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Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Prasugrel and Aspirin in Tablet Dosage Form

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ABSTRACT

The present research work discusses the two new, simple, accurate, precise and reproducible UV spectrophotometric methods have been developed and validated for the simultaneous determination of Prasugrel (PRASU) and Aspirin (ASP) in their combined dosage form. Method- I is based on simultaneous equation method using two wavelengths, 254 nm (λmax of PRASU) and 276 nm (λmax of ASP). Method - II Q-absorption ratio method using two wavelengths, 274.7 nm (Isoabsorptive point) and 254 nm (λmax of PRASU). Methanol was the solvent used in all methods. This method obeyed Beer's law in the concentration range of 5-60 µg /ml for PRASU and 20-140 µg/ml ASP. All methods were validated statistically and recovery studies were carried out. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage form.

Keywords: Prasugrel, Aspirin, Simultaneous equation method, Q-absorbance ratio method, Validation

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INTRODUCTION

Prasugrel(PRSU),[(RS)-5-[2- cyclopropyl-1- (2-fluorophenyl)-2- oxoethyl] -4,5,6,7- tetrahydro thieno [3,2-c] pyridin-2-yl acetate] (figure. 1), is an thienopyridine which inhibits ADP receptors by irreversibly acting on the P2Y12 receptor on platelets which is not official in any pharmacopoeia and Aspirin (ASP), Acetylsalicylic acid (figure 2), is a anti-inflammatory and antiplatelet which is official in many pharmacopoeia which recommends a titrimetric method[1,2] and HPLC[3] for its analysis The combination formulation is used for the treatment of coronary heart disease. The chemical structures of PRASU and ASP are shown in Figure.1 and 2.

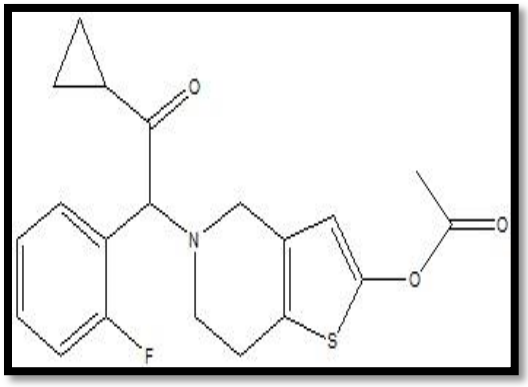

Figure. 1: Structure of Prasugrel (PRASU)

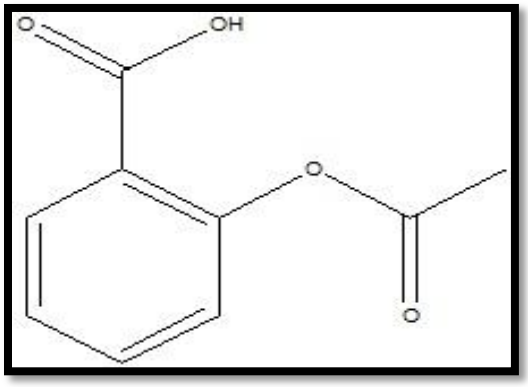

Figure. 2: Structure of Aspirin (ASP)

Literature survey reveals that many analytical methods are reported for determination of PRASU[4-16] and ASP[17-18] individually and aspirin with other antiplatelet drug[19-20]. According to detailed survey of analytical literature none of the reported analytical procedures describes a simple and satisfactory UV spectrophotometric method for simultaneous determination of PRASU and ASP in their combined dosage form. So the objective of this work was to develop simple, precise and rapid spectrophotometric methods for combination drug products containing PRASU and ASP.

MATERIALS AND METHODS

Instrumentation

A Shimadzu model 1700(Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31). An Electronic analytical balance (Acculab) and an ultrasonic bath were used in the study.

Materials and reagents

PRASU and ASP bulk powder was gifted by Zydus Cadila Health Care Pvt. Ltd., Ahmedabad, India and Enaltec lab Pvt. Ltd., Mumbai, India respectively. Methanol AR Grade was procured from S.D. Fine Chemicals Ltd., Mumbai, India.

Preparation of standard solution:

An accurately weighed quantity of PRASU (10 mg) and ASP (100 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with Methanol to obtain standard solution having concentration of PRASU (100 µg/ml) and ASP (1000 µg/ml).

