pharmacie, 146, rue Le´o Saignat, 33076 Bordeaux Cedex, France.
8. Angel A.J. Torriero, Juan M. Lucoa, Leonides Sereno, Julio Rabaa, "Voltammetric determination of salicylic acid in pharmaceuticals formulations of acetylsalicylic acid", Journal of Chromatography A, Talanta 62 (2004) 247–254.
9. Lewis, H D; J W Davis, D G Archibald, W E Steinke, T C Smitherman, (1983-08-18). "Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. Results of a Veterans Administration Cooperative Study". The New England journal of medicine 309 (7): 396–403
10. Monika Bakshi, Saranjit Singh, "Development of validated stability-indicating assay methods-critical review", Journal of Pharmaceutical and Biomedical Analysis 28 (2002) 1011–1040
11. Mei Chena, Camille Granvil, Qin C. Ji, Zhi-Yi Zhanga, Mahesh V. Padval a, Vikram V. Kansraa, "Development and validation of a liquid chromatography–tandem mass spectrometry assay for the simultaneous quantitation of prednisolone and dipyridamole in human plasma and its application in a pharmacokinetic study", Journal of Pharmaceutical and Biomedical Analysis.
12. Szabolcs Feketea, Jeno˝ Fekete, Imre Molnar, Katalin Ganzler, "Rapid high performance liquid chromatography method development with high prediction accuracy, using 5 cm long narrow bore columns packed with sub-2_m particles" , Journal of Chromatography A. "An Introduction to Chromatography," (1994).
13. Guidelines for Submitting Samples and Analytical Data for Methods Validation U.S.FDA. (Center for Drug Evaluation and Research) Feb.1987, U.S.G.P. O: 1990, 281- 794, 20818.
14. USP 32, NF 27, (2009), The United State Pharmacopoeia, The National Formulary US pharmacopoeial Convention Inde., Rockyvilley.
15. ICH (International Conference on Harmonization) –Guidelines Q2A, Validation of Analytical Procedures: Definition and terminology (CPMP III/5626/94) March (1995) Geneva, Switzerland
16. ICH (International Conference on Harmonization) –Guidelines Q2B, Validation of Analytical Procedures: Methodology (CPMP/ICH/281/95) November (1996) Geneva, Switzerland.
17. Lloyd R. Snyder, "Practical HPLC Method Development", 2nd ed. A-Wiley- Inter Science publication; 1997.
18. International Conference on Harmonization Stability testing of new drug substances and products Q1A(R2)
19. Bakshi M, Singh S. Development of validated stability-indicating assay methods critical review. J Pharm Biomed Anal. 2002;28:1011-1040.