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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND LANSOPRAZOLE BY RP-HPLC

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Abstract

A new, rapid, sensitive, accurate RP-HPLC method has been developed for the simultaneous estimation of Aspirin and Lansoprazole. The best chromatographic separation was found to be on a Phenomenex Luna C18 (100Å 250×4.60mm 5µ) column. Elution was carried out with a mobile phase consisting of Acetonitrile:Water:Triethylamine (pH-7.6 adjusted with o-phosphoric acid) (700:300:15). The UV detection wavelength was 265 nm and 283 nm for Aspirin and Lansoprazole respectively.

The retention time for Aspirin and Lansoprazole was found to be around 2.41 and 5.54 minutes respectively with a flow rate of 1ml/min. Developed method was validated according to ICH Q2 (R1) guidelines. The method was found to be linear between the range of 1-10 µg/ml for Aspirin and Lansoprazole. The precision (intra-day, inter-day, repeatibility) data of this method was found to be within limits (% RSD < 2%).

Keywords: Aspirin, Lansoprazole, Method development, Validation, RP-HPLC method.

Introduction:

Aspirin 2-(acetoxy) benzoic acid (fig. 1). Acetylsalicylic acid directly and irreversibly inhibits the activity of both types of cyclooxygenase (COX-1 and COX-2) to decrease the formation of precursors of prostaglandins and thromboxanes from arachidonic acid. Cyclooxygenase is required for prostaglandin and thromboxane synthesis. Aspirin is official in IP, BP, and USP. Acetylsalicylic acid is an analgesic, antipyretic, antiplatelet, antirheumatic, and anti-inflammatory agent.

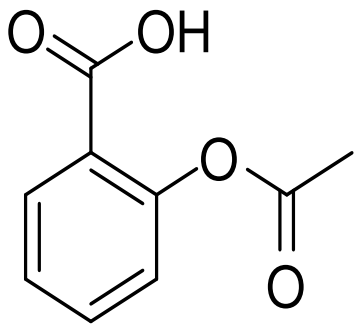


Figure-1: Structure of aspirin.

Lansoprazole 2-{[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methanesulfinyl}-1Hbenzimid – azole (fig.2). Lansoprazole is a substituted benzimidazole pro-drug with selective and irreversible proton pump inhibitor activity. Lansoprazole pro-drug is converted to an active sulphonamide derivative in the acidic environment of the gastric parietal cell; the sulphonamide derivative binds to the gastric proton pump H^+/K^+ ATPase and forms a stable disulphide bond with the sulphydryl group near the potassium binding site on the luminal side, resulting in inactivation of the ATPase and a reduction in gastric acid secretion.[1,2,3]

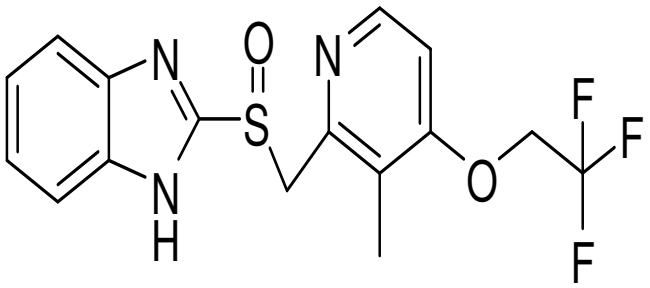


Figure-2: Structure of lansoprazole.

The number of patients taking low-dose Aspirin for prevention of the recurrence of cerebral infarction or myocardial infarction is increasing along with the rapidly aging population. As administration of low-dose Aspirin may cause gastric or duodenal ulcers, the gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of cyclooxygenase 1 (COX-1), which is essential for the production of these prostaglandins. [4,5,6]

Literature survey revealed that various analytical methods like UV spectroscopy[7], RP-UPLC[8,9], RP –HPLC[10,11,12,13], LC-MS available for the estimation of lansoprazole and aspirin as single drug or in combination with other drugs. There are few analytical methods available for selectiveestimation of this combination. But, No method was reported for

simultaneous estimation of these two drugs in combinaion. Therefore, the focus of present work is to develop a simple, accurate, precise method for the simultaneous estimation of Aspirin and Lansoprazole in combination.

