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High Performance Liquid Chromatography with PDA Detector for Combined Determination of Lansoprazole, Amoxicillin and Clarithromycin

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ABSTRACT

A simple, precise and accurate high performance liquid chromatography method is developed and validated for the estimation of lansoprazole, amoxicillin and clarithromycin as the bulk drug and in pharmaceutical dosage forms. Chromatographic separation of the drugs was performed on Symmetry Shield C8 (250 x 4.6 mm; 5 µm particle size) analytical column as the stationary phase. The solvent system consisted of 0.1% OPA and Methanol in the ratio of 50:50 (v/v) as mobile phase. Evaluation of the separated drugs was performed using a PDA detector covering the range of 200-400 nm. All the three drugs were resolved with the retention time of 2.546 min, 3.564 min and 4.923 min for lansoprazole, amoxicillin and clarithromycin, respectively. The method was validated with respect to linearity, sensitivity, precision, accuracy and robustness in accordance with ICH guidelines. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding good and reproducible result.

Keywords: *Lansoprazole; Amoxicillin; Clarithromycin; High-performance liquid chromatography; Simultaneous Determination.*

INTRODUCTION

Lansoprazole [1, 2] is a proton pump inhibitor which prevents the stomach from producing acid belongs to a class of antisecretory compounds, the substituted benzimidazoles. Chemically, amoxicillin is described as 2-{[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methane sulfinyl}-1H-1,3-benzodiazole. Lansoprazole suppress gastric acid secretion by specific inhibition of the (H^+,K^+)-ATPase enzyme system at the secretory surface of the gastric parietal cell. Because this enzyme system is regarded as the acid (proton) pump within the parietal cell. It blocks the final step of acid production. This effect is dose-related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus.

Amoxicillin [3, 4] is a broad-spectrum semi synthetic antibiotic belonging to o the class of organic compounds known as penicillins. Chemically, amoxicillin is described as (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino-2-(4-hydroxy phenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabi cyclo[3.2.0]heptane-24-carboxylicacid. Amoxicillin binds to penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell well. Penicillins acylate the penicillin-sensitive transpeptidase C-terminal domain by opening the lactam ring. This inactivation of the enzyme prevents the formation of a cross-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that amoxiclin interferes with an autolysin inhibitor.

Clarithromycin [5, 6] a semisynthetic macrolide antibiotic derived from erythromycin, inhibits bacterial protein synthesis by binding to the bacterial 50S ribosomal subunit. Chemically, clarithromycin is described as (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R) -6-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-eth yl-12,13-dihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy -4,6-dimethyloxan-2-yl]oxy}-7-methoxy-3,5,7,9,11,13-hexa methyl-1-oxacyclotetradecane-2,10-dione. Clarithro mycin is as a Cytochrome P450 3A4 Inhibitor, Cytochrome P450 3A Inhibitor, and P-Glycoprotein Inhibitor. Fig. 1, 2 and 3 shows the chemical structure of lansoprazole, amoxicillin and clarithromycin, respectively.

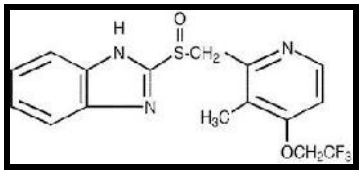

Fig. 1: Chemical structure of lansoprazole

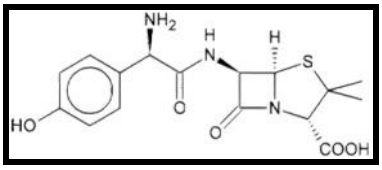

Fig. 2: Chemical structure of amoxicillin

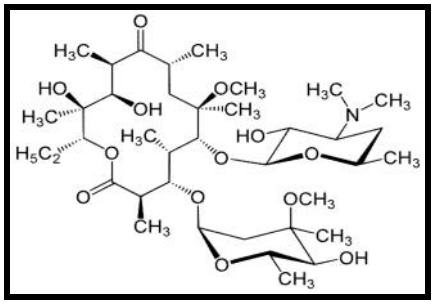

Fig. 3: Chemical structure of clarithromycin

The literature reports, many methods for simultaneous quantitative determination of lansoprazole, amoxicillin and clarithromycin in bulk, tablet dosage form, capsule dosage form and human plasma. These methods include simultaneous estimation of lansoprazole, amoxicillin and clarithromycin by UV spectrophotometry [10-13], HPLC [14-16] and LC-MS/MS [18-19].