Preparation of test solution:

Take tablet powder equivalent to PRASU(10 mg) and ASP(75 mg) was transferred in 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was sonicated for 15 minutes. Filter the solution through Whatman filter paper no.42 and discard first few drops of filtrate. Pipette out 1ml of the above solution in 10ml volumetric flask and diluted to mark with methanol to obtain standard solution having concentration of PRASU(10µg/ml) and ASP(75µg/ml).

Selection of wavelength

Simultaneous Equation Method:

In this method, working standard solutions having concentration 5-60 µg/ml for PRASU and 20-140 µg/ml for ASP were prepared in methanol. The concentration of two drugs in the mixture can be calculated using following equations

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \quad \text{..... (1)}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \quad \text{..... (2)}$$

Where, A_1 and A_2 are absorbance of mixture at 254 nm (λ_1) and 276 nm (λ_2) respectively, ax_1 and ax_2 are absorptivities of PRASU at λ_1 and λ_2 respectively, ay_1 and ay_2 are absorptivities of ASP at λ_1 and λ_2 respectively, Cx and Cy are concentrations of PRASU and ASP respectively.

Q-Absorption Ratio Method:

This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbance at any two wavelengths are a constant value, independent of concentration or pathlength[21-24]. The concentration of individual components, PRASU and ASP may be calculated using the following equations

$$C_x = \frac{Qm - Qy}{Qx - Qy} \times \frac{A1}{ax1} \quad \text{..... (3)}$$

$$C_y = \frac{A1}{ay1} - C_x \quad \text{.....(4)}$$

Where,

$$Q_m = \frac{\text{Absorbance of sample at 274.7 nm}}{\text{Absorbance of sample at 254 nm}}$$

$$Q_x = \frac{\text{Absorptivity of PRASU at 274.7 nm}}{\text{Absorptivity of PRASU at 254 nm}}$$

$$Q_y = \frac{\text{Absorptivity of ASP at 274.7 nm}}{\text{Absorptivity of ASP at 254 nm}}$$

A_1= Absorbance of sample at isoabsorptive point

ax_1 and ay_1 are absorptivitities of PRASU and ASP at isoabsorptive point respectively.

Q_x and Q_y are Q value of PRASU and ASP respectively.

Cx is concentration of PRASU, Cy is concentration of ASP.

Method validation

All the methods were validated as per ICH guidelines for parameters like linearity, accuracy, precision, limit of detection, limit of quantitation[25].

❖ **Linearity (Calibration Curve for Prasugrel and Aspirin)**

Calibration Curve of PRASU

The linearity response was determined by analyzing 7 independent levels of calibration curve in the range of 5-60 µg/ml. The solutions were prepared by pipette out 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of the standard solution of Prasugrel (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. Absorbance of each solution against methanol was measured at 254 nm. The calibration curve of absorbance vs. concentration was plotted and correlation co-efficient and regression line equation for Prasugrel were determined.

Calibration Curve of ASP

The linearity response was determined by analyzing 7 independent pipette levels of calibration curve in the range of 20-140 µg/ml. The solutions were prepared by pipette out 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml of the standard solution of Aspirin (1000 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. Absorbance of each solution against methanol was measured at 276 nm. The calibration curve of absorbance vs. concentration was plotted and correlation co-efficient and regression line equation for Aspirin were determined.

❖ **Precision**

➢ **Repeatability**

Test solution analyzed six times and %RSD was calculated.

- **Intra-Day Precision**

  Intra-Day Precision was determined by analyzing 4, 10 and 16 µg/ml Prasugrel with 30, 75, 120 µg/ml Aspirin for three times in the same day and %RSD was calculated.

- **Inter-Day Precision**

  Inter-Day Precision was determined by analyzing 4, 10 and 16 µg/ml Prasugrel with 30, 75, 120 µg/ml Aspirin for three days and %RSD was calculated.

❖ **Accuracy**

The accuracy of the method was determined by calculating recoveries of PRASU and ASP by the standard addition method. Accuracy is performed at three levels 20, 40 and 60%. Known amount of standard stock solutions of PRASU (2, 4 and 6 µg/ml) and ASP (15, 30 and 45µg/ml) were added to a pre-quantified test solution of PRASU (10 µg/mL) and ASP (75 µg/mL). The absorbance of PRASU and ASP were recorded at 274.7 nm, 254 nm and 276 nm and the percentage recovery was calculated.

❖ **Limit of Detection (**LOD**) and Limit of Quantitation (**LOQ**)**

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity.

