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Research Article

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lansoprazole, Clarithromycin and Tinidazole in Combined Dosage Form

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A B S T R A C T

Analytical chemistry is one of the supreme branches of science which deals with the separation, identification and quantification of various natural and chemical compounds. It includes both qualitative and quantitative analysis. Quantitative analysis is having importance from the point of pharmaceutical chemistry. Quantification methods include chromatographic techniques, spectrophotometery, fluorimetery etc. One of the most popular method for quantitative analysis is High Performance Liquid Chromatography (HPLC). In this article, a reverse phase – HPLC method was developed for the simultaneous determination of Lansoprazole, Clarithromycin and Tinidazole in combined dosage form and validation of the developed method. The development of method includes selection of mobile phase, chromatographic method and wavelength whereas validation involves the parameters like linearity, accuracy, precision, Limit of detection (LOD), Limit of Quantification (LOQ), robustness, system suitability of the developed method. The result showed that the developed method is best fitted to the simultaneous determination of Lansoprazole, Clarithromycin and Tinidazole and validated as per standards.

Keywords: Validation, HPLC, accuracy, precision

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INTRODUCTION

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. There are many reasons for the development of newer methods of drug analysis as a proper analytical procedure for the drug may not be available in the literature due to patent regulations. Analytical methods for the quantification of the drug in biological fluids may not be available. Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients. [1] Analytical methods for a drug in combination with other drugs may not be available; the existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable etc. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Analytical techniques that are generally used for drug analysis are biological and microbiological methods, radioactive methods, physical methods and miscellaneous techniques like conventional titrimetric, gravimetric and polarimetric methods. Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures. Mainly two modes are defined depending on the relative polarity of the two phases: normal and reversed-phase chromatography. Method development and design of separation method depends on

selection of best mobile phase, detector, column length and diameter, buffer, pH of buffer, type of stationary phase etc. In the chromatographic techniques all factors like detector, mobile phase, column length, and stationary phase should be fixed and must be validated. If any changes occurs in the chromatographic conditions various fine parameter like Resolution, Capacity factor, Selectivity, Column efficiency, Peak asymmetry factor could be affected and may change the end result. To avoid these type of errors one should follow proper validation guidelines of ICH in which some typical parameters are used for validation like specificity, accuracy, precision, linearity, limit of detection, limit of quantification, robustness. Literature assessment showed that various analytical methods has been reported for the estimation of Lansoprazole, Clarithromycin and Tinidazole and with combination also but combination does not fulfill regulatory requriment and with other drugs combination by HPLC in pharmaceutical dosage form. The aim of present study was to develop HPLC method for simultaneous estimation of Lansoprazole, Clarithromycin and Tinidazole and validate it. [5,9]

MATERIALS AND METHODS:

The present work aimed to develop assay method that is simple, accurate, rapid, precise, sensitive and reliable for the estimation of Lansoprazole, Clarithromycin and Tinidazole combination in different stress conditions as per ICH guideline Q2 (B). [5]

Drugs and chemicals

Working standards of Lansoprazole, Clarithromycin and Tinidazole were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples.

HPLC grade Water, Methanol and Acetonitrile were procured from Merck chemical division, Mumbai and tablets of PYLOKIT containing Tinidazole 10 mg, Lansoprazole 25 mg, Clarithromycin 5 mg were procured from the pharmacy store. [8]

Instrument

2996 series of Waters Photodiode array detector attached to 2995 series of Waters HPLC, which is having Hamilton syringe and autosampler was opted for chromatography. After the selection of the drug combination, both the drugs were dissolved in suitable diluent to get a clear solution. Based on the literature, phase chromatography was identified as a suitable chromatographic separation method. Mobile phase was optimized by modifying different combinations of buffers and organic solvents. The pka value of both the drugs was also considered for optimisation of pH of the buffer. The resolution and the peak shape of both the drugs found significant with the mobile phase composition of Orthophosphoric acid buffer and Acetonitrile at a flow rate of 1.0ml/min was used, excellent elution of the three drugs with low retention and run times was observed. Different columns like Xterra, Inertsil, Inspire columns were tried and finally X-bridge C18, 4.6x150 mm, 5µ column was finalized as the stationary phase. All the three drugs were scanned at different wave lengths in the range 205 nm-280 nm. Finally, 210 nm is identified as the iso-absorptive point for all the three drugs and hence it is used as detection wavelength in the present assay. The developed HPLC method is validated for simultaneous assay of Lansoprazole, Clarithromycin and Tinidazole in combined formulation in accordance to ICH guidelines by using the parameters like System Suitability, Specificity, Linearity, Accuracy, Repeatability,