The aim of the present investigation is to develop and

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validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of lansoprazole, amoxicillin and clarithromycin in bulk and in its combined pharmaceutical formulation.

MATERIALS AND METHODS

Apparatus:

A Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used in the present investigation.

Mobile phase:

The solvents and chemicals used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used was 0.1% OPA and Methanol in the ratio of 50:50 *v/v*. Before use, the mobile phase was filtered through millipore membrane filter and degassed for 15 minutes by sonication.

Chromatographic conditions:

Symmetry Shield C8 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of lansoprazole, amoxicillin and clarithromycin. The column temperature was maintained at 30±1^{0}C. The separation was carried out under isocratic elution. The flow rate was maintained as 1.0 ml/min. The injection volume was 10 µl. The eluents were detected at 322nm.

Standard solutions:

The standard stock solution was prepared by dissolving 30mg lansoprazole,500 mg of amoxicillin, and 500 mg of clarithromycin in 100 ml mobile phase. Working standard solutions equivalent to 3-9 µg/ml lansoprazole, 50-150 µg/ml amoxacillin and 50-150 µg/ml clarithromycin was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

Sample Solution:

Ten tablets were weighed and crushed to a fine powder. The powder equivalent of 30mg lansoprazole, 500mg of amoxicillin and 500mg of clarithromycin was taken in a 100ml volumetric flask containing 20ml of mobile phase, sonicated for 20minute and made up to mark with the same solvent. The resultant mixture was filtered through 0.45µm filter paper. The filtrate was diluted appropriately with the mobile phase to get a final concentration of 30mg lansoprazole, 500mg of amoxicillin and 500mg of clarithromycin.

RESULTS AND DISCUSSION

HPLC parameters optimization:

The main aim of this study is to simultaneously analyze lansoprazole, amoxicillin, and clarithromycin with sufficient resolution in reasonable analysis time. To obtain a good chromatographic condition, two different stationary phases were tested by name Inertsil C8 (250 x 4.6mm; 5 µm particle size) and Symmetry Shield C8 (250 x 4.6 mm; 5 µm particle size). Similarly, various mobile phases with isocratic elution were also tested to obtain good chromatographic condition:

1. 0.1M NaH2PO4: Methanol (60:40, *v/v*)
2. 0.1M KH_2PO_4: Methanol (60:40, *v/v*)
3. 0.1% OPA: Methanol (60:40, *v/v*)
4. 0.1% OPA: Methanol (60:40, *v/v*)
5. 0.1% OPA: Methanol (50:50, *v/v*)

The peak shape and system suitability parameters of lansoprazole, amoxicillin, and clarithromycin were good with Symmetry Shield C8 (250 mm × 4.6 mm, 5 µm) column. Hence this analytical column was selected. The good performance and better separation was achieved with the mobile phase combination 0.1%OPA and methanol in the ratio of 50:50*v/v* using Symmetry Shield C8 (250 mm × 4.6 mm, 5 µm) column. The isocratic elution with a flow rate of 1 mL/min was optimized.

Under the optimized chromatographic conditions, the chromatogram (Fig. 4) obtained, demonstrated a good separation of the lansoprazole (RT = 2.546 min), amoxicillin (RT = 3.564 min) and clarithromycin (RT = 4.923 min) from each other.

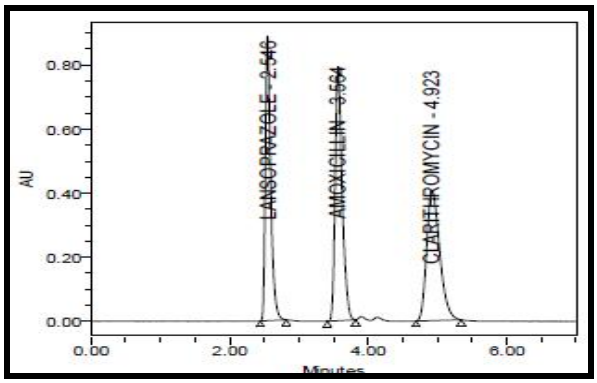

Fig. 4: Typical chromatogram of lansoprazole, amoxicillin, and clarithromycin

Method validation:

The optimized RP-HPLC method for simultaneous assay of lansoprazole, amoxicillin, and clarithromycin was validated according to ICH guidelines [24] with respect to system suitability, linearity, sensitivity, accuracy, precision and robustness.