LOD= 3.3(σ/S) and

LOQ= 10(σ/S)

Where, σ = the standard deviation of y-intercepts of regression lines

S = the slope of the calibration curve

RESULTS AND DISCUSSION

In the present work, Two methods, namely, Simultaneous equation method and Q-absorption ratio method were developed for the simultaneous spectroscopic estimation of PRASU and ASP in commercially available tablet dosage form. Methanol was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of the tablet were observed.

Selection of Wavelength

By appropriate dilution of two standard drug solutions with methanol, solutions containing 40 µg/ ml of PRASU and 40 µg/ ml of ASP were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs PRASU and ASP showed absorbance maxima at 254 nm and 276 nm respectively. So for simultaneous equation method λ_1 254 nm (λ-max of PRASU) and λ_2 276 nm (λ-max of ASP) and for Q absorbance ratio method

λ1 274.7 nm which is isoabsorptive points of PRASU and ASP and λ2 254 nm which is λmax of PRASU were selected. Overlay Spectra of PRASU and ASP was shown in figure. 3.

Method validation

The developed methods were validated for parameters like linearity, precision, accuracy, LOD, LOQ. The data for which are presented in the fig 4, 5 and tables 1 - 5. The low value of R.S.D. value indicates that all the methods are precise and accurate.

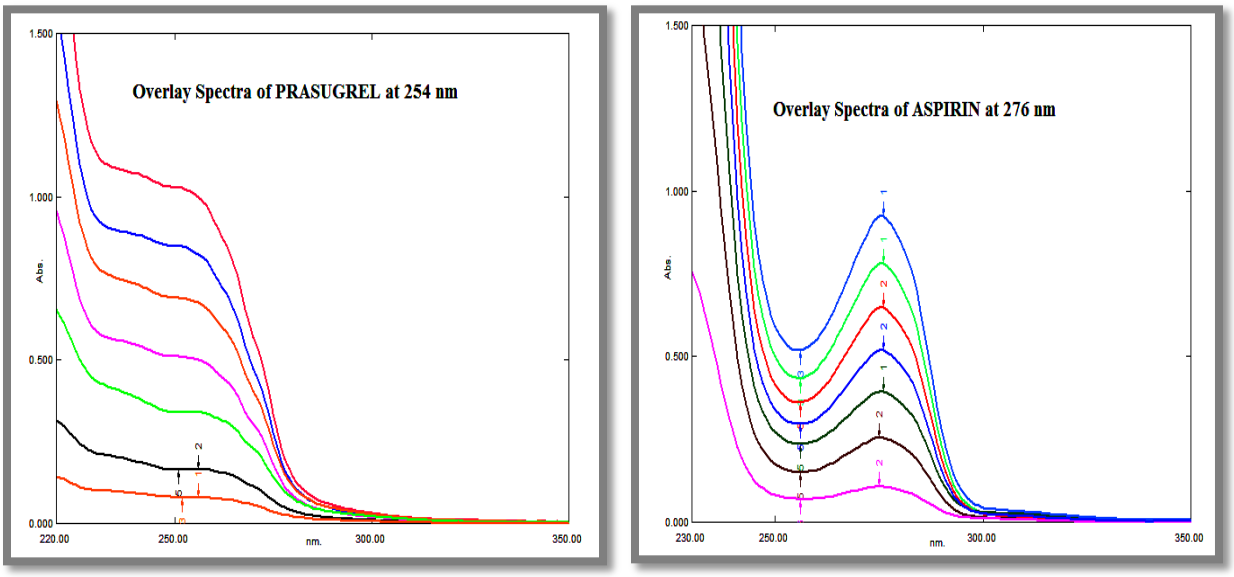

Figure 3 & 4: Overlay Linearity Spectra of Prasugrel and Aspirin

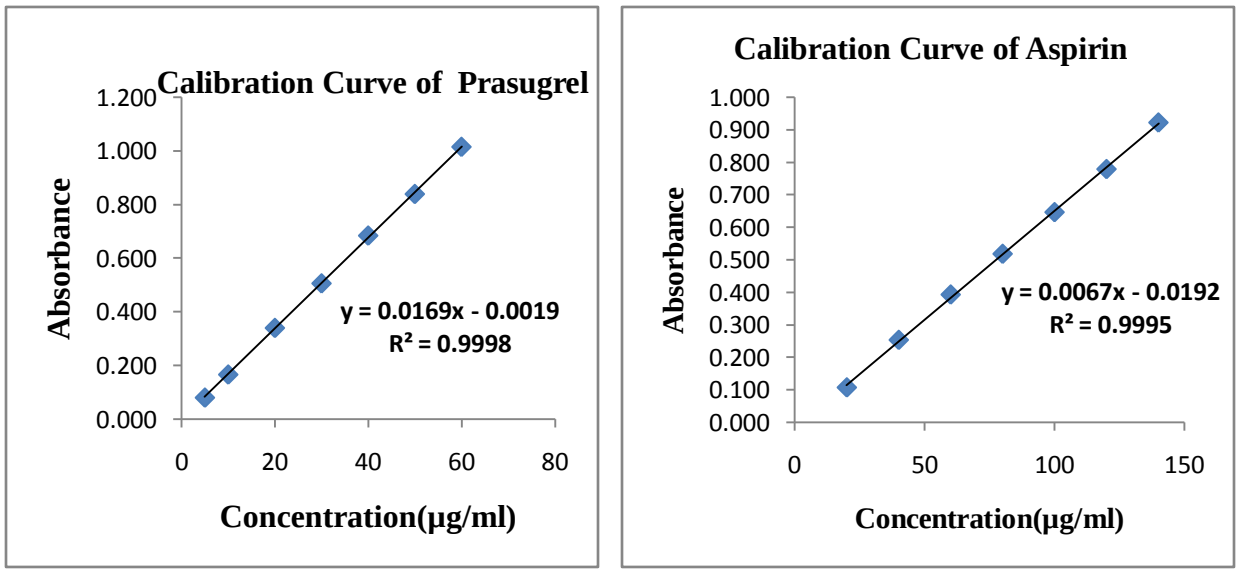

Figure. 5: Calibration Curve of Prasugrel and Aspirin

Table 1: Data showing linearity of the developed methods

Methods	Simultaneous equation method		Q-Absorption ratio method	
Parameters	**PRASU**	**ASP**	**PRASU**	**ASP**
Linearity Range	5-60 µg/ml	20-140 µg/ml	5-60 µg/ml	20-140 µg/ml
