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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF PANTOPRAZOLE AND ASPIRIN USING REVERSE PHASE HPLC METHOD IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

RP-HPLC method was developed for the determination of Pantoprazole & Aspirin (ASP) in bulk and dosage form. Mobile phase use for the separation of Panto & ASP is methanol and 0.05%OPA in water (pH= 3.5) with ratio of 60:40. The Colum used as C18 (Cosmosil) 4.6×150mm and flow rate 0.7mL/min. UV detector is used and the detection wavelength is 231nm. Retention time of Panto and ASP are 4.61 & 8.03 min, respectively. This method was validated as per ICH guidelines. Linearity was observed at 10-50µg/mL of Panto and 20-100µg/mL of ASP. The % RSD is found to be less than 2%. The resolution between Panto and ASP is 11.55 and the tailing factors of both are less than 2.0. Theoretical plates for Panto and ASP are 5060, and 9367, respectively. Total run time is 15min. The developed RP- HPLC method was accurate, precise, selective and rapid for simultaneous estimation of Pantoprazole and Aspirin in the pharmaceutical dosage form."

KEYWORD: Pantoprazole, Aspirin, RP-HPLC validation.

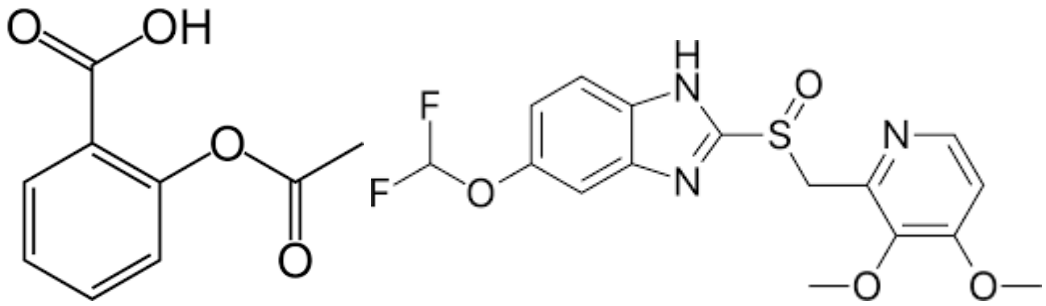
INTRODUCTION

Aspirine (ASP) (Fig. 1a), chemically 2-acetoxy benzoic acid is a cyclo oxygenase inhibitor which is used as an analgesic, antipyretic, anti-inflammatory and anti thrombic agent.[1] It is one of the most commonly used anionic drugs in the world.[2] After ingestion, it rapidly hydrolyzes to salicylic acid which is primarily responsible for its pharmacological action.[3] It is included in the official monograph of both USP-NF[4] and BP.[5] US Food and Drug

administration has approved this drug for use in secondary prevention of heart attacks and stroke due to its anti-platelet activity.[4-5]

Pantoprazole Sodium is chemically sodium 5- (difluoro methoxy)-2- [(3, 4- dimethoxy-2pyridyl) Methyl] sulphinyl] 1H- benzimidazole sesquihydrate which has been widely used in the treatment of peptic ulcer. Pantoprazole is an irreversible proton pump inhibitor which, at the therapeutic dose of 40mg, effectively reduces gastric acid secretion. Pantoprazole causes irreversible inhibition of proton pump (H+, K+-ATPase) function. It is chemically more stable than omeprazole or lansoprazole under neutral to mildly acidic conditions, but is rapidly activated under strongly acidic conditions. This pH-dependent activation profile underlies the improved in vitro selectivity of pantoprazole against parietal H+, K+-ATPase compared with omeprazole.[6]

Literature survey revealed several assay methods for ASP and PANTO individually or in combination with other drugs by UV spectrophotometry[7-9], High performance liquid chromatography (HPLC)[10], RP HPLC-UV[11], Reverse phase -Ultra fast liquid chromatography (RP-UFLC)[12-13], Gas Chromatography-Mass Spectrometry (GC-MS)[14] and LC-MS/MS.[15] However, every method has its own limitation. Thereby, an attempt has been taken to develop and validate a simple, economic, reliable, reproducible, accurate and precise RP-HPLC based method for simultaneous estimation of ASP and PANTO in combined tablet dosage form where validation of the analytical method has been performed in accordance with ICH guideline.[16]



(A) **(B)**

Figure 1: Chemical Structures of (a) Aspirine (b) Pantoprazole.