Robustness and Limit of Detection (LOD)/Limit of Quantification (LOQ) [11,12]

RESULTS

Table 1: Optimized chromatographic conditions

Mobile Phase	Column	Detector wavelength	Injection Volume	Flow Rate
OPA buffer: Acetonitrile in gradient flow	Xbridge C-18 4.6 x150 mm, 5µ	210 nm	10 µL	1.0 mL/min

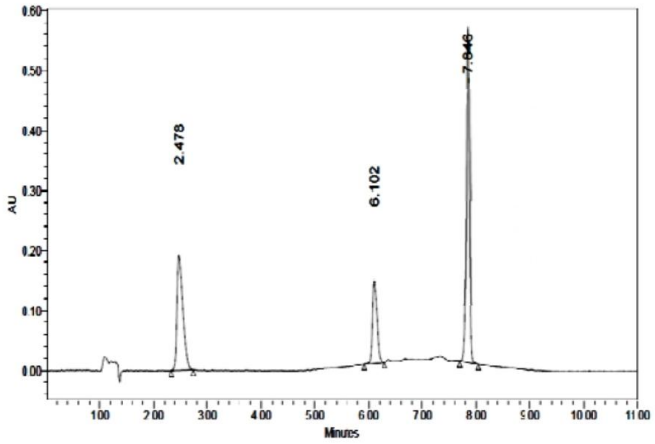

Figure. 1: Typical Chromatogram of LAN, CLA and TIN

Method Validation

The developed HPLC method is validated for simultaneous assay of Lansoprazole, Clarithromycin and Tinidazole in combined formulation in accordance to ICH guidelines by using the following parameters. [6]

System Suitability

System suitability test was performed by injecting six replicate injections 100% target solution of Lansoprazole (LAN), Clarithromycin (CLA) and Tinidazole (TIN). The parameters such as a number of theoretical plates, area and peak tailing were determined and were observed that all the parameters were within the limits. Results were shown in Table 2. [8]

Table 2: System suitability parameters

PARAMETERS	LAN	CLA	TIN
Area	1408485	786520	2195769
Tailing Factor	1.58	1.21	0.95
No of theoretical plates	2492	24368	91113

Specificity

The results showed no interference at the retention time of Lansoprazole, Clarithromycin and Tinidazole.

