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Simultaneous Estimation of Clarithromycin, Pantoprazole and Metronidazole in Bulk and Pharmaceutical Formulations by RP-HPLC Method

P.Satyanarayana[1]*, K.L.N.N.S.V.K.Pavankumar[1], A.Srinivasa Rao[1], G. Subrahmanya Sastry[2]

1. Department of chemistry, Andhra Loyola College, Acharya Nagarjuna University, Vijayawada, India.

2.Head Of The Department of chemistry, Andhra Loyola College, Acharya Nagarjuna University, Vijayawada, India.

ABSTRACT

A simple, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of clarithromycin, pantoprazole and Metronidazole in bulk and pharmaceutical formulations. Separation of clarithromycin, pantoprazole and Metronidazole was successfully achieved on a YMC Pack Pro C18 (250 mm x 4.6mm x 5µ) in an isocratic mode utilizing sodium dihydrogen phosphate buffer and methanol (65:35 *v/v*) at a flow rate of 1.0 mL/min. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 31-93.75 mg/mL for clarithromycin and 5-15 mg/mL for pantoprazole and 50-150 mg/mL for Metronidazole. The correlation coefficient was found to be 0.999 for both the drugs. The limit of detection (LOD) was 0.228, 0.0309 and 0.743 for clarithromycin, pantoprazole and Metronidazole respectively. The limit of quantification (LOQ) was 0.758, 0.1030 and 2.475 for clarithromycin, pantoprazole and Metronidazole respectively. The relative standard deviation (RSD) of six replicates is less than 2%. This HPLC method is applied successfully to the simultaneous quantitative analysis of clarithromycin, pantoprazole and Metronidazole in commercial tablets.

Keywords: RP-HPLC, clarithromycin, pantoprazole and Metronidazole, pharmaceutical formulation, analysis.

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*Corresponding Author Email: satya498@gmail.com
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INTRODUCTION

Clarithromycin[1,2] 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-ethyl-12,13-di hydroxyl-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-7-methoxy -3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione. Clarithromycin is as a Cytochrome P450 3A4 Inhibitor, Cytochrome P450 3A Inhibitor, and P-Glycoprotein Inhibitor. The chemical structure of the clarithromycin is shown in Figure 1. In the literature, several analytical techniques were reported for the quantification of clobetasol. They include spectrophotometry[3,4], HPLC[5,6,7] and LC-MS[8,9] methods are reported for the determination of clarithromycin.

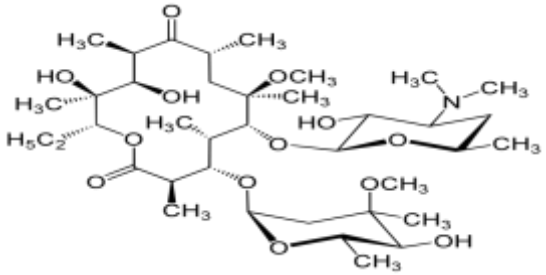

Figure 1: Chemical structure of clarithromycin

Pantoprazole[10,11] is a proton pump inhibitor (PPI) drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease. Chemically, pantoprazole is described as 6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methanesulfinyl]-1H-1,3-benzodiazole.It suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H^+,K^+)- ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect is dose- related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus. The chemical structure of the pantoprazole is shown in Figure 2. In the literature, several analytical techniques were reported for the quantification of pantoprazole. They include spectrophotometry[12,13,14], HPLC[15] and HPTLC[16].

