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RESEARCH ARTICLE

SIMULTANEOUS ESTIMATION OF ASPIRIN AND LANSOPRAZOLE BY RP–HPLC METHOD

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ABSTRACT

A Simple, sensitive, rapid, precise and accurate RP-HPLC method has been developed and validated for simultaneous estimation Aspirin and Lansoprazole in synthetic mixture. The Chromatographic separation was achieved on a Reversed-phase Phenomenax-luna C18 (250 x 4.6mm, 5 µm) column using a mobile phase consisting of Acetonitrile : Tris buffer : Methanol (30:40:30 % v/v/v) (pH 4.0) at a flow rate of 1.2 mL/min and UV detection at 280 nm. Developed method was validated according to ICH Q2 (R1) guidelines. The method was found to be linear between the range of 13.2 – 66.0 µg/ml for Aspirin and 2 - 10 µg/ml for Lansoprazole. The precision (intra-day, inter-day) data of this method was found to be within limits (% RSD < 2%). Accuracy was determined by recovery studies and % recovery found between 98 to 102%.

INTRODUCTION

Aspirin, 2-(acetyloxy) benzoic acid, (Figure 1), acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins. It also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis. Aspirin is official in IP, BP, and USP. Acetylsalicylic acid is an analgesic, antipyretic, antiplatelet, antirheumatic, and anti-inflammatory agent.

Lansoprazole, chemically known as 2-([3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl]methylsulfinyl)-1 *H*-benzimidazole, is a strong anti-secretory agent that acts on gastric H^+/K^+ ATPase of parietal cells. It is used to treat ulcers, gastroesophageal reflux disease and peptic ulcer caused by stress, non-steroidal inflammatory disease. In addition to its acid-suppressing effects, Lansoprazole has been shown to modulate the inflammatory status, oxidative stress in the esophagus, intestine and lungs.

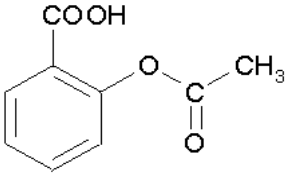

Fig. 1 structure of aspirin

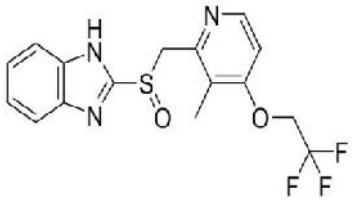

Fig. 2structure of lansoprazole

Aspirin is used to prevent the thrombus, embolism, and the reocurrence of cerebral infarction or myocardial infarction or treatment of rheumatoid arthritis and osteo arthritis in low-dose. Lansoprazole used to treat ulcers, peptic ulcer. Due to longer use of Aspirin, there is possibility of the onset of ulcer. So, to prevent inflammation and to prevent gastric or duodenal ulcer administration of Aspirin and Lansoprazole is given in one combination. So, combination of these two drugs shows synergic effect.

Based on literature survey it is found that various analytical methods like UV spectroscopy, High Performance Thin Liquid Chromatography (HPTLC), Reverse Phase High performance Liquid Chromatography (RP–HPLC), Liquid Chromatography and Mass Spectroscopy (LC-MS) have been reported for the determination and estimation of as Aspirin individual drug and combination with other drug. Similarly, various methods like

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RP-HPLC, HPTLC, UV spectroscopy were reported for the analysis of Lansoprazole as individual drug and in combination with other drug. But, No method was reported for selective estimation of these two drugs in combination.

Therefore, it is a thought of interest to develop simple, accurate and precise methods for the simultaneous estimation Aspirin and Lansoprazole in combination.

MATERIALS AND METHODS

Instrumentation

HPLC of Shimadzu (LC-20AD Prominence Liquid Chromatography) with Phenomenax-luna C18 (250 x 4.6 mm, 5 µm) (Spinotech Pvt. Ltd.) Column was used for chromatographic separation. It contain Rhenodyne valve with 20 µl fixed loop injector and UV Detector (LC-20AD). The Ultrasonic bath of Equitron Agilent 1200 Infinity Series was used for sonication. Analytical balance of Wensar DAB- 220 was used for the study.

Materials

Aspirin and Lansoprazole sample were received as a gift sample by West-coast Pharma, Ahmedabad, Gujarat.