MATERIALS AND METHODS

Chemicals and reagents: Aspirin (AR grade), Pantoprazole (AR grade), HPLC grade methanol purchase from Merck ltd, Mumbai. Ortho phosparic acid water - 0.1mL OPA in

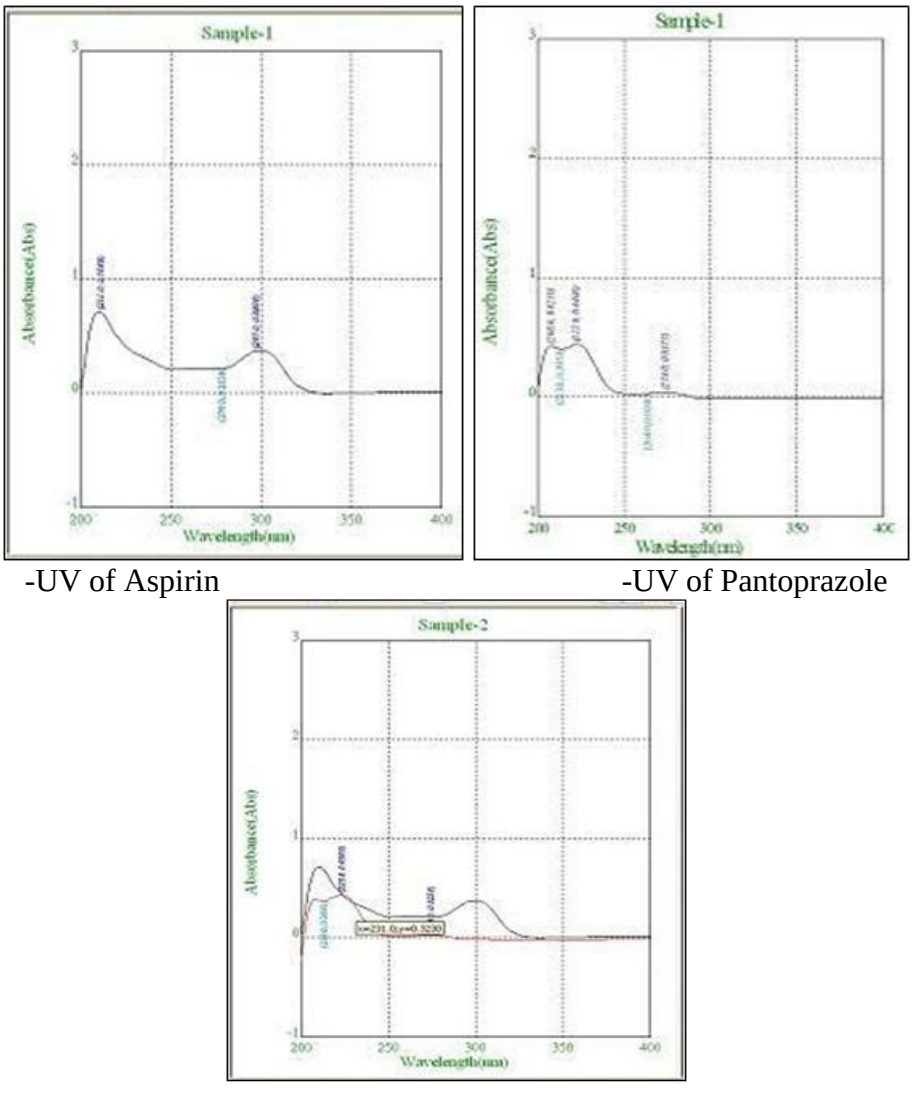
200mL water (0.05%), HPLC grade water was prepared from Millipore Milli-Q water purification system. All other materials and reagents were of analytical grade.

Identification of Aspirin & Pantoprazole

Melting point was determined using digital melting point apparatus with one end open capillary method. The reference melting point of aspirin and pantoprazole is 136°C and 150°C respectively.

Determination of wavelength maxima

Both the standard solution was scanned between 400nm to 200nm.The overlain spectrum of both drugs was recorded. From the overlain spectrum, 297nm (λmax of pantoprazole) and 222 nm (λmax of Aspirin) were selected for estimation of drugs and the isosbestic point was found to be at 231 nm and it is shown in figure.[17-18]



-UV of Aspirin -UV of Pantoprazole

Figure 5: Isosbestic point of Pantoprazole & Aspirin.

Name of sample	Wavelength	Absorbance
Pantoprazole	296nm	0.3698
Aspirin	222nm	0.4408
Isosbestic Pantoprazole & Aspirin	231nm	0.3230

Selection of chromatographic mode

Proper selection of the method depends upon the nature of the sample (ionic, ionisable and neutral, its molecular weight and solubility. Both the drugs are freely soluble in organic. Hence, reversed phase HPLC was selected for the initial separations because of its simplicity and suitability.