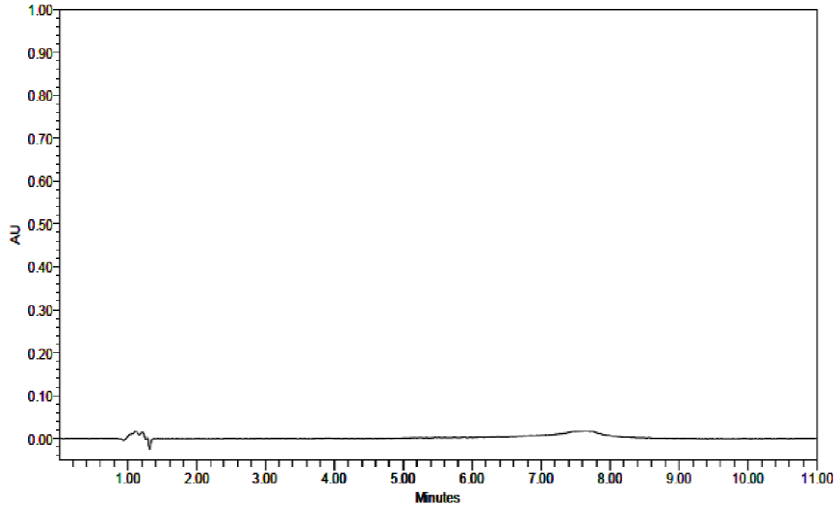

Figure 2: Placebo Chromatogram

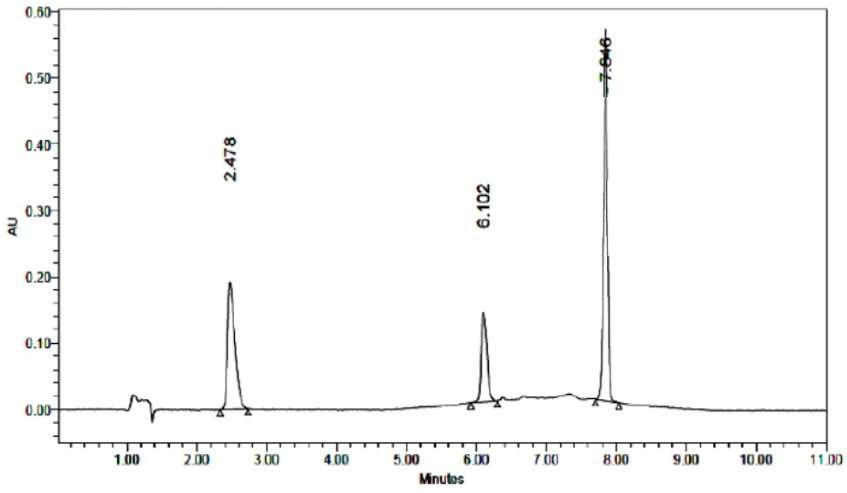

Figure 3: Standard Chromatogram of LAN, CLA and TIN.

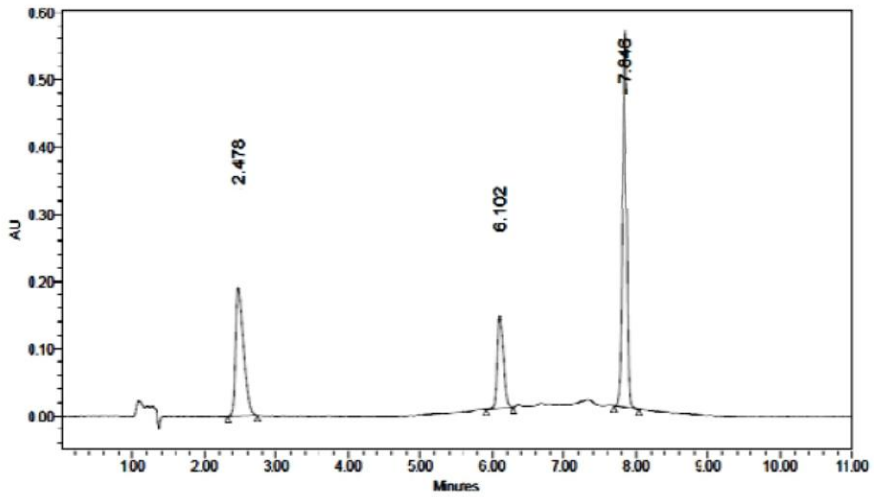

Figure 4: Sample Chromatogram of LAN, CLA and TIN

Linearity

Standard solutions of Lansoprazole (62.5-625.0 µg/mL), Clarithromycin(6.25-62.5µg/mL) and Tinidazole (12.5 µg/mL-125 µg/mL) respectively were prepared and injected under the chromatographic conditions described above. Calibration curves were drawn the concentration of drug versus corresponding peak areas obtained at 210 nm. The results showed a significant correlation between detector response and concentration level of each drug within the concentration range. The equation Y = (mx+c) was used to represent the linearity as follows:

Y (LAN) =5636.x +29570, Y (CLA) = 29436.x+20510 & Y (TIN) = 44950.x +35874

The results are given in Table 3-5 and the resulted chromatograms.[07]

Table 3: Linearity Table of Lansoprazole

PPM	Reponse_1	Reponse_2	Response_3	Average
0	0	0	0	0
62.5	407087	405661	406140	406296
125	766319	753091	758238	759216
250	1454784	1436190	1450549	1447174
312.5	1749146	1742191	1742202	1744513
375	2169142	2175661	2125178	2156660
625	3553455	3549114	3565993	3556187