Materials and Methods

Instrumentation:

HPLC of Shimadzu (LC-2010CHT) with Phenomenex-luna C18 (250 × 4-60mm, 5µ 100Å) column was used for chromatographic separation. UV (UV 1800). Bath sonicator (Pci analytics 6-5L 200H) was used for sonication. Vortex mixer (REMI CM101). pH meter (Labindia Pico+). Analytical balance (Shimadzu AUX220) was used for the study.

Materials:

Aspirin and Lansoprazole sample was received as gift samples by a Paras Pharmaceuticals Baddi and Medico remedies pvt. Ltd. Mumbai.

Preparation of Mobile phase:

Mobile phase was prepared by mixing 50 volumes of Acetonitrile, 50 volumes of Water and 0.5 ml of Triethylamine pH 7.6 adjusted with ortho-phosphoric acid. The mobile phase was sonicated and filtered through 0.45 µm membrane filter.

Stock solution of Aspirin and Lansoprazole:

Accurately weigh 10mg of Aspirin and 10mg of Lansoprazole tranfered to 1ml epindroff and dissolves in Dimethy sulfoxide (DMSO) and further dilutions in HPLC grade Water.

Preparation of blank:

10 ml water and 10 ml acetonitrile were mixed in (1:1). The water and acetonitrile mixed in 1:1 were taken as blank.

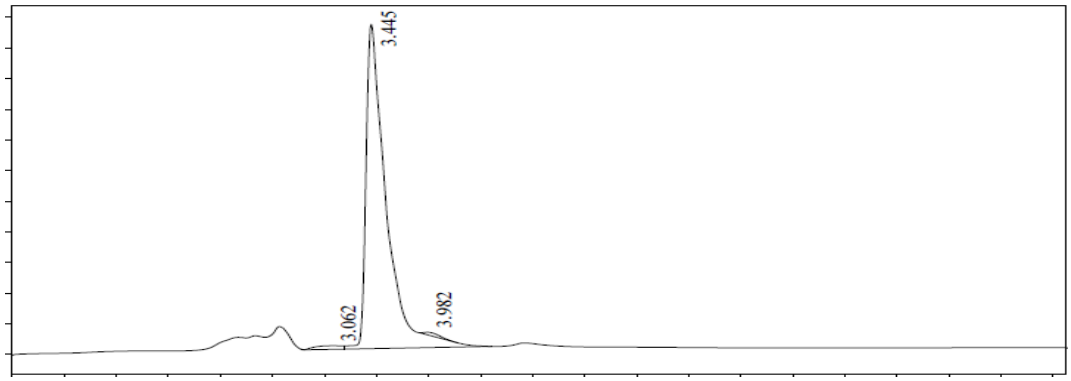
Chromatographic conditions:

Mobile phase A: water, Mobile phase B: acetonitrile, Mobile phase C: triethylamine (A:B:C = 50:50:0.5); pH 7.6 (adjusted with ortho-phosphoric acid). Wave length for Aspirin is 265 nm and for Lansoprazole is 283 nm. Column used was C-18 Phenomenex (100Å, 250×4.60mm 5 micron) with a flow rate of 1.0 ml/min.