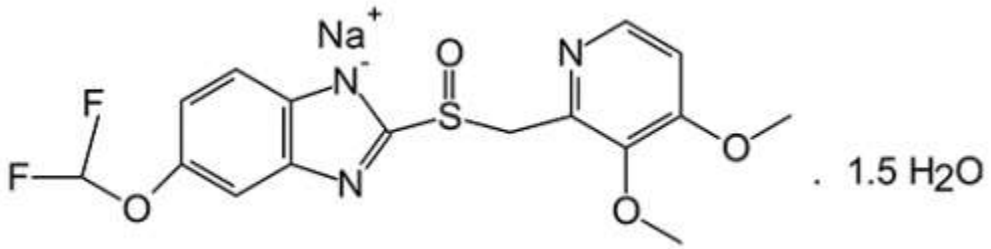

Figure 2: Chemical structure of pantoprazole

Metronidazole[17,18] is a nitroimidazole used to treat amebiasis; vaginitis; trichomonas infections; giardiasis; anaerobic bacteria; and treponemal infections. Chemically, Metronidazole is described as 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethan-1-ol. Metronidazole is a prodrug. Unionized metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduce metronidazole to its active form. This reduced metronidazole then covalently binds to DNA, disrupt its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death. The chemical structure of the Metronidazole is shown in Figure 3. In the literature, several analytical techniques were reported for the quantification of clobetasol. They include spectrophotometry[19] and HPLC[20].

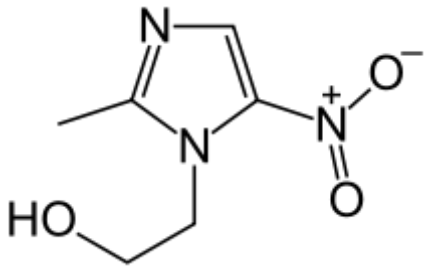

Figure 3: Chemical structure of Metronidazole

The detailed literature has indicted that there is no HPLC method for the simultaneous determination of clarithromycin, pantoprazole and Metronidazole in pharmaceutical formulation. Hence, there is a need for developing a HPLC method for the simultaneous estimation of both drugs in pharmaceutical formulation. Therefore, the present study was focused on the development of simple and rapid RP-HPLC method for the routine simultaneous analysis of clarithromycin, pantoprazole and Metronidazole in pharmaceutical formulations. The developed method was validated by following ICH guidelines[21].

MATERIALS AND METHOD

Chemicals and Reagents

Clarithromycin, pantoprazole and Metronidazole were obtained as a gift sample from Lara drugs pvt Ltd., Hyderabad. Sodium dihydrogen phosphate and methanol of HPLC grade was purchased

from Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from Sd Fine Chemicals Ltd., Mumbai. Mille Q water was used throughout the process.

Chromatographic Apparatus and Conditions

The development and validation of the assay was performed on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software. The analytical column used to achieve chromatographic separation was YMC Pack Pro C18, (250 mm × 4.6; 5µm) column. The mobile phase consisting of sodium dihydrogen phosphate buffer (pH 5.8) and methanol was degassed and pumped from the solvent reservoir in the ratio of 65:35 *v/v*. The flow rate was 1.0 mL/min. The column temperature was maintained at 30°C. The detection was performed at 264 nm and the run time was 12 min. Injection was carried out using a 10 µL loop. Prior to injection of the drug solution the column was equilibrated for at least 15 minutes with the mobile phase.

Standard Solution

250mg clarithromycin,40mg pantoprazole and 400mg Metronidazole was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 mL volumetric flask. Pipette out 2.5 mL of the above standard stock standard stock into 100 mL volumetric flask and dilute to volume with mobile phase.

Sample Solution

Accurately weigh 915.50 mg of sample. Transfer the sample powder into 100 mL volumetric flask. Add 10 mL mobile phase and sonicate for 20 minutes. The resulting solution was made up to the volume with mobile phase. Filter through the 0.45 µm filter paper. Transfer 2.5 mL of the above solution into a 100 mL volumetric flask and made up to the volume with mobile phase.

METHOD VALIDATION

System Suitability

System suitability tests are an integral part of liquid chromatographic method. System suitability was checked on each day of validation to evaluate the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established are number of theoretical plates, resolution and tailing factor.

Linearity

The linearity of the proposed method was constructed for clarithromycin, pantoprazole and Metronidazole standard solutions by plotting the concentrations of the compound versus peak area response. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried on the selected drugs at three different concentration levels (50%, 100% and 150%). The percentage recovery and standard deviation of the percentage recovery were calculated. The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage relative standard deviation were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage relative standard deviation were calculated.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as composition of mobile phase ratio and temperature of the column, and studying its effects on the performance of the method.

LOD and LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of clarithromycin, pantoprazole and Metronidazole. The LOQ and LOD values were calculated by using the following formula

1. LOQ = 10 σ / S
2. LOD = 3.3 σ / S

Where σ = residual standard deviation of response; S = slope of the calibration curve.