Preparation of Tris Buffer pH 8.5

The Calcium chloride was accurately weighed about 7.35 mg and dissolved in HPLC Grade water and add 302.75 mg of Tris (hydroxymethyl)aminomethane was added and dissolved and volume was made up to mark with HPLC grade Water in 1000 mL volumetric flask. Adjust the pH with 1% Ortho-phosphoric acid.

Preparation of Mobile Phase

Mobile phase was prepared by mixing 30 volumes of Acetonitrile and 40 volumes of Tris buffer and 30 volume of Methanol and pH 4.0 adjusted with ortho-phosphoric acid. The mobile phase was sonicated and filtered through 0.45 µm membrane filter.

Preparation of standard solutions

For Stock solution of Aspirin

Accurately weigh 100 mg of Aspirin and transferred into a 100 ml volumetric flask and diluted with Acetonitrile (1000 µg/ml).

For Stock solution of Lansoprazole

Accurately weigh 10 mg of Lansoprazole and transferred into a 100 ml volumetric flask and diluted with Acetonitrile (100µg/ml).

Preparation of synthetic mixture of Aspirin and Lansoprazole

The synthetic mixture of Aspirin and Lansoprazole was prepared in the ratio 6.66:1. Accurately weighed Aspirin (100 mg) and Lansoprazole (15 mg) were transferred in 100 ml volumetric flask and dissolved in Acetonitrile (70 ml). Common excipients like, Lactose, Corn starch, Hydroxyl Propyl Methyl cellulose, Talc were added in this mixture and sonicated for 20 mins. This solution was filtered through What man filter paper (0.45µm) and residue was washed thoroughly with Acetonitrile. The filterate and washing were combined and diluted to the mark with Acetonitrile to get solution of Aspirin (1000 µg/ml) and Lansoprazole (150 µg/ml).

Selection of wavelength

The solution of Aspirin was prepared in Methanol by pipette out 0.99 ml from the stock solution (1000 µg/ml) in 10 ml volumetric flask and dilute up to the mark with Methanol (99.99 µg/ml). and scanned in the wavelength range of 200-400 nm.

Lansoprazole was also prepared in Methanol by pipetting out 1.5 ml from stock solution (100 µg/ml) in10 ml volumetric flask and dilute upto the mark with Methanol (15 µg/ml). and scanned in the wavelength range of 200-400 nm.

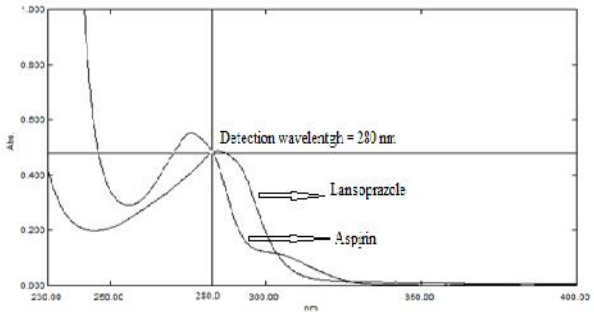

Fig 3 Overlain Spectra of Aspirin and Lansoprazole

Method validation

Calibration curve for aspirin and lansoprazole

For Aspirin

An aliquots of stock solution of Aspirin (0.13, 0.26 ,0.39, 0.52, and 0.6 ml) were pipetted out from 1000 µg/ml stock solution in five different 10 ml volumetric flasks and further diluted with Acetonitrile to attain concentration of 13.2, 26.4, 39.6, 52.8, 66.0 µg/ml respectively.

Graph of peak Area Vs Concentration was plotted.

For Lansoprazole

An aliquots of stock solution of Lansoprazole (0.2, 0.4, 0.6, 0.8, and 1.0 ml) were pipetted out in five different 10 ml volumetric flasks and further diluted with Acetonitrile to attain concentration of about 2, 4, 6, 8, 10 µg/ml respectively.
Graph of peak area Vs Concentration was plotted.

Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of Aspirin

and Lansoprazole in the linear range were analyzed in 3 independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). The precision of the analysis was determined by calculating the relative standard deviation (RSD %).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degrades etc. A solution of placebo in mobile phase and Mobile phase were injected and the chromatogram showed no inferring peaks at retention time of the two drugs. Further Aspirin and Lansoprazole individually were injected which confirm that Asprin was eluted at 3.7 min and Lansoprazole was eluted at 6.9 min.