System suitability:

Prior to analysis, the chromatographic system must satisfy system suitability test requirements. System suitability test was assessed from five replicate injections of the standard solution containing 30, 500 and 500µg/mL lansoprazole, amoxicillin, and clarithromycin, respectively. All the three peaks were well resolved and the precision of injections for all the peaks were acceptable. The percent relative standard deviation of the lansoprazole, amoxicillin, and clarithromycin peaks area responses were determined to be less than 1. The USP tailing factor and USP plate count were also calculated. The results of system suitability in comparison with the required limits are shown in Table 1 and are found to be within the accepted limits.

Table No. 1: System suitability test of the HPLC method

Parameters	Results			Recommended limits
	Lansoprazole	Amoxicillin	Clarithromycin	
Retention time	2.546	3.564	4.923	-
Peak area	4733134 (%RSD – 0.9)	5502562 (%RSD – 0.6)	5262795 (%RSD – 0.6)	RSD ≤ 1
USP resolution	-	6.10	4.92	> 1.5
USP plate count	5579	6104	3224	> 2000
USP tailing factor	1.29	1.26	1.31	≤ 2

Linearity and range:

The linearity of the method was determined by analyzing five different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 3-9 µg/ml lansoprazole, 50-150 µg/ml amoxacillin and 50-150 µg/ml clarithromycin. The parameters such as a regression equation and regression coefficient are given in Figures 4 and 5. The results show a good correlation between the peak areas of the drugs and their corresponding concentrations.

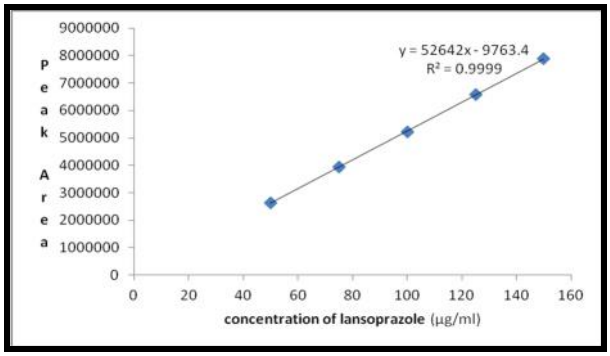

Fig. 5: Linearity curve of lansoprazole

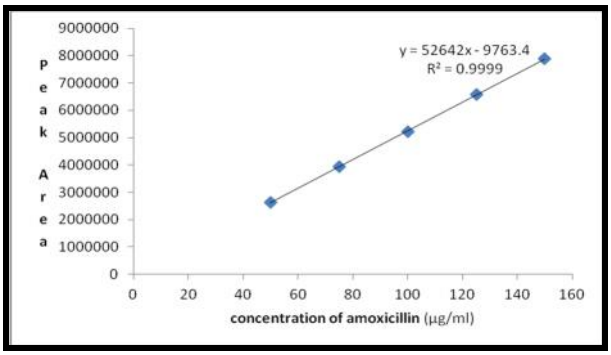

Fig. 6: Linearity curve of amoxicillin

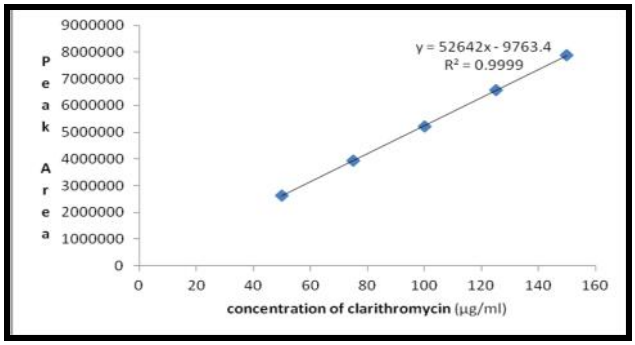

Fig. 7: Linearity curve of clarithromycin

Sensitivity:

The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ) according to ICH guidelines. The results are summarized in Table 2. The low values of LOD and LOQ demonstrate the sufficient sensitivity of the method. The chromatograms of lansoprazole, amoxicillin and clarithromycin at LOD and LOQ levels are presented in Fig. 8 & 9.