Table 4: Linearity Table of Clarithromycin

PPM	Response_1	Response_2	Reponse_3	Average
0	0	0	0	0
6.25	202872	206396	202904	204057.3
12.5	401099	400729	404765	402197.7
25	763462	764974	762768	763734.7
31.25	946387	949931	948964	948427.3
37.5	1115248	1115314	1153691	1128084
62.5	1846458	1849315	1849344	1848372

Table 5: Linearity Table of Tinidaz**ole**

PPM	Reponse_1	Reponse_2	Response_3	Average
0	0	0	0	0
12.5	624247	624225	624694	624388.7
25	1193581	1157884	1186283	1179249
50	2298643	2273440	2283748	2285277
62.5	2894945	2831920	2803525	2843463
75	3345691	3361230	3484429	3397117
125	5617984	5674040	5670763	5654262

Accuracy

Known amounts of reference solution for all the three drugs Lansoprazole, Clarithromycin and Tinidazole equivalent to 50%, 100% and 150% of the label claim were added to the tablet solutions of Lansoprazole, Clarithromycin and Tinidazole. The percentage of analyte recovery was calculated, and the results were summarized in Table 6. The percent mean recovery for Lansoprazole, Clarithromycin and Tinidazole were 100.82, 100.05 and 100.70 respectively, indicating that the method was accurate[10.]

Table 6: Recovery Experiments of LAN, CAL and TIN

Amount Spiked			Standard Amount added			% Recovered		
LAN	CLA	TIN	LAN	CLA	TIN	LAN	CLA	TIN
125	12.5	25	250	25	50	102.29	99.30	101.31
125	12.5	25	250	25	50	102.19	99.61	101.74
125	12.5	25	250	25	50	102.28	100.91	98.50
250	25	50	250	25	50	99.89	99.88	99.87
250	25	50	250	25	50	100.43	101.12	100.37
250	25	50	250	25	50	100.21	98.43	101.33
375	37.5	75	250	25	50	99.87	100.44	101.01
375	37.5	75	250	25	50	99.99	100.74	100.89
375	37.5	75	250	25	50	100.25	100.02	101.31
					AVG	100.82	100.05	100.70
					SD	1.09	0.86	1
					%RSD	1.08	0.86	0.99

Repeatability

The precision of the developed method was assessed for intra-day (Precision) and inter-day (by varying the analyst and HPLC column called as intermediate precision). The% RSD for Lansoprazole, Clarithromycin and Tinidazole were calculated, which found to be within the acceptable limits (RSD < 2). [3]

Table 7: Precision data for LAN, CLA and TIN

	(% Assay)					
	Intra Day			Inter Day		
	LAN	CLA	TIN	LAN	CLA	TIN
Sample 1	99.17	102.28	98.92	99.19	99.2	98.88
Sample 2	99.15	100.98	99.12	98.97	98.33	99.14

Sample 3	100.36	99.029	100.08	100.39	98.67	100.07
Sample 4	100.56	100.55	102.09	100.61	101.18	102.13
Sample 5	99.85	99.19	99.28	99.89	101.92	99.27
Sample 6	100.89	97.95	100.49	100.93	100.68	100.49
%Mean	100.00	100.00	100.00	100.00	100.00	100.00
SD	0.73	1.57	1.19	0.79	1.47	1.21
%RSD	0.73	1.57	1.19	0.79	1.47	1.21

Robustness

Robustness is performed by making slight variations in the Flow rate, column temperature and concentration of the mobile phase. The changes and the results were tabulated No significant effect was observed with the above changes indicating the robustness of the method.

Table 8: Robustness table of LAN, CLA and TIN

	Changed value	Retention time			Tailing factor			% Assay		
		LAN	CLA	TIN	LAN	CLA	TIN	LAN	CLA	TIN
Column	25	2.314	6.054	7.475	1.27	0.93	0.92	101.8	101.5	101
Temperature	35	2.415	6.115	7.870	1.37	0.83	0.72	100.7	100.9	101.3
Flow Rate	0.9 mL	2.116	6.044	7.435	1.51	1.13	0.96	98.5	99	98.9
	1.1 mL	2.006	5.945	7.335	1.51	1.13	0.96	100.1	99.2	99.2
Mobile- phase	-5	2.125	5.995	7.415	1.51	1.13	0.96	98.8	99	99.4
	+5	2.015	5.884	7.315	1.12	1.88	0.97	101.2	101.9	101.6
		Average						100.18	100.25	100.20
		Std Dev						1.32	1.34	1.19
		% RSD						1.31	1.33	1.19

