



RP-HPLC Method for Simultaneous Estimation of Aspirin and Prasugrel in Binary Combination

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ABSTRACT

A simple, reliable, rapid, precise, sensitive and validated RP-HPLC method has been developed to determine Aspirin and Prasugrel in synthetic mixture form. Chromatographic separation achieved isocratically on Luna C₁₈ column (5 μ m, 150mm $\times$ 4.60mm) and acetonitrile: 0.05M ammonium acetate buffer (pH 4.5) in the ratio of 75:25 (v/v) as the mobile phase, at a flow rate of 0.6 ml/min. Detection was carried out at 245 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention times for Aspirin and Prasugrel was found to be 2.25 $\pm$ 0.5 and 8.72 $\pm$ 0.5 min respectively. Linearity for Aspirin and Prasugrel was in the range of 75-375 μ g/ml and 10-50 μ g/ml respectively. The mean recoveries obtained for Aspirin and Prasugrel were 99.58 and 99.48 % respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Aspirin and Prasugrel.

Keywords: Aspirin, Prasugrel, RP-HPLC, Validation.

INTRODUCTION

Aspirin (ASP), 2-acetoxy benzoic acid [Figure 1A] is cyclooxygenase inhibitor. It is used as an analgesic, antipyretic, anti-inflammatory and anti thrombotic agent. [1] It is one of the most widely used anionic drugs in the world. Once ingested, aspirin is rapidly hydrolyzed in the body to produce salicylic acid, which is the compound that is primarily responsible for the pharmacological activity of aspirin. [2] Aspirin is official in IP [3], BP [4] and USP. [5] Several methods have been employed for the estimation of aspirin alone and combination with other drugs such as UV, [6-7] RP-HPLC, [8-19] HPTLC [20-21] spectrofluorometric, [22] spectrometric method. [23-24] A number of acute coronary syndrome (ACS) trials have demonstrated significant regional variation in clinical outcomes and treatment effects. [25-27] Dual anti-platelet therapy with aspirin and a thienopyridine is a cornerstone of treatment to prevent thrombotic coronary intervention (PCI). [28-29] In the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-thrombolysis in myocardial infarction 38 (TRITON-TIMI 38), more intensive and consistent anti-

platelet therapy with the third-generation thienopyridine prasugrel resulted in a reduction in ischemic events, increase in bleeding and on balance, an improved net clinical outcome. [30] Prasugrel (PRA) chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4, 5, 6, 7-tetrahydrothieno [3, 2- c] pyridin-2-yl acetate [Figure 1B]. It is a member of the thienopyridine class of ADP receptor inhibitors, like ticlopidine and clopidogrel. These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to P2Y₁₂ receptors. Prasugrel inhibits adenosine diphosphate-induced platelet aggregation more rapidly, more consistently and to a greater extent than do standard and higher doses of clopidogrel in healthy volunteers and in patients with coronary artery disease. Literature survey revealed that only a few analytical methods such as UV, [31-32] HPLC, [33-34] LC-MS, [35-37] HPTLC [38] have been reported.

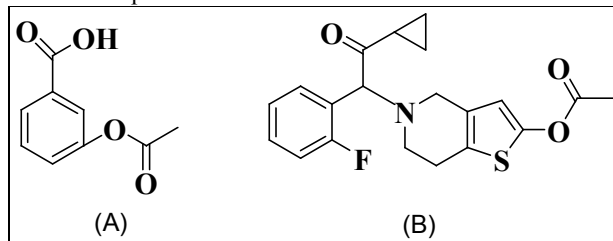


Fig. 1: Chemical structures of (A) Aspirin (B) Prasugrel

According to the information collected from literature there is no reported method for simultaneous determination of ASP

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and PRA. In the present work we are therefore focused on to achieve the optimum chromatographic conditions for the simultaneous determination of ASP and PRA in a synthetic mixture. We describe in this paper a simple, sensitive and validated HPLC method with total run time less than 9 minutes for the simultaneous determination of ASP and PRA. The developed method can be applied successfully to quality control and for other analytical purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [39-40], which is mandatory also.

MATERIALS AND METHODS

Instrumentation

Liquid chromatographic system from Shimadzu (LC-20AT) comprising of manual injector, double reciprocating plunger pump LC-20ATVp for constant flow and constant pressure delivery and Photodiode array detector SPD-M20A connected to software LC solution for controlling the instrumentation as well as processing the data generated was used.

Chemicals and reagents

Analytically pure sample of ASP was a generous gift from Lupin Laboratories Mumbai, India and PRA was an obtained from Aurbindo Pharma Ltd., Hyderabad. Ammonium acetate, glacial acetic acid and acetonitrile (HPLC Grade) was purchased from E. Merck Ltd. Worli, Mumbai, India. The 0.45µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. All excepients used were of pharmaceutical grade. Triple distilled water was generated in house.