Table 2: Sensitivity of the HPLC method

Parameters	Results		
	Lansoprazole	Amoxicillin	Clarithromycin
LOD	2.552	3.559	4.927
LOQ	2.554	3.562	4.933

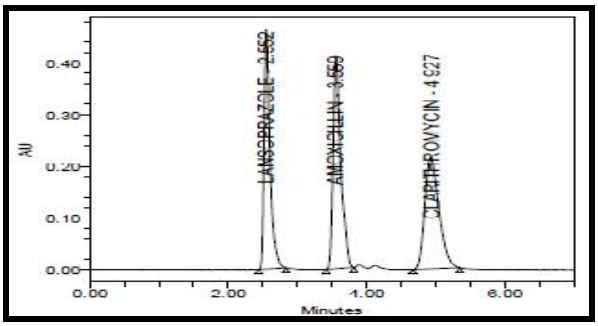

Fig. 8: Chromatogram of lansoprazole, amoxicillin and clarithromycin at LOD level

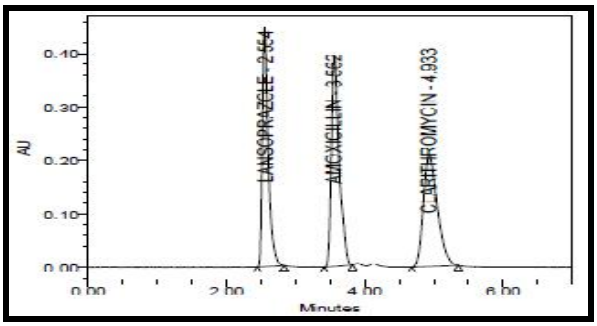

Fig. 9: Chromatogram of lansoprazole, amoxicillin and clarithromycin at LOQ level

Precision:

Precision was determined by injecting six standard solutions of lansoprazole (30µg/ml), amoxicillin (500µg/ml), clarithromycin (500µg/ml). The peak areas were determined. Relative standard deviationn of peak areas of the two drugs was then calculated to represent precision. The results are summarized in Table 3. The low % RSD values indicated that the method was precise.

Table No. 3: Precision of the HPLC method

Lansoprazole		Amoxicillin		Clarithromycin	
Peak area	%RSD	Peak area	%RSD	Peak area	%RSD
4731954	0.06	5500483	0.06	5260973	0.06
4735502		5501154		5268080	
4731343		5506233		5260706	
4736594		5507969		5264094	
4738023		5508260		5267111	
4733104		5501994		5265103	

Accuracy:

Accuracy of the method was evaluated by recovery studies at three concentration (50%, 100%, and 150%) levels by standard addition method. The mean percentage recoveries obtained were shown in Table 5. The good % recovery values showed that the method was highly accurate.

Table No. 4: Accuracy of the HPLC method

Drug	Spiked Level	µg/ml added	µg/ml found	% Recovery	% Mean
	50%	2.970	2.97	100	100
	50%	2.970	2.98	100	
	50%	2.970	2.97	100	
	100%	5.940	5.96	100	100
Lansoprazole	100%	5.940	5.96	100	
	100%	5.940	5.97	100	
	150%	8.910	8.93	100	100
	150%	8.910	8.93	100	
	150%	8.910	8.93	100	
	50%	50.000	50.03	100	100
	50%	50.000	49.92	100	
	50%	50.000	49.93	100	
	100%	100.000	99.88	100	100
Amoxicillin	100%	100.000	99.91	100	
	100%	100.000	99.87	100	
	150%	150.000	149.77	100	100
	150%	150.000	149.72	100	
	150%	150.000	149.75	100	
	50%	49.500	49.52	100	100
	50%	49.500	49.34	100	
	50%	49.500	49.64	101	
	100%	99.000	99.11	100	100
Clarithromycin	100%	99.000	99.49	100	
	100%	99.000	99.53	100	
	150%	148.500	148.48	100	100
	150%	148.500	148.76	100	
	150%	148.500	148.89	100	