LOD and LOQ

In the present chapter LOD and LOQ of LAN, CLA and TIN were determined by linearity curve method. LOD and LOQ were calculated by using the equations. LOD = 3.3 σ/S and LOQ= 10 σ/S. Where"σ" is the standard deviation of the response, and "S" is the slope of the linearity curve. The LOD values were 2.29 µg/mL, 0.05 µg/mL and 1.08 µL for LAN, CLA and TIN respectively. The LOQ values were 6.95µg/mL, 0.15 µg/mL, and 3.26 µg/mL for LAN, CLA and TIN respectively. [5-7]

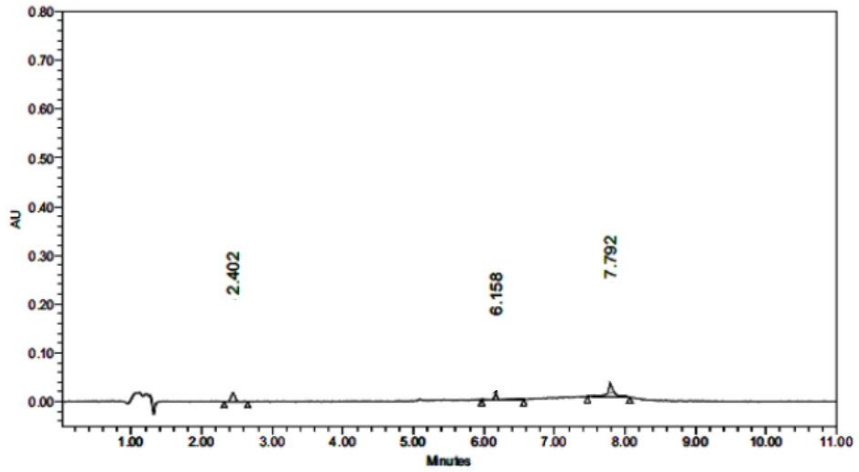

Figure. 5: LOD Chromatogram of LAN, CLA and TIN

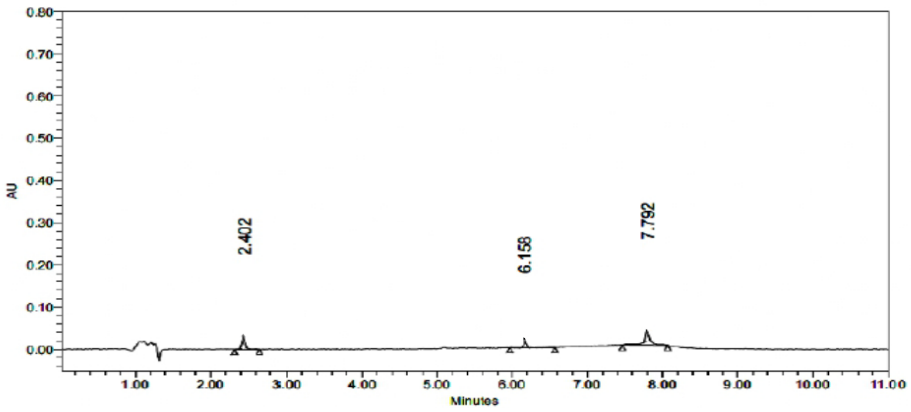

Figure: 6 LOQ Chromatogram of LAN, CLA and TIN

Analysis of formulations

The proposed method was applied for the estimation of Lansoprazole, Clarithromycin and Tinidazole in tablet dosage form and the results are reported in Table 9.The high recovery with low RSD value confirmed the appropriateness of the proposed method for the routine analysis of Lansoprazole, Clarithromycin and Tinidazole in tablet dosage forms.