RESULTS AND DISCUSSION

System Suitability Studies

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fall within ±2 % Relative standard deviation range during routine performance of the method.

Table 1: System suitability

Parameter	Clarithromycin	Pantoprazole	Metronidazole
Retention time	2.531	4.222	8.711
Theoretical plates	3853	4325	4760
Tailing factor	1.33	1.18	1.13
% RSD	0.7	1.0	0.9

Linearity and range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. clarithromycin, pantoprazole and Metronidazole exhibited linearity of the concentration range of 31.25-93.75 µg/mL, 5-15 µg/mL and 50-150 µg/mL (Figures 4, 5 and 6).The regression equations for the selected drugs are:

Clarithromycin: y = 27017x +2686 (R2 = 0.9998)

Pantoprazole : y = 33001x + 25189(R2 = 0.9999)

Metronidazole : y = 64152x - 35747(R2 = 0.9999)

Where y = peak area and x = concentration of the drug in µg/mL

The results show an excellent correlation exists between areas and concentration of drugs. The results for calibration data are shown in Table 2 and calibration curves are given in Figure 4, 5, 6.

Table 2: Linearity data of clarithromycin, pantoprazole and Metronidazole

Clarithromycin		Pantoprazole		Metronidazole	
Area	**Amount of drug (µg/mL)**	**Area**	**Amount of drug (µg/mL)**	**Area**	**Amount of drug (µg/mL)**
1351921	31.25	1668462	5	3191468	50
2030781	46.88	2496736	7.5	4746137	75.00
2718170	62.50	3335606	10	6382126	100.00
3353519	78.125	4167707	12.5	7987997	125
4067723	93.75	4958142	15.00	9589539	150