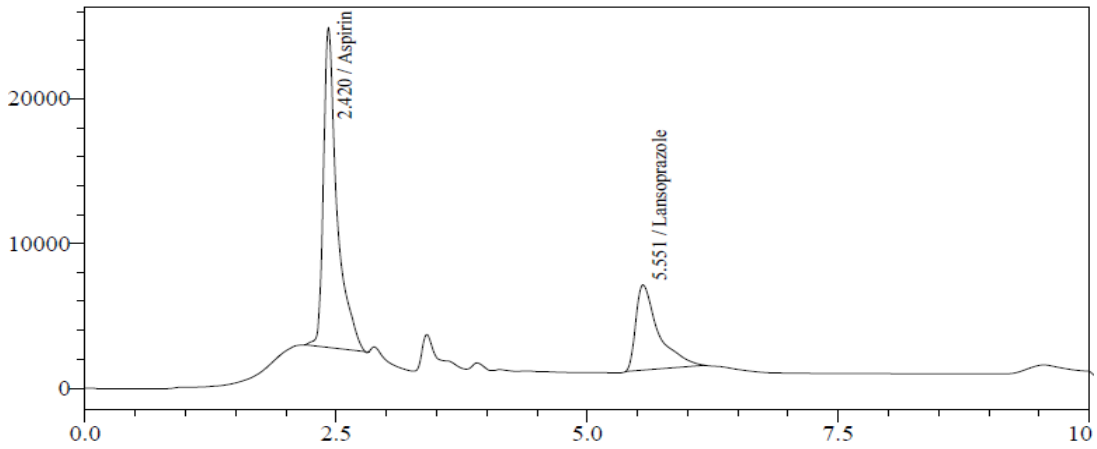
Method Validation

Stock solution of Aspirin and Lansoprazole (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml) were pipetted out from 10 µg/ml stock solution in ten different epindroffs and further diluted with blank to attain concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml respectively.

Linearity: The linearity of analytical procedure is its ability to produce a response, which are directly proportional to the concentration of analyte in sample. Ten concentration solutions ranging from 1-10 µg/ml were run to obtain a plot of area v/s conc. for linearity.



(A)



(B)

Fig. 3 (A) Chromatogram of Blank, (B) Chromatogram of linearity.

Table 1: Linearity Data.

Conc. (µg/ml)	Area	
	ASP	**LAN**
1	43802	18549
2	87502	37315
3	134939	56968
4	174188	76188
5	207739	92063
6	262434	115056
7	286907	135621
8	340337	159055
9	376969	172118
10	421875	196012

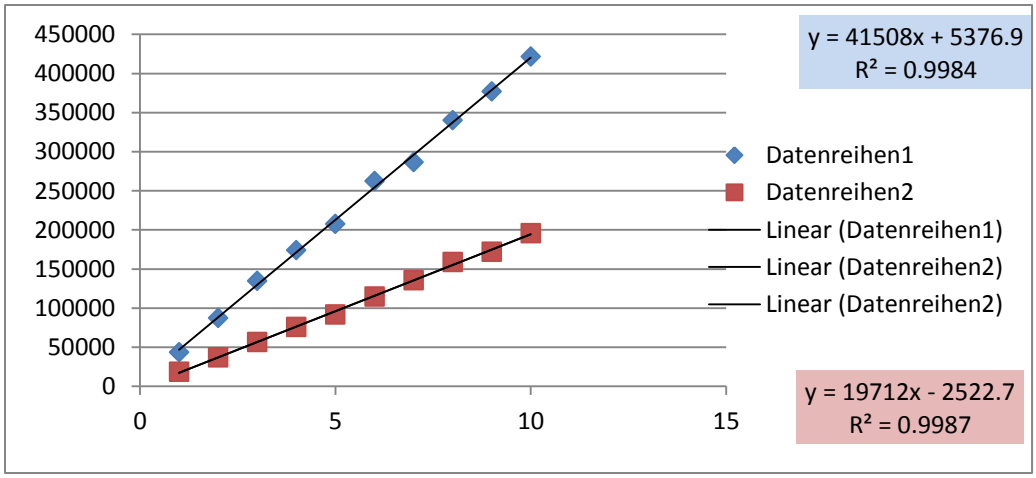


Fig. 4 Area v/s Conc. Plot for linearity

Accuracy: The accuracy of a measurement is defined as the closeness of the measured value to the true value. Three different concentration solutions (80%, 100%, 120%) were prepared from standard stock solution to determine the accuracy.

Table 2: Accuracy Data.

S.No.	Accuracy Test conc.	Results (%RSD)	
		Aspirin	Lansoprazole
1.	80%	0.03	1.09
2.	100%	0.70	0.02
3.	120%	0.26	0.23

Precision: It demonstrates that the analytical method is capable to yield closeness of data values between a series of measurements obtained from multiple sampling of the same homogenous sample. Standard concentration sample was analyzed three times on day to day interval (interday precision), three times on different time intervals (morning and evening) in same day (intraday precision), repeatibility was evaluated by performing six replicate injections of standard test solution.

Table 3: Precision Data.

S.No.	Precision	Results (%RSD)	
		Aspirin	Lansoprazole
1.	**Interday** • Day 1 • Day 2	 0.68 1.06	 0.49 1.66
2.	**Intraday**		

	• Morning • Evening	0.19 0.27	1.35 0.17
3.	**Repeatibility**	0.90	1.24

Robustness: Robustness of the method was determined by small, deliberate changes in mobile phase ratio and detection wavelength. Influence of small changes in chromatographic conditions such as change in flow rate, that is, ± 0.1 ml/min, pH ± 0.1, wavelength of detection ± 5 nm and column change was studied to determine the robustness.