Chromatographic conditions

The isocratic mobile phase consisted of acetonitrile: 0.05M ammonium acetate buffer (pH 4.5) in the ratio of 75:25 (v/v), flowing through the column at a constant flow rate of 0.6 ml/min. A Luna C₁₈ column (5 µm, 150mm × 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 245nm was selected as the detection wavelength for UV-PDA detector. The HPLC system was operated at room temperature 25°C.

Standard preparation

Standard stock solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of acetonitrile and ammonium acetate buffer in the ratio of 50:50 (pH 4.5) to get concentration of 1000µg/ml.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 75-375, 10-50µg/ml for ASP and PRA respectively.

Sample preparation

The content of twenty tablets where taken and weighed. Powdered equivalent to 300mg aspirin and 10 mg prasugrel was dissolved in 100ml diluents and then sonicated for 15 min. and filtered through watmann paper no. 41. Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analysed.

RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A

mobile phase consisting of acetonitrile: 0.05M ammonium acetate buffer (75:25, v/v, pH 4.5) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 ml/ml were studied. A flow rate of 0.6 ml/ml gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed-phase C₁₈ column, the retention times for ASP and PRA were observed to be 2.25±0.5 and 8.72±0.5 min respectively. Total time of analysis was less than 9 min. The maximum absorption of ASP and PRA together as detected at 245 nm and this wavelength was chosen for the analysis Fig. 2.

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for ASP and PRA were 1543 and 9261 respectively.

Linearity

The calibration curve was linear over the concentration range of 75-375µg/ml for ASP and 10-50µg/ml for PRA.

Table 1: System suitability parameters

Parameter	Aspirin	Prasugrel
Retention time*	2.25 ± 0.5	8.72 ± 0.5
No. of theoretical plate*	1543.26±26.29	9261.33±16.72
Tailing factor*	0.89±0.01	0.98±0.01
HETP*	0.16±0.01	0.027±0.02
Calibration Range	75-375 µg/ml	10-50 µg/ml

* Each value is the Mean ± S.D of six determinations

Table 2: Result of recovery studies with statically evaluation

S. No	Conc. of drug in preanalyzed samples (µg/ml)		Std. drug sol. Added (µg/ml)		Recovered amount* (µg/ml)		% Recovered	
	ASP	PRA	ASP	PRA	ASP	PRA	ASP	PRA
1	150	20	120	16	266.78	36.03	98.80	100.08
2	150	20	150	20	300.05	39.45	100.01	98.62
3	150	20	180	24	329.85	43.89	99.95	99.75
						Mean	99.58	99.48
						S.D	0.063	0.467
						%R.S.D	0.063	0.469

* Mean of Nine determinations (3 replicates at 3 concentration level)

Table 3: Result of precision and robustness

Validation Parameter	Percentage Mean ± S.D*(n=15)		Percentage RSD*	
	ASP	PRA	ASP	PRA
Repeatability	98.23±1.12	99.02±1.41	98.74±1.46	98.63±1.25
Intermediate precision				
Day to Day	97.96±0.21	98.34±0.55	98.01±1.68	97.67±1.29
Analyst to Analyst	98.29±0.56	98.75±0.53	99.07±1.06	98.43±1.01
Reproducibility	99.03±0.42	99.31±0.24	98.72±0.23	99.08±1.06
Robustness	98.61±0.06	99.46±0.87	98.22±0.27	98.75±1.08

Table 4: Stability data of ASP and PRA

Hours	ASP	PRA
0	746489	239584
6	748652	243565
12	751234	246854

Table 5: Statistical evaluation of synthetic mixture analysis

Parameter	Sample	
	ASP	PRA
Mean % estimated	98.61	98.1
Standard deviation(S.D.)	0.45	0.12
% Coefficient of variation	0.456	0.122
*Standard error (SEσ)	0.106	0.028

* Mean of Nine determinations (3 replicates at 3 concentration level)