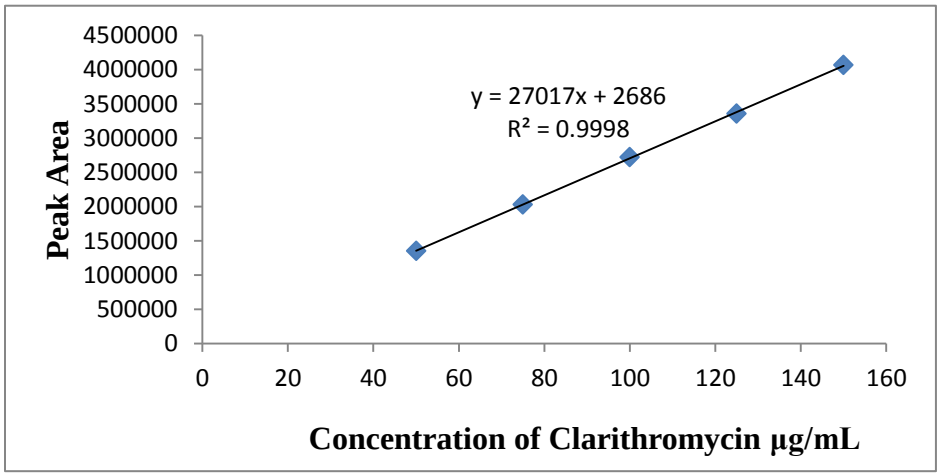

Figure 4: Linearity curve for clarithromycin

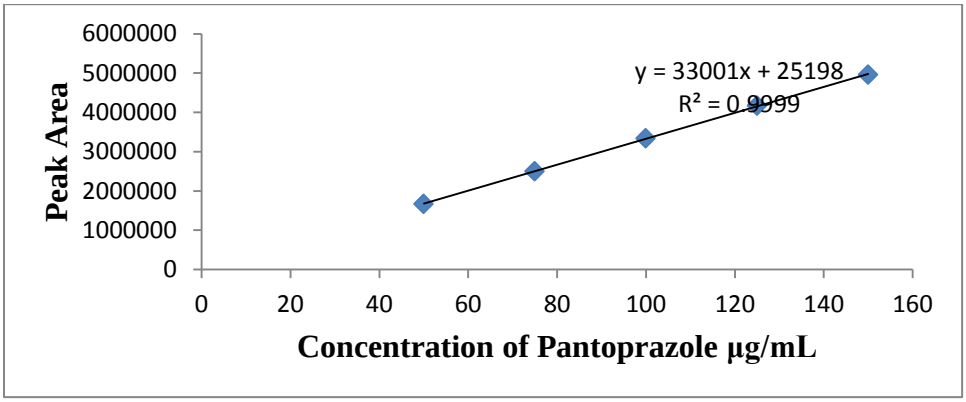

Figure 5: Linearity curve for pantoprazole

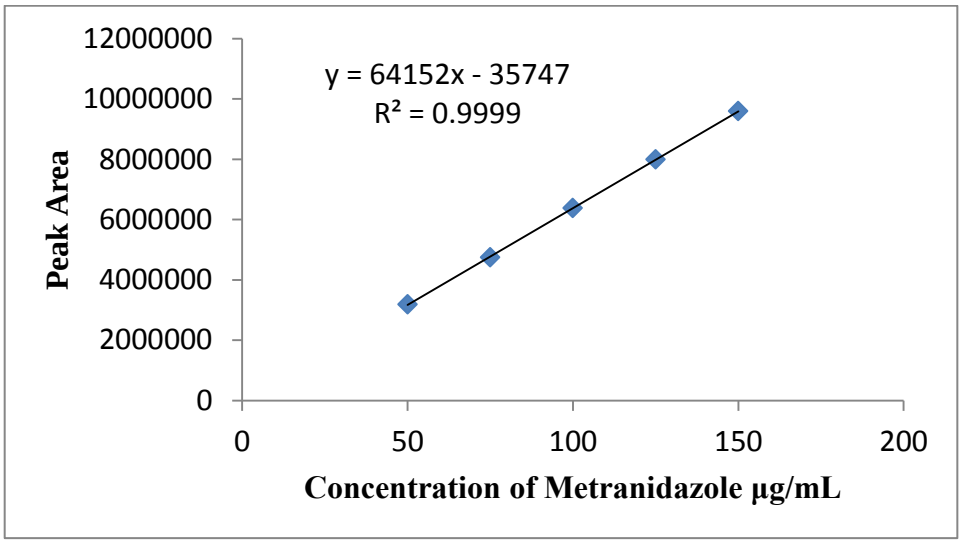

Figure 6: Linearity curve for Metronidazole

Accuracy and Precision

The results of accuracy of proposed methods at three different concentration levels are summarized in Tables 3, 4 and 5. The chromatograms at three different levels are shown in Figures 7, 8 and 9. From the results obtained, added recoveries of standard drugs were found to be accurate.

Table 3: Accuracy for clarithromycin

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	457.75	30.938	30.93	100	100
	457.75	30.938	30.94	100	
	457.75	30.938	30.92	100	
100%	915.50	61.875	62.13	100	100
	915.50	61.875	62.15	100	
	915.50	61.875	62.26	101	
150%	1373.25	92.813	93.03	100	100
	1373.25	92.813	93.06	100	
	1373.25	92.813	93.13	100	

Table 4: Accuracy for pantoprazole

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	457.75	5.000	4.99	100	100
	457.75	5.000	4.99	100	
	457.75	5.000	4.98	100	
100%	915.50	10.000	9.98	100	100
	915.50	10.000	9.99	100	
	915.50	10.000	9.99	100	
150%	1373.25	15.000	14.94	100	100
	1373.25	15.000	14.95	100	
	1373.25	15.000	14.95	100	

Table 5: Accuracy for Metronidazole

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	457.75	49.500	49.74	100	100
	457.75	49.500	49.75	101	
	457.75	49.500	49.65	100	
100%	915.50	99.000	99.40	100	100
	915.50	99.000	99.35	100	
	915.50	99.000	99.43	100	
150%	1373.25	148.500	149.23	100	100
	1373.25	148.500	149.24	100	
	1373.25	148.500	149.16	100	