Selection of Mobile phase

Pantoprazole and Aspirin are freely soluble in HPLC grade of methanol. Not freely soluble in orthophosphoric acid and used ultrasonication. Both drugs are soluble in methanol and orthophosphoric acid.

Mobile Phase	60+40 (Methanol+(0.05%) OPA)
Selection of column	4.6×150mm
Wavelength	231nm
Flow rate	0.7ml/min
Column temp	Ambient
Sample size	20µL
Retention time	4.61 pantoprazole, 8.35 aspirin
Conclusion	Satisfactory Resolution, Theoretical Plate, Tailing Factor.

Analytical Method Validation of the proposed method.

The proposed method was validated as per current regulatory guidelines.[19-21]

System suitability. To assess system suitability of the proposed method (repeatability, theoretical plates, tailing factor, and retention time of six replicate) vials containing the working standard of pantoprazole and aspirin of nominal concentration (50 µg/ml) were used and percentage relative standard deviation (% RSD) values were calculated in each case.

Linearity. The linearity was evaluated by analyzing five working solutions of pantoprazole and aspirin over the concentration range 10-50 µg/ml. A calibration curve was prepared and the linearity was evaluated by linear regression analysis, which was then evaluated by the least-square regression analysis. The regression line was calculated as $y = mx + c$; where, y, m, x and c represent the response (peak area expressed as mAU), the slope of the regression line, the concentration of sample in µg/ml and the intercept of the regression line, respectively.

Specificity. The specificity of the developed method was determined by blank analysis. A blank sample was prepared for this study and was treated in the same manner as the test samples.

Accuracy (recovery test). Accuracy of the proposed method was studied by recovery experiments for both standard and sample solutions. It was assured by the standard addition technique. The result obtained for both were compared to those expected.

Precision. The precision intraday and inter day Precision system result showed good reproducibility. Intraday precision study was carried out by preparing drug solution from stock solution, aliquots of Pantoprazole and Aspirin 0.2, 0.3, 0.4 ml were taken a diluted to 10 ml with diluents such that the final concentration of Pantoprazole-20, 30, 40 µgm/mL and Asp-40, 60, 80 µg/mL and analyzed. The same procedure was followed for second day also to determine inter-day precision. The results were expressed as % RSD of the measurements.

Sensitivity. To perform this, a blank sample (MP) was run in the HPLC system and the pump pressure was monitored. When the pressure fluctuation became negligible, highly diluted standard solutions of pantoprazole and aspirin were run through the same chromatographic condition. The LOD and LOQ were determined based on the standard deviation (SD) of the response and slope (S) of the regression line as per ICH guidelines. Equation i and ii represent the formulas for determining LOD and LOQ, respectively.

$$LOD = 3.3 \times SD/SLOQ = 10 \times SD/S$$

Robustness. The robustness is the ability of a method to remain unaffected by small deliberate changes in chromatographic parameters. To determine the robustness of the current method, pH of the buffer solution, mobile phase compositions and flow rate were changed and % RSD of those changed conditions were calculated.

RESULT AND DISCUSSION

Orthophosparic acid water - 0.1mL OPA in 200mL water. (0.05%).

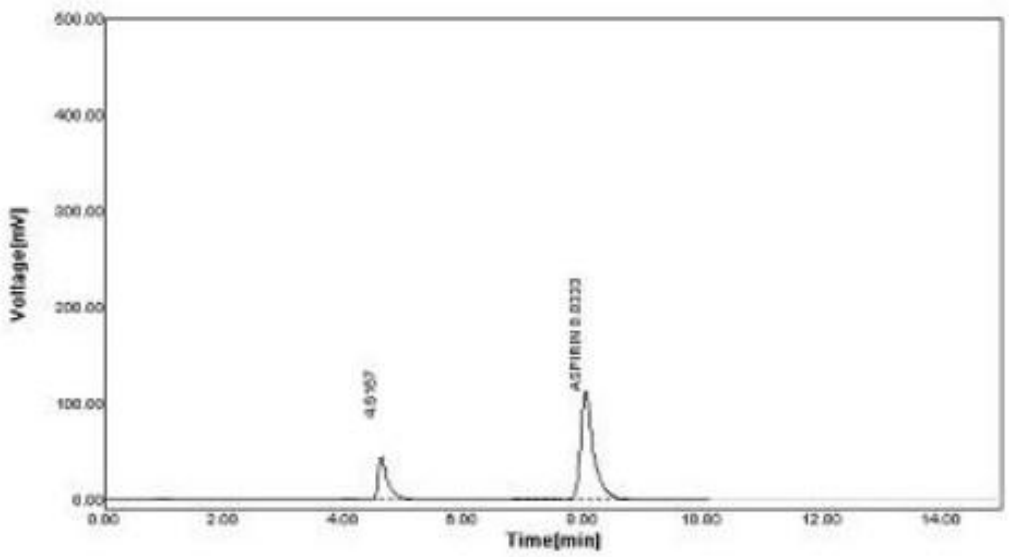


Figure 6: Chromatogram of Pantoprazole & Asp.