Limit of Detection (LOD)

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

$LOD = 3.3 * SD/Slope$

Where,

SD = Standard deviation of Y- intercept of 5 calibration curves.

Slope = Mean of slope of the 5 calibration curves.

Limit of Quantification (LOQ)

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

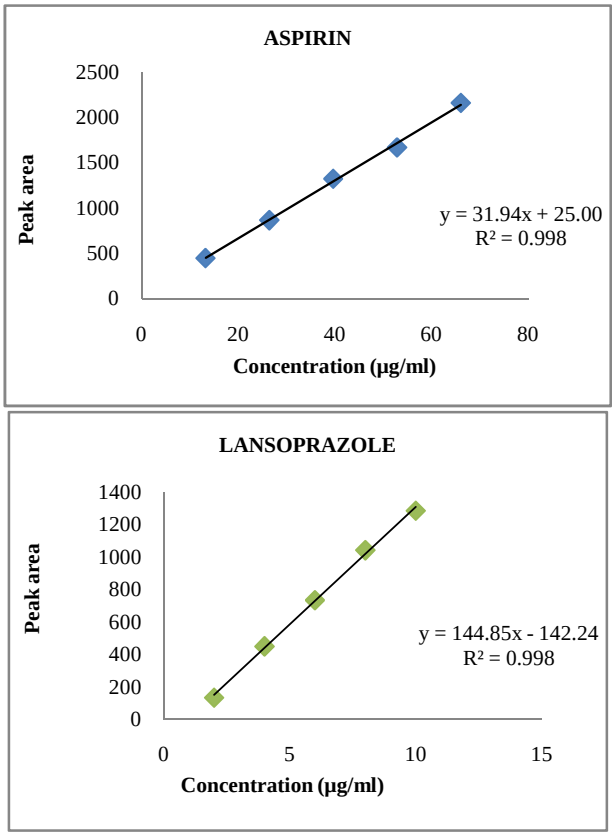

Fig. 4 calibration curve of Aspirin and Lansoprazole

$LOQ = 10 * SD/Slope$

Where, SD = Standard deviation of Y- intercept of 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out. Different amounts (80%, 100%, and 120%) from standard mixture of Aspirin and Lansoprazole within the linearity range were taken and added to the pre-analyzed synthetic mixture of concentration 100 µg/ml Aspirin and 15µg/ml Lansoprazole. From that percentage recovery values were calculated.

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, ± 0.2 mL/mins and wavelength of detection ± 2 nm, was studied to determine the robustness.

RESULTS AND DISCUSSION

Table 1 Calibration data for (n=3) Aspirin

Aspirin		
Conc. (µg/ml)	**Mean Area (mV*s) ± SD**	**% RSD**
13.2	446.88 ± 3.23	0.72
26.4	864.17 ± 7.71	0.89
39.6	1323.96 ± 6.88	0.52
52.8	1662.92 ± 9.37	0.56
66.0	2155.18 ± 12.99	0.60

Table 2 Calibration data for (n=3) Lansoprazole

Lansoprazole		
Conc. (µg/ml)	**Mean Area (mV*s) ± SD**	**% RSD**
2	131.25 ± 1.00	0.76
4	447.43 ± 4.26	0.95
6	723.05 ± 2.70	0.37
8	1040.30 ± 9.93	0.95
10	1283.37 ± 5.96	0.46

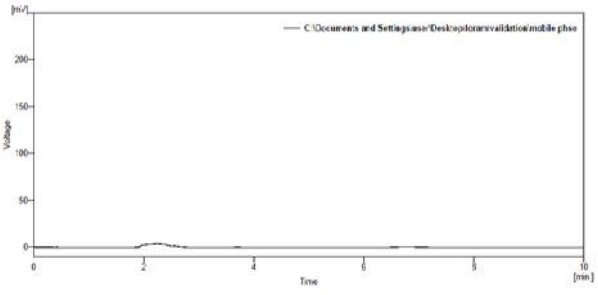

Fig. 5 Chromatogram of Blank (Mobile Phase)