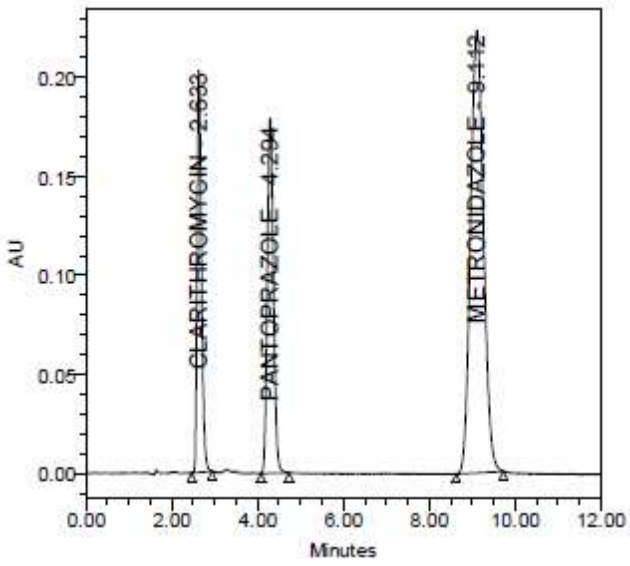

Figure 7: Chromatogram of clarithromycin, pantoprazole and Metronidazole at 50% level

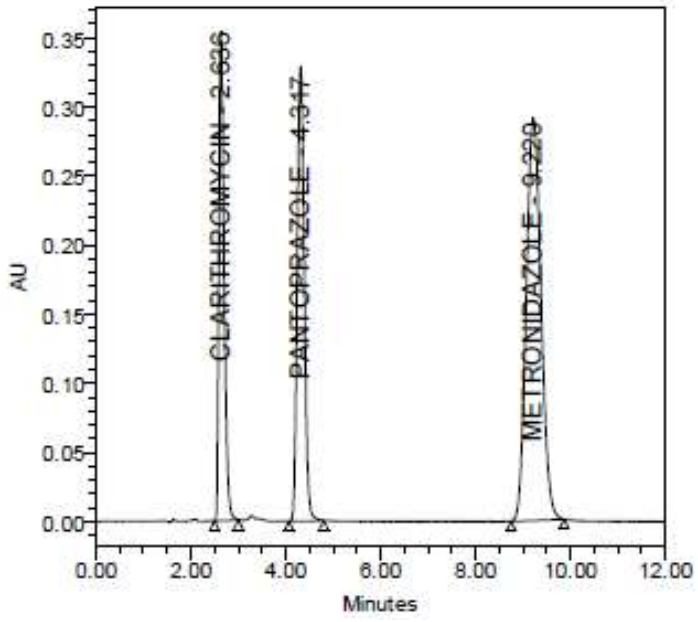

Figure 8: Chromatogram of clarithromycin, pantoprazole and Metronidazole at 100% level

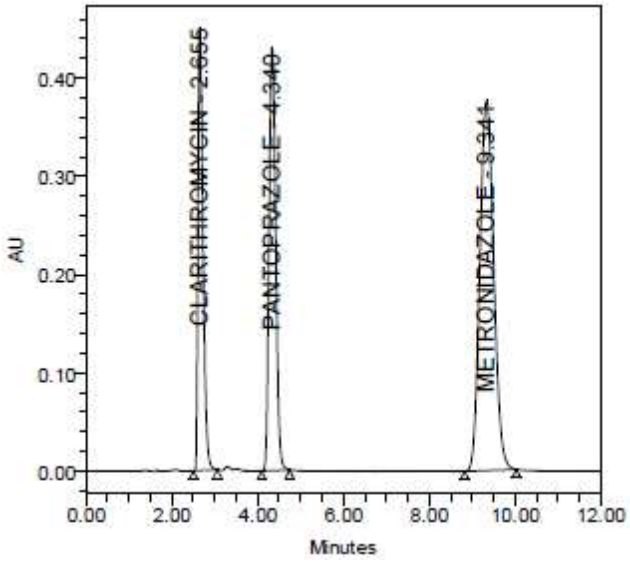

Figure 9: Chromatogram of clarithromycin, pantoprazole and Metronidazole at 150% level

The precision of the method was demonstrated by inter-day and intra-day variation studies. The results of the precision studies are tabulated in the Table 6. From the results obtained, the developed method was found to be precise for the simultaneous determination of clarithromycin, pantoprazole and Metronidazole.