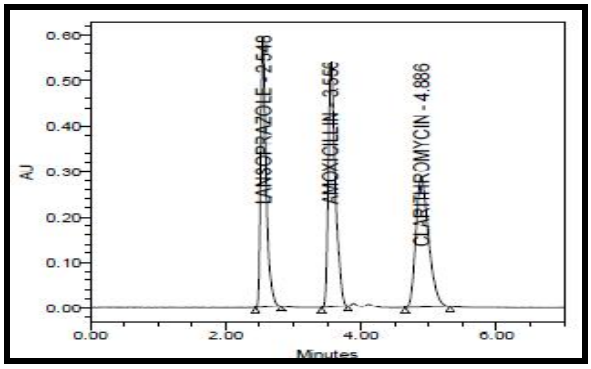

Fig. 10: Chromatogram of lansoprazole, amoxicillin and clarithromycin at 50% level

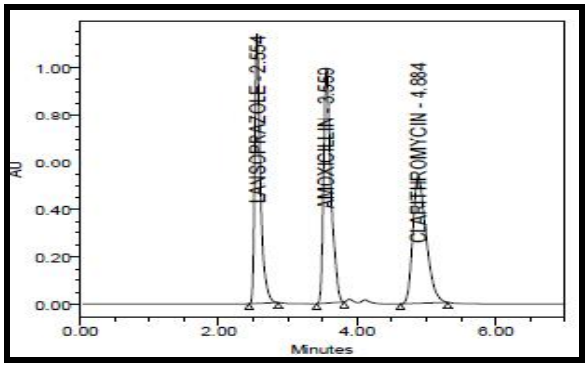

Fig. 11: Chromatogram of lansoprazole, amoxicillin and clarithromycin at 100% level

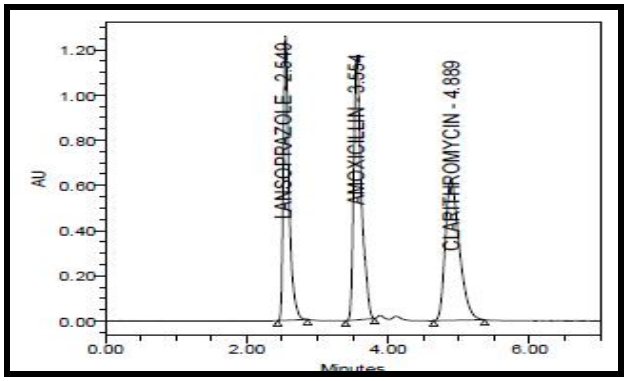

Fig. 12: Chromatogram of lansoprazole, amoxicillin and clarithromycin at 150% level

Robustness:

In order to show the robustness of the method, system suitability parameters were evaluated at different flow rate and different column temperature. The parameters used to define robustness are retention time, USP tailing factor and USP plate count. The results showed (Table 5) that slight variations in method parameters had a negligible effect on the analysis.

Table No. 5: Robustness of the method

Drug	Parameter	Retention time	Peak area	USP Plate Count	USP Tailing
Lansoprazole	**Flow 1**	2.554	5439682	4735	1.23
	Flow 2	2.547	5566472	4792	1.30
	Temperature 1	3.126	6881412	5265	1.31
	Temperature 2	2.169	4742454	4560	1.28
Amoxicillin	**Flow 1**	3.560	6326668	5280	1.33
	Flow 2	3.533	6412512	5495	1.33
	Temperature 1	4.366	7995323	5049	1.33
	Temperature 2	3.019	5463317	5373	1.34
Clarithromycin	**Flow 1**	4.890	6127584	3217	1.28
	Flow 2	4.760	6267833	3026	1.29
	Temperature 1	6.030	7714631	3343	1.28
	Temperature 2	4.160	5270176	3043	1.28

CONCLUSION

A RP-HPLC method has been reported for simultaneous estimation lansoprazole, amoxicillin and clarithromycin. The proposed method gives good resolution of the above said drugs. The validation of developed method was done as per ICH guidelines and proved that method to be simple, sensitive, precise, accurate and robust. The validated method was successfully applied to the determination of commercially available pharmaceutical dosage form. Hence, the method can be used for the routine quality control analysis of pharmaceutical dosage forms containing lansoprazole, amoxicillin and clarithromycin.

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