Precision

Table 3 Repeatability Data (n=6)

Drug Name	Aspirin	Lansoprazole
Concentration	39.6 µg/ml	6 µg/ml
Mean area (mV*s) ± SD (n=6)	1316.67 ± 1.51	725.27 ± 1.00
%RSD	0.11	0.13

Table 4 Intraday Precision Data

Aspirin			Lansoprazole		
Conc. (µg/ml)	**Mean Area (mV*s) ± SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Mean Area (mV*s) ± SD (n=3)**	**% RSD**
13.2	454.63± 4.13	0.90	2	131.24 ± 1.00	0.76
39.6	1335.55 ± 9.63	0.72	6	722.52 ± 2.91	0.40
66.0	2187.11 ± 10.57	0.48	10	1285.04 ± 5.31	0.41

Table 5 Interday Precision Data

Aspirin			Lansoprazole		
Conc. (µg/ml)	**Mean Area (mV*s) ± SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Mean Area (mV*s) ± SD (n=3)**	**% RSD**
13.2	455.30 ± 5.06	1.11	2	131.97 ± 1.544	1.17
39.6	1336.62 ± 11.28	0.84	6	728.74 ± 7.64	1.04
66.0	2177.06 ± 15.21	0.69	10	1276.71 ± 15.21	1.19

Specificity

It is proven by comparing the chromatogram of blank (mobile phase), and Mobile phase with Excipients, standard solution and test preparation solution to show that there was no any interference of excipients with the peak of Aspirin and Lansoprazole.