Table 4: Robustness Data.

S.No.	Robustness	Results (%RSD)	
		Aspirin	**Lansoprazole**
1.	**Change in flow rate** • Decreased (0.9ml) • Increased (1.1ml)	 1.31 0.52	 0.29 0.45
2.	**Change in wavelength** • Decreased (260,278) • Increased (270,288)	 0.17 0.29	 0.29 0.35
3.	**Change in pH** • Decreased (7.5) • Increased (7.7)	 1.22 0.65	 1.61 0.43
4.	**Column Change**	1.46	1.05

Ruggedness: Ruggedness was determined by assaying sufficient number of aliquots of homogenous sample to be able calculate statistically valid estimates of standard deviation or relative standard deviation. Ruggedness was determined by studing the variation in sample analyzed by two different analyst.

Table 5: Ruggedness Data.

S.No.	Ruggedness	Results (%RSD)	
		Aspirin	**Lansoprazole**
1.	**Analyst to Analyst variation** • Analyst 1 • Analyst 2	 0.10 0.05	 0.79 0.10

Specificity:

It is the ability of the analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be presents in the sample matrix. In a test solution of 5µg/ml diluents were added and volume made upto 1ml.

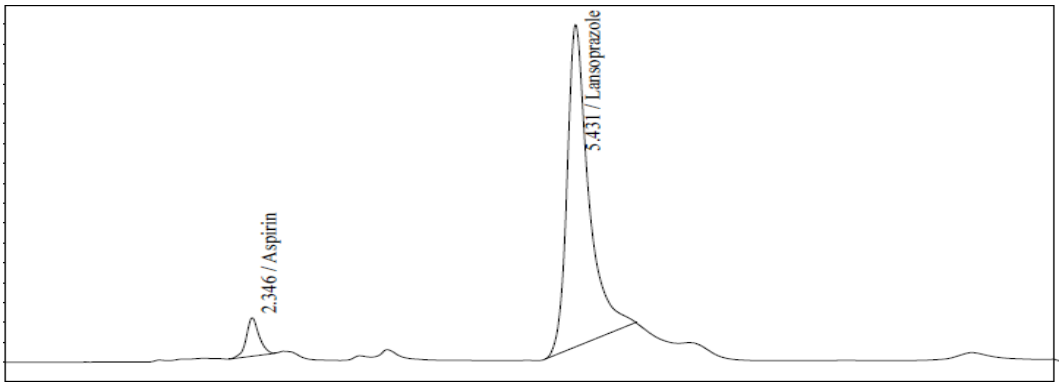


Fig. 5 Chromatogram for Specificity

Results and Discussion: Method is sensitive as the limit of detection for Aspirin and Lansoprazole was found to be 0.1229 and0.3724 *ppm* respectively. The method was found to be accurate and precise as indicated by the results %RSD not more than 2%. The corelation coeficient (r^2) obtained was 0.998 and 0.999 for Aspirin and Lansoprazole respectively. This shows that the method and system both are suitable for the simultaneous estimation of Aspirin and Lansoprazole. Validation results show satisfactory precision, specificity, accuracy, linearity, robustness which were found to be passing all the acceptance criteria.

Conclusion: A new RP-HPLC method has been developed for the simultaneous estimation of Aspirin and Lansoprazole. In this study, different chromatographic conditions were used to develop the method. The best chromatographic separation was found to on a Phenomenex Luna C18 (100Å 250×4.60mm 5µ) column. Elution was carried out with a mobile phase consisting of Acetonitrile : water : Triethylamine (pH-7.6 adjusted with o-phosphoric acid) (700:300:15). The UV detection wavelength was 265 nm and 283 nm for Aspirin and Lansoprazole respectively. The retention time for Aspirin and Lansoprazole was found to be around 2.41 and 5.54 minutes respectively with a flow rate of 1ml/min. The method is validated as outlined in USP and ICH guidelines. Therefore, method may be useful for analysis of Lansoprazole and Aspirin in pharmaceutical preparations.

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