Table 6: Precision of the method

	Clarithromycin		Pantoprazole		Metronidazole	
Sample wt (mg)	**Area**	**%Assay**	**Area**	**%Assay**	**Area**	**%Assay**
915.50	2717220	100	3337999	100	6388399	99
915.50	2715662	99	3339325	100	6385175	99

915.50	2718143	100	3336279	100	6383473	99
915.50	2716505	99	3331184	100	6385571	99
915.50	2712240	99	3336902	100	6386220	99
915.50	2715111	99	3333144	100	6388474	99

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions such as column temperature and mobile phase flow rate. It was observed that there were no marked changes in the analytical performance of the method. The results are shown in Table 7. The results demonstrated that the proposed method is robust.

Table 7: Robustness of the method

Sample no.	Sample Name	Retention time	Peak area	Theoretical plates	USP Tailing
Clarithromycin					
1	Temp-1	2.728	2707137	4131	1.29
2	Temp-2	2.657	2688049	3351	1.32
3	Flow-1	3.388	3416553	4977	1.30
4	Flow-2	2.289	2257297	3754	1.25
Pantoprazole					
1	Temp-1	4.390	3445287	4156	1.18
2	Temp-2	4.282	3425874	3377	1.23
3	Flow-1	5.508	4342208	4252	1.09
4	Flow-2	3.619	2850528	3441	1.24
Metronidazole					
1	Temp-1	9.670	6269893	3209	1.11
2	Temp-2	8.776	6348317	3725	1.15
3	Flow-1	12.009	7866323	4458	1.09
4	Flow-2	7.957	5206920	3517	1.21

Limit of quantification and limit of detection

Limit of quantification (LOQ) and limit of detection (LOD) gives information about the sensitivity of the method. The LOD and LOQ values for the clarithromycin, pantoprazole and Metronidazole are presented in Table 8. The chromatograms of LOD and LOQ are shown in Figures 10 and 11, respectively. The results indicated that the proposed method possess sufficient sensitivity.

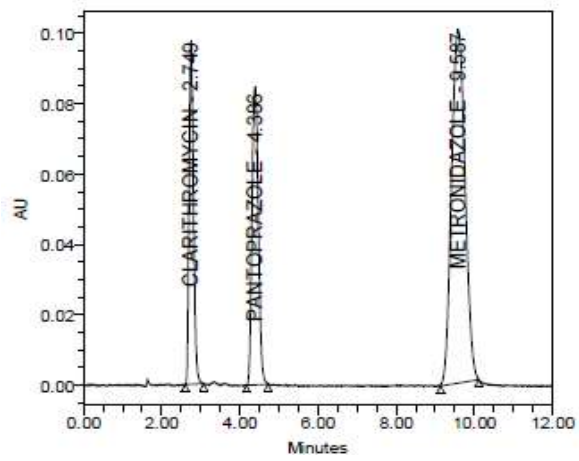

Figure 10: Chromatogram of LOD

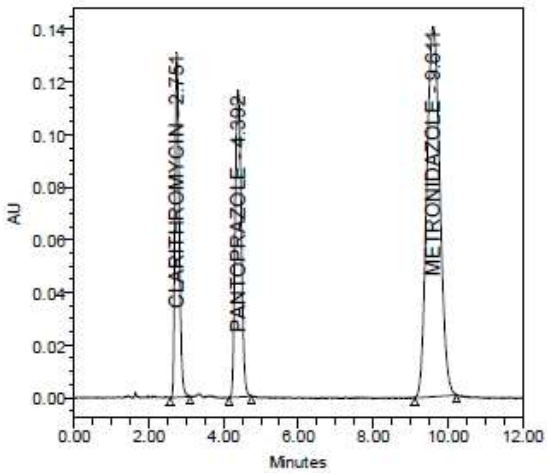

Figure 11: Chromatograms of LOQ

Table 8: LOD and LOQ for clarithromycin, pantoprazole and Metronidazole

Sample Type	Sample name	RT	Area	Value
LOD	Clarithromycin	2.749	764067	0.228
LOQ	Clarithromycin	2.751	1043095	0.758
LOD	Pantoprazole	4.386	967735	0.0309
LOQ	Pantoprazole	4.392	1344067	0.1030
LOD	Metronidazole	9.587	2404261	0.743
LOQ	Metronidazole	9.611	3410521	2.475

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of clarithromycin, pantoprazole and Metronidazole pharmaceutical formulations. Hence, this method can easily and conveniently adopt for routine quality control analysis of clarithromycin, pantoprazole and Metronidazole in pure and in its pharmaceutical formulations.

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