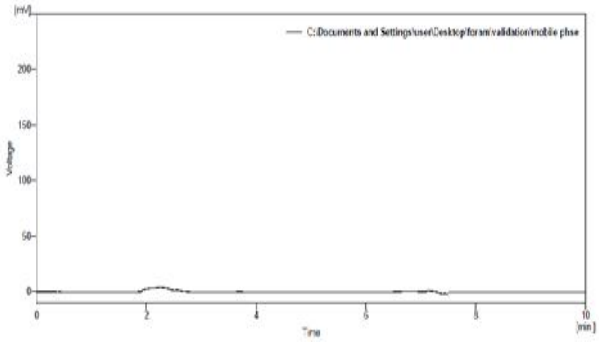

Fig. 6 Chromatogram of Mobile Phase with Excipients

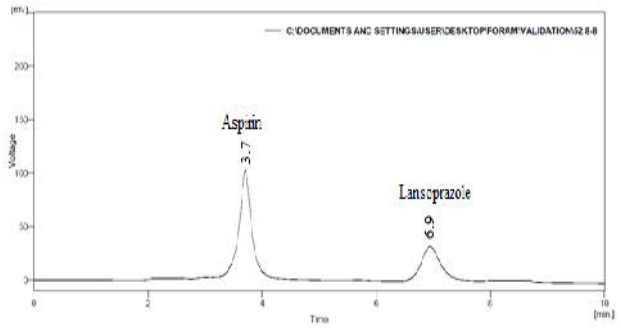

Fig. 7 Optimized chromatogram of Aspirin and Lansoprazole

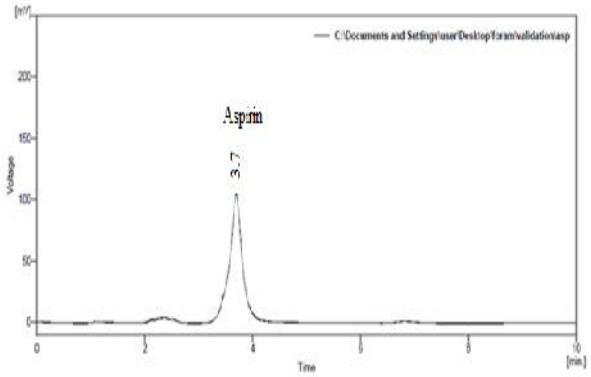

Fig. 8 Chromatogram of Aspirin

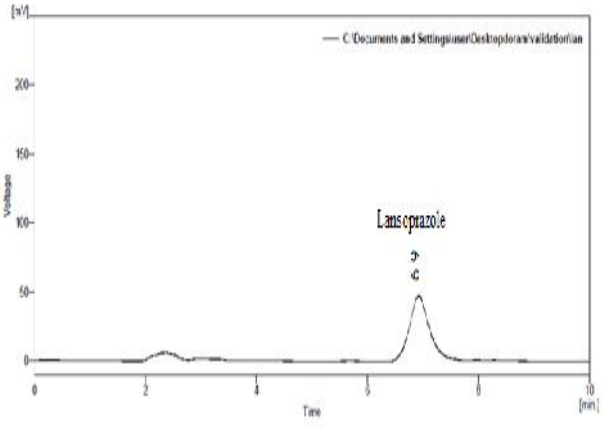

Fig. 9 Chromatogram of Lansoprazole

Table 6 System Suitability parameter

System Suitability Parameters	Proposed Method		Standard Values
	Aspirin	**Lansoprazole**	
Retention time (min)	3.70 ± 0.01	6.9 ± 0.01	-
Theoretical plates (N)	2243 ± 0.8	2354.00 ± 1.6	Greater than 2000
Resolution (R_S)	6.71 ± 0.08		Greater than 2
Tailing factor	0.96 ± 0.07	1.24 ± 0.04	Not greater than 2.0

Table 7 LOD and LOQ data

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Aspirin	0.0174	0.0517
Lansoprazole	0.2716	0.8450

Table 8 Robustness data

Parameter	Aspirin (%RSD)	Lansoprazole (%RSD)
Flow rate (± 0.2 mL/min)	0.390	0.532
Wavelength (± 2 nm)	0.570	0.307

Accuracy

Table 9 Accuracy Data

Drug name	Level of addition	Amount spiked (µg/ml)	Total amount (µg/ml)	Total amount obtained (n=3) ±SD	% Recovery±SD
Aspirin (26.4 µg/ml)	80 %	7.0	15.8	15.66±0.21	99.72 ±0.09 %
	100 %	8.8	17.6	17.68±0.12	100.60 ± 0.48 %
	120 %	10.5	19.3	19.34±0.10	99.73 ±0.13 %
Lansoprazole (4 µg/ml)	80 %	1.0	2.3	2.35 ±0.05	101.12 ±0.24%
	100 %	1.3	2.6	2.67 ±0.04	99.56 ±0.58%
	120 %	1.6	2.9	2.91 ±0.03	99.73 ±0.86 %

Table 10 Optical Regression Characteristics And Validation Parameters.

Parameter	Aspirin	Lansoprazole
Beer's Law Limit (µg/ml)	13.2-66.0	2-10
Regression equation (y = mx +c)	y = 31.949x + 25.002	y = 144.85x - 142.24
Correlation Coefficient (r^2)	0.998	0.998
Repeatability (% RSD, n=6)	0.11	0.13
Interday (n=3) (% RSD)	0.69 – 1.11	1.04-1.19
Intraday(n=3) (% RSD)	0.48-0.90	0.40-0.76
LOD(µg/ml)	0.0174	0.2716
LOQ(µg/ml)	0.0517	0.8450
Accuracy	99.72 -100.60%	99.56 – 101.12%

Table 11 Applicability to the synthetic mixture of Aspirin and Lansoprazole

Drug	Label claim	Amount found(mg) (n=3) ± SD.	%Label Claim ± SD.
Aspirin	100 mg	99.86±0.66	99.86%±0.66
Lansoprazole	15mg	14.93±0.15	100.44%±1.01

Application To Synthetic Mixture

Applicability of proposed method was tested by analysing the synthetic mixture of Aspirin and Lansoprazole.

CONCLUSION

Rapid, accurate and precise RP-HPLC method was developed and validated for simultaneous estimation of Aspirin and Lansoprazole in synthetic mixture. This method developed in Acetonitrile : Tris buffer : Methanol (30:40:30 % v/v/v) (pH 4.0) . The plot of area versus respective concentration was found to be linear in the concentration range of 13.2- 66.0 µg/ml for Aspirin and 2-10 µg/ml for Lansoprazole. The precision (intra-day, inter-day) of method were found within limits (% RSD <2%). Accuracy was determined by recovery studies and % recovery found between 98 to 102 %. This method can be successfully applied for the simultaneous estimation of Aspirin and Lansoprazole.

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