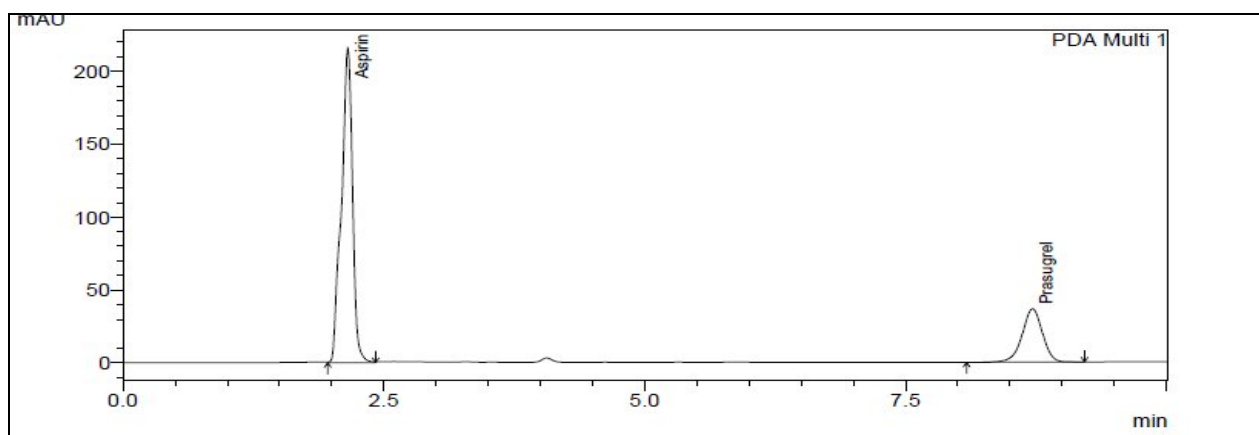


Fig. 2: Chromatograms of ASP (150µg/ml) and PRA (20µg/ml) reference substance

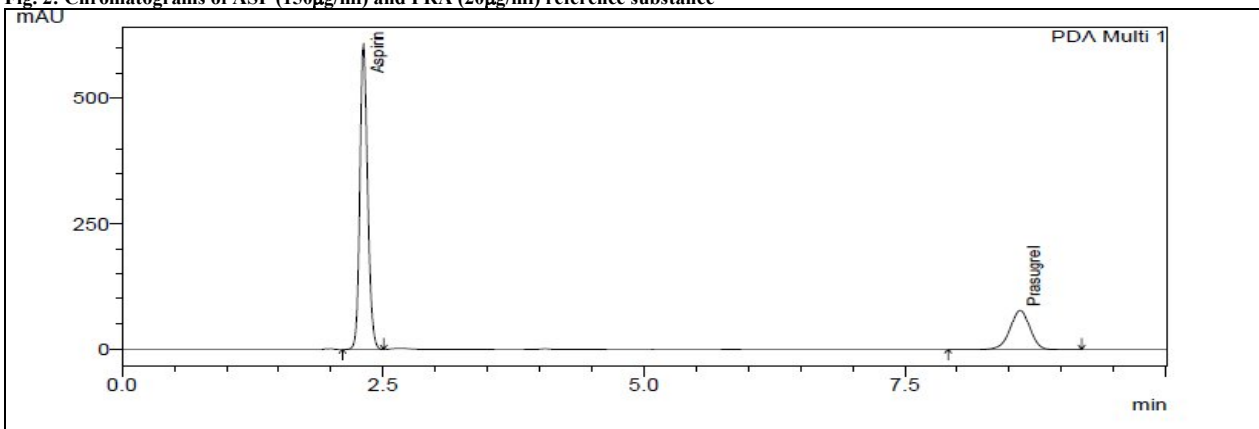


Fig. 3: Chromatograms of ASP (300µg/ml) and PRA (40µg/ml) in a synthetic mixture

The linearity was represented by a linear regression equation as follows.

$$Y (\text{ASP}) = 11420.56 \text{conc.} - 65319.14 \quad (r^2=0.998)$$

$$Y (\text{PRA}) = 28537.15 \text{conc.} - 11811.28 \quad (r^2=0.999)$$

Where Y is area under curve and r^2 is correlation coefficient.

Accuracy

Method accuracy was performed by adding known amounts of ASP and PRA to the pre-analysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80, 100 and 120 % of the nominal analytical concentration (150µg/ml for ASP and 20µg/ml for PRA). Each level was made in triplicate Table 2. The mean percentage recoveries obtained for ASP and PRA were 99.58 and 99.48 % respectively and RSD was less than 2.

Repeatability

Five dilutions in three replicates were analyzed in same day for repeatability and results were found within acceptable limits ($\text{RSD} < 2$) as shown in Table 3.

Intermediate Precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits ($\text{RSD} < 2$) as shown in Table 3.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of acetonitrile: phosphate buffer (pH 4.5) (75:25v/v), acetonitrile: phosphate buffer (pH 4.5) (70:30 v/v), was used

as a mobile phase. Results of analysis were summarized in Table 3.

Stability of sample solution

The sample solution injected after 12 h do not show any appreciable change. Results are shown in Table 4.

Specificity & selectivity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate, lactose,) were spiked in to a pre weighed quantity of drugs .The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 3. Where complete separation of aspirin and prasugrel in presence of tablet excipients.

Synthetic mixture analysis

Content of ASP and PRA found in the synthetic mixture by the proposed method are shown in Table 5. The low values of R.S.D. indicate that the method is precise and accurate.

A simple precise, reliable, rapid, sensitive and accurate reverse phase HPLC method has been developed for the simultaneous determination of aspirin and prasugrel. The developed method is suitable for the identification and quantification of binary combination of aspirin and prasugrel. A high percentage of recovery and the run time of less than nine minutes allow its application for the routine determination of aspirin and prasugrel in tablet dosage form.

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