Table 9: HPLC Data for Analysis of Tablets

Sample No	Peak Area			% Assay		
	LAN	CLA	TIN	LAN	CLA	TIN
1	1429632	775736	2211971	100.26	100.48	99.26
2	1412020	757810	2191829	99.02	98.16	98.35
3	1403625	759296	2194494	98.44	98.35	98.47
4	1403266	759840	2193044	98.41	98.42	98.41
5	1401293	759386	2199506	98.27	98.36	98.70
6	1402562	757759	2189486	98.36	98.15	98.25
			AVG	98.79	98.65	98.57
			SD	0.77	0.90	0.37
			%RSD	0.78	0.91	0.37

DISCUSSION

Method development

Several mobile phase compositions were tried to get good optimum resolutions of Lansoprazole, Clarithromycin and Tinidazole peaks. The mobile phase OPA buffer: Acetonitrile in gradient flow was selected because it gave sharp peaks with good resolution, minimum tailing and satisfactory retention time. The drugs having appreciable absorbance at 210 nm and therefore 210 nm was selected as the detection wavelength. The working standard solutions of Lansoprazole, Clarithromycin and Tinidazole were injected separately. The retention time of Lansoprazole, Clarithromycin and Tinidazole was found to be 2.4 min, 6.1 min and 7.8 min respectively when injected as individual compounds.

Validation of the method: The validation of the method was done by various methods in terms of linearity, precision, accuracy, robustness, LOD/LOQ, specificity and ruggedness/system suitability as per ICH guidelines.

To evaluate the linearity of method, six replicate measurements were done. The linearity range of Lansoprazole,Clarithromycin and Tinidazole were found 62.5-625 PPM, 6.25-62.5 PPM and 12.5-125 PPM respectively. The obtained data demonstrates that method has sufficient sensitivity to the concentrations of analytes.

The precision of the method was determined by performing six independent assays of the test samples and inter-day precision was checked by doing same procedure on different days by another person under the

same experimental conditions. The %RSD for intra-day precision for Methionine, Pyridoxine hydrochloride, Nicotinamide was found to be 0.73, 1.57, 1.19 respectively. The %RSD for inter-day precision for Lansoprazole,Clarithromycin and Tinidazole was obtained 0.79, 1.47, 1.21 respectively. Hence the method was showing high degree of precision. [7,9]

The accuracy of the method was evaluated by doing recovery studies. The recovery experiments were performed at three concentrations levels i.e. 50, 100 and 150%. Each level was repeated for six times. The recovery for Lansoprazole,Clarithromycin and Tinidazole were found to be 100.82%, 100.05% and 100.70% respectively. So the method is adequately accurate.

The LOD/LOQ was determined by serial dilutions of Methionine, Pyridoxine hydrochloride, Nicotinamide stock solutions by the proposed method. [10=[-] The LOD values for Lansoprazole,Clarithromycin and Tinidazole were found to be 2.40, 6.15 and 7.79 respectively and the LOQ values for Lansoprazole,Clarithromycin and Tinidazole were 2.40, 6.15 and 7.79 respectively. The robustness of the method was checked by doing certain experiments using changing conditions like flow rate and temperature. No significant effect was seen on chromatographic resolution and hence the developed method was found to be robust.

The system suitability was checked by injecting seven replicates of working standard solution at six min interval. The parameters such as a number of theoretical plates, area and peak tailing were determined and were observed that all the parameters were within the limits.

CONCLUSION

For the first time, a new HPLC method was developed and validated for simultaneous estimation of Lansoprazole, Clarithromycin and Tinidazole in tablet dosage form. The calibration curve was found to be linear over a concentration range of 62.5-625 µg/mL, 6.25-62.5 µg/mL and 12.5-125 µg/mL for Lansoprazole, Clarithromycin and Tinidazole respectively. A linear equation was established to provide the best fit for the concentration vs. detector response. The goodness of fit was consistently found to be 0.99 during the course of validation. No interference or overlapping of the peaks either due to excipients or diluents was observed during the Selectivity experiment at the retention time of Lansoprazole, Clarithromycin and Tinidazole. The obtained value of % RSD for precision studies which was < 2 confirms that proposed method is effectively precise. Furthermore, the separation of the above analytes was completed within 11minutes respectively, making the proposed HPLC method conveniently adopted for the routine quality control analysis of other combination formulations containing these drugs.

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