Table 4: Final chromatographic conditions.

Peak Name	Retention time	Area	Plate count	T F
Panto	4.6167	507.77	5060.1	1.1297
Asp	8.0333	1753.2	9367.1	1.3266

Preparation of stock solution

Accurately weighed of Pantoprazole 10mg and 20mg Aspirin was taken in 10mL volumetric flask and dissolved in 10 mL methanol. To get standard stock solution.1000 µg/mL Pantoprazole and 2000 µg/mL Aspirin. STOCK -I.

Preparation of mobile phase

Pipette out 60 ml of concentrated methanol and 40 ml of 0.05% OPA make up the volume up to 100ml and Sonicate 5 min.

1) Take 0.1mL from the stock I and make up the volume with mobile Phase 10 mL = 10 µg/mL Pantoprazole and 20 µg/mL Aspirin.
2) Take 0.2 mL from the stock I and make up the volume with mobile Phase 10 mL = 20 µg/mL Pantoprazole and 40 µg/mL Aspirin.
3) Take 0.3 mL from the stock I and make up the volume with mobile Phase 10 mL = 30 µg/mL Pantoprazole and 60 gm/mL Aspirin.
4) Take 0.4 mL from the stock I and make up the volume with mobile Phase 10 mL = 40 µg/mL Pantoprazole and 80 µg/mL Aspirin.
5) Take 0.5 mL from the stock I and make up the volume with mobile Phase 10 mL = 50 µg/mL Pantoprazole and 100 µg/mL Aspirin.

Tab solution Preparation

Weigh and finely powder 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 10mg of Pantoprazole and 20mg of Aspirin into a 10 ml clean dry volumetric flask, add 10ml methanol and sonicate to dissolve it completely and make volume up to the mark with the diluents (1000 µg/mL Panto and 2000 µg/mL. From these solutions 0.1ml was pipette out and transferred into 10ml volumetric flask and makeup the volume up to the mark with methanol & Orthophosphoric acid (10 µg/mL Pantoprazole and 20 µg/mL Aspirin) and measured the absorbance at 231nm. The % purity of the drug was calculated by comparing the absorbance of test solution with standard.

Tab Assay

0.4 ml from tab stock and make up 10 ml with mobile phase and produced 40 µg/mL Pantoprazole + 80 µg/mL Asp for assay.

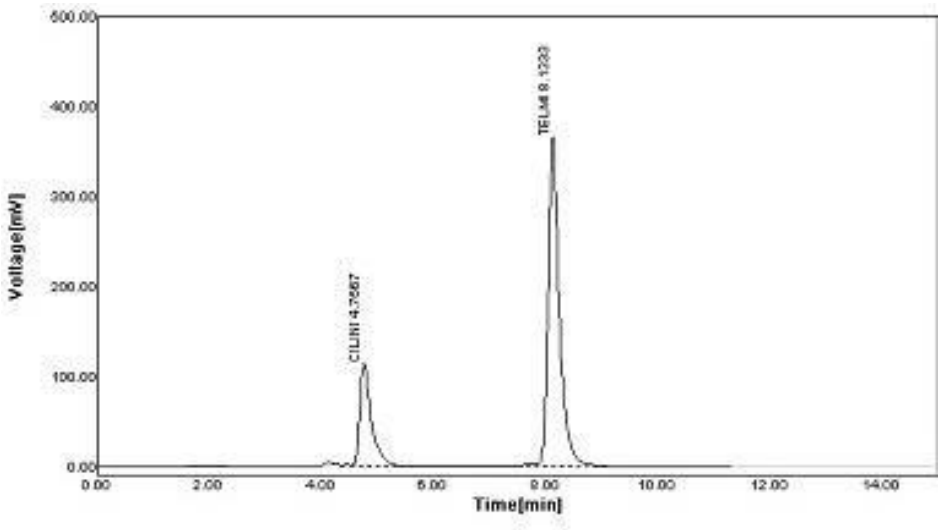


Figure 7: Chromatogram for Assay.