Slope	0.0169	0.0067	0.0169	0.0067
Intercept	0.0019	0.0192	0.0019	0.0192
R^2 value	0.9998	0.9995	0.9998	0.9995

Table 2: Data showing Accuracy of the developed methods

DRUG	Amt. taken (µg/ml)	Amt. added (µg/ml)	% Amt. added	% mean recovery(± S.D.) n=3	
				Simultaneous equation method	Q-Absorption ratio method
PRASU	10	2	20 %	100.93±0.87	99.78±0.79
	10	4	40 %	100.79±0.98	100.36±0.83
	10	6	60 %	99.16±1.09	99.54±0.61
ASP	75	15	20 %	99.04±0.50	100.07±1.55
	75	30	40 %	98.53±0.58	98.25±0.96
	75	45	60 %	98.31±0.63	98.24±0.32

(n = number of repetition)

Table 3: Data showing Precision of the developed methods

Methods		Simultaneous equation method		Q-Absorption ratio method	
		PRASU	**ASP**	**PRASU**	**ASP**
Precision (%RSD)	Repeatability,(n=6)	0.83%	0.25%	0.95%	0.33%
	Intraday,(n=3)	0.24-0.55%	0.31-0.47%	0.28-0.97%	0.37-0.85%
	Interday,(n=3)	0.29-0.84%	0.47-0.98%	0.41-1.41%	0.56-1.15%

(n = number of repetition)

Table 4: Data showing LOD and LOQ of the developed methods

Methods	Simultaneous equation method		Q-Absorption ratio method	
	PRASU	**ASP**	**PRASU**	**ASP**
LOD(µg/ml)	0.40	0.92	0.37	0.93
LOQ(µg/ml)	1.21	2.79	1.11	2.83

Table 5: Result of Analysis of formulation

Methods	Simultaneous equation method		Q-Absorption ratio method	
	PRASU	**ASP**	**PRASU**	**ASP**
%Assay ± SD (n=6)	93.87± 0.78	97.55±0.25	92.55± 0.88	98.63±0.32

(n = number of repetition)

CONCLUSION

The developed spectroscopic methods are found to be simple, accurate and precise and can be used for routine analysis of PRASU and ASP. The developed methods were validated as par ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis

of PRASU and ASP in combination as a single drug in bulk as well as in pharmaceutical formulation.

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