Table 5: Assay.

Name	RT (min)	TP	TF	Area
Pantoprazole	4.7667	3827.0	1.33	1265.54
Asp	8.1333	9876.3	1.37	5033.44

Validation Parameter

Linearity: The con. of panto and asp solution take in different concentration respect. 10-50ug/mL, 20-100ug/mL. At selected wavelength 231nm.

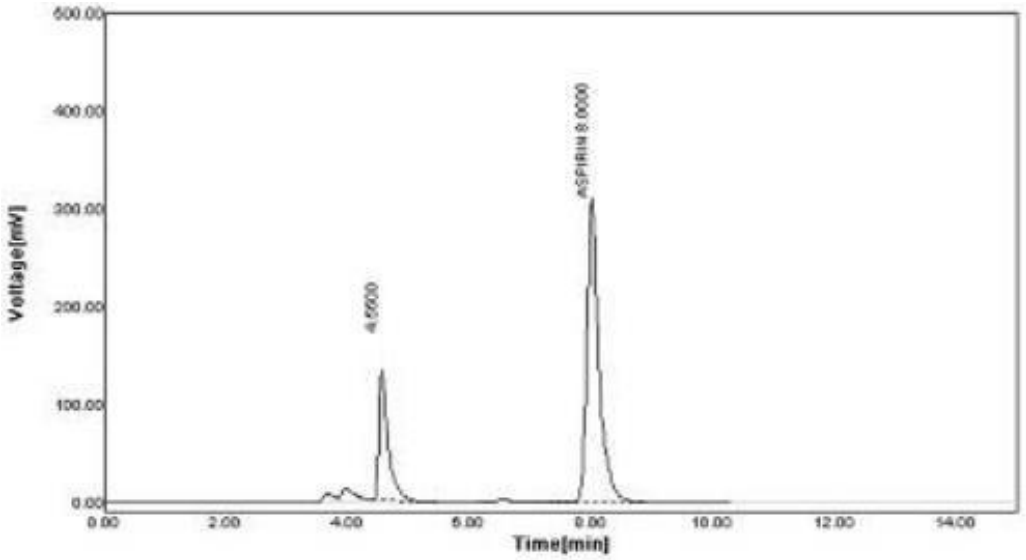


Figure 8: Linearity 50 µg/mL Panto & 100 µg/mL.

Peak Name	Retention time	Area	Plate count	T F
Panto	4.6000	1230.7	5503.1	1.1472
Asp	8.0167	5042.1	9537.0	1.1699

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The three triplicates of stock solution of Pantoprazole and Aspirin equivalent to 10ppm, 20ppm, 30ppm and 20ppm, 40ppm, 60ppm respectively were preparing by using standard solution. From stock solution aliquots of Panto and Aspirin 0.1 ml, 0.2ml, 0.3ml were taken and diluted to 10ml mobile phase with diluted such that the final concentration of Pantoprazole-10, 20, 30 µg/mL and Aspirin-20, 40, 60 µg/mL.

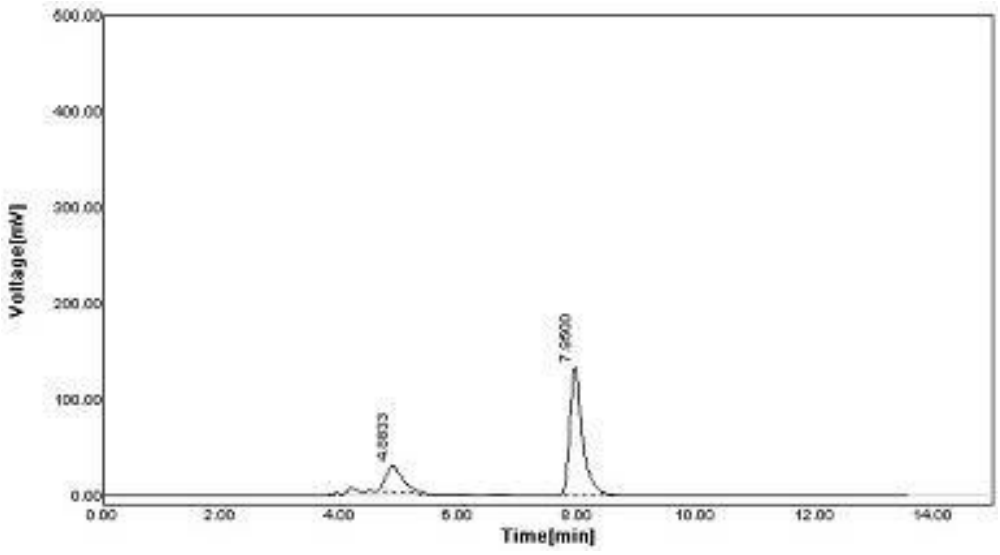


Figure 9: Chromatogram of accuracy Pantoprazole 30µg/mL Aspirin 60 µg/mL.

Table 6: Accuracy of Pantoprazole.

Conc. (µg/ml)	Standard Deviation		% RSD
	Area Mean	SD	% RSD
10	288.91	2.63	0.91
20	525.86	7.71	1.47
30	778.18	3.34	0.43

Table 7: Accuracy of Aspirin.

Conc. (µg/ml)	Standard Deviation		% RSD
	Area Mean	SD	% RSD
20	706.42	5.03	0.71
40	1741.04	7.25	0.99
60	2863.04	3.78	0.13

Precision: The precision intraday and inter day Precision system result showed good reproducibility. Intraday precision study was carried out by preparing drug solution from stock solution, aliquots of Pantoprazole and Aspirin 0.2, 0.3, 0.4 ml were taken a diluted to 10 ml with diluents such that the final concentration of Panto-20, 30, 40 µgm/mL and Asp-40, 60, 80 µg/mL and analyzed. The same procedure was followed for second day also to determine inter-day precision.

Table 8: Intra-day precision.

Conc. µg/mL	Area Mean	SD	%AmtFound	% RSD
Pantoprazole				
20	530.32	6.74	100.42	1.27
30	748.75	6.60	97.98	0.88
40	996.01	5.40	99.84	0.54
Aspirin				
40	1765.1	10.9	99.87	0.58
60	2880.9	6.19	100.95	0.22
80	3913.4	1.44	98.58	0.21

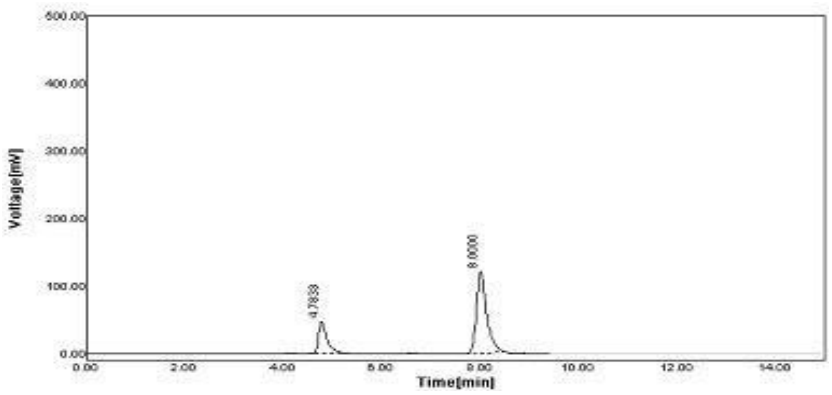


Figure 10: Precision-20ug/ml and 40ug/ml Intra-dayTable 9: Inter day precision.

Conc.µg/mL	Area Mean	SD	%AmtFound	% RSD
Pantoprazole				
20	530.32	6.74	100.42	1.27
30	763.32	4.88	100.15	1.57
40	996.01	5.40	99.84	0.54
Aspirin				
40	1765.5	10.1	99.87	0.58
60	2880.9	6.19	100.95	0.22
80	3913.4	1.44	99.58	0.04

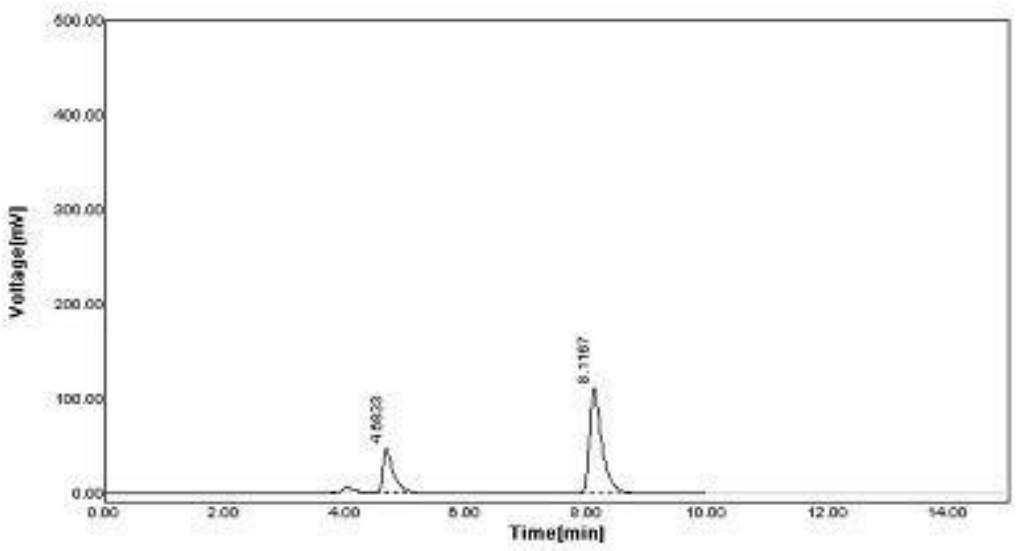


Figure 11: Precision-20ug/mL and 40ug/ml interday.

% Recovery

The recovery of an analytical method is determined by applying the method to analysed samples to which known amounts of analyte have been added. The Recovery is calculated from the test results as the percentage of analyte recovered by the assay. Three replicate injections, each of three different test concentrations in the range of 80%, 100% and 120% of labelled claim of tablet under study yielded the result within 98 to 102% of true concentration of each drug. The results indicated that the method is accurate.

Table 10: Statistical Validation of Recovery Studies Panto and Asp.

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation	% RSD
80%	**Panto**	98.20	1.70	1.73
	Asp	99.10	0.75	0.64
100%	**Panto**	101.16	1.78	0.44
	Asp	100.75	1.51	0.27
120%	**Panto**	101.58	1.07	0.37
	Asp	99.23	0.41	0.11

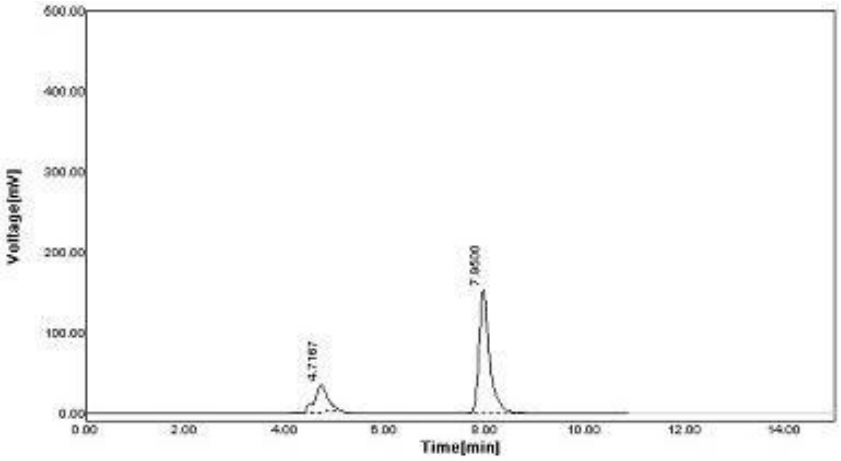


Figure 12: Recovery 120 %.

Robustness

The robustness of an analytical method 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. To verify the robustness of the method, the analysis was done under variables flow rate, mobile phase ratio and wavelength.

Table 11: Robustness study of Panto & Asp.

Parameterchange	Conc.ug/ml	Amt. Mean±SD	%RSD
Flow rate 0.6ml	32	1217.7±5.69	0.88
0.8ml	32	1812.21±4.68	0.26
69ml+31ml Me + OPA	32	2099.53±24.9	1.19
59ml+41ml Me + OPA	32	1320.56±7.42	0.56
230nm	32	2099.53±5.69	0.47
232m	32	1855.70±32.8	1.77

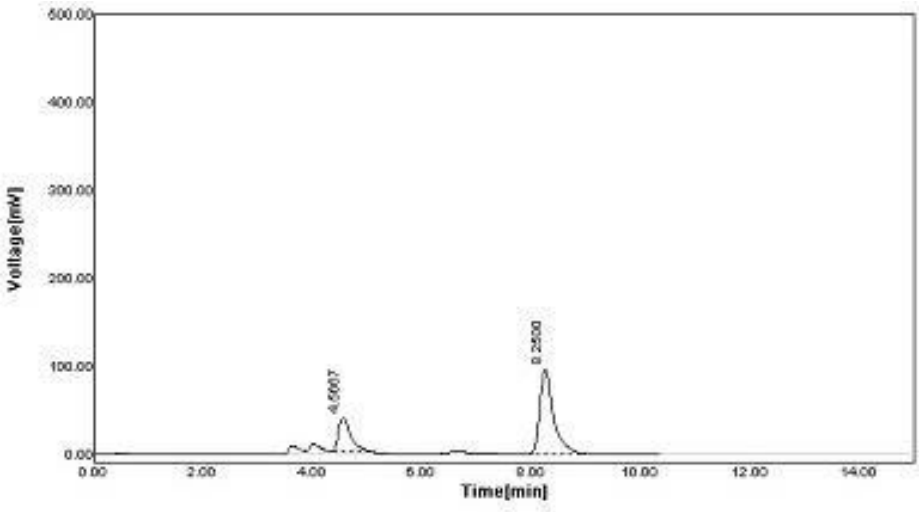


Figure 13: Change in wavelength 230 nm.

System suitability parameter

The system was evaluated by analyzing repeatability, retention time, tailing factor and theoretical plats of the column.

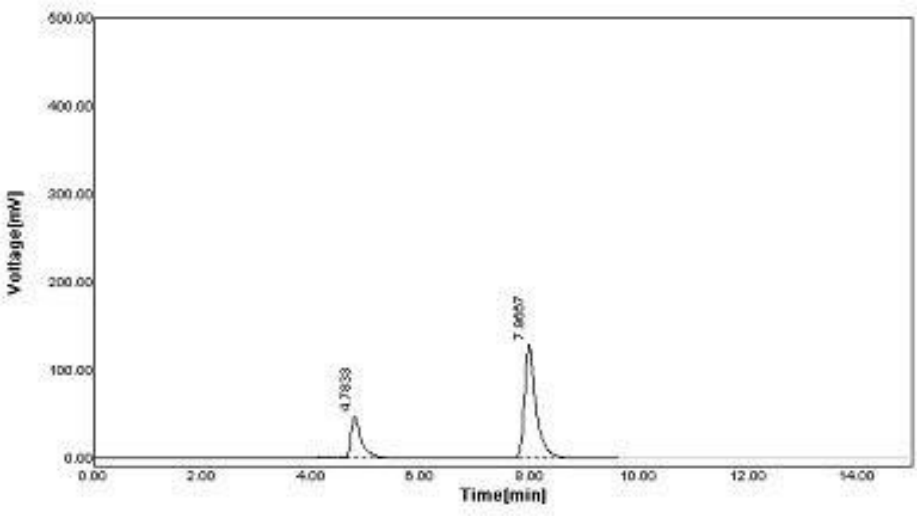


Figure 14: Change in wavelength 232 nm.

Limit of Detection (LOD)

The LOD is the lowest limit that can be detected. Based on the S.D. of the response and the slope. The limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ (SD)/S$$

Where, SD= Standard deviation of the Y interceptS = Slope

LOD = 3.3 x 4.65/ 23.46 = 0.5946 (µg/ml) (Pantoprazole)LOD = 3.3 x 8.81/ 54.08 = 0.5375 (µg/ml) (Aspirin)

The LOD of Pantoprazole and Aspirin was found to be 0.5946 (µg/ml) and 0.5375 (µg/ml) respectively.

Limit of Quantitation

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope.

The quantitation limit (LOQ) may be expressed as$LOQ = 10\ (SD)/S$

Where, SD = standard deviation of Y interceptS = Slope

LOQ = 10 x 4.65/23.46 = 1.9820 (µg/ml) (Pantoprazole)LOQ = 10 x 8.81/54.08 = 1.6290 (µg/ml) (Aspirin)

The LOQ of Pantoprazole and Aspirin was found to be 1.9820 (µg/mL) and 1.6290 (µg/mL) respectively.

Table 12: Summary of validation parameter.

Parameter (Unit)		Panto	Asp	Accep. Criteria
Linearity range (µg/ ml)		10-50	20-100	-----
Correlation Coefficient		0.9995	0.9987	0.999
% Recovery		98.20-101.58%	99.10- 99.23%	98%- 102%
Precision %RSD	**Intraday**	1.27%	0.58 %	% RSD NMT 2
	Interday	1.27%	0.58 %	
Robustness		Robust	Robust	Robust
LOD		0.5946 µg /ml	0.5375 µg /ml	NMT 2
LOQ		1.9820 µg /ml	1.6290 µg/ ml	NMT 2

CONCLUSION

RP-HPLC method has been developed for the simultaneous estimation of Pantoprazole and Aspirin. These methods have good resolution both the drugs good and short analysis time. Literature survey revealed that several methods have been reported for determination of Pantoprazole and Aspirin individually or in combination with other drugs in pharmaceutical dosage forms. The developed method was validated. It was found to be simple, precise, accurate and robust. The proposed method can be used for routine analysis of Pantoprazole and Aspirin in combined dosage form.

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