RESEARCH PAPER

A RP-HPLC Method Development, Validation And Estimation Of Clarithromycin

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ABSTRACT

New RP-HPLC method was developed for the simultaneous estimation of clarithromycin. RP-HPLC method was a simple, reliable and acceptable and it confirmed that method is suitable for the intended use for routine quality control and assay of drugs. This method is successfully applied for the determination of commercial dosage form preparation. This method is validated as per ICH (International conference on harmonization) guidelines.

Keywords: *RP-HPLC, Clarithromycin, Method development, Method validation.*

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1. INTRODUCTION

Peptic ulcers are localized erosions of the mucous membranes of the stomach and duodenum. The pain associated with ulcers is caused by irritation of exposed surfaces by the stomach acids. The current approach for treating ulcers caused by *Helicobacter pylori* is to use combination of drugs, which includes a proton pump inhibitor and two antimicrobials, such as tinidazole and amoxicillin or clarithromycin.

1.1 Structure of Clarithromycin

Clarithromycin represented in Fig.1is a semi-synthetic macrolide antibiotic derived from erythromycin A. It consists of a 14 membered lactone ring as well as cladinose and desosamine residues at positions 3 and 5 of the ring, respectively. Like erythromycin, it has no conjugated double bond in the lactone ring, hence significant UV absorbance is only obtained at wavelengths below 210 nm.[1,2] It is white or almost white crystalline powder, practically insoluble in water, slightly soluble in alcohol and acetonitrile and freely soluble in acetone. It may be bacteriostatic or bactericidal depending on the organism and drug concentration and it mainly acts by inhibiting bacterial protein synthesis. The drug is used to treat pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia, skin infections, etc.[3] Literature survey revealed that various methods have been developed for the estimation of clarithromycin from laboratory prepared mixtures, pharmaceutical preparations and biological matrices (such as human plasma) through automated solid phase extraction and electrochemical detection, liquid chromatographic electrospray tandem mass spectrometry and high performance liquid chromatography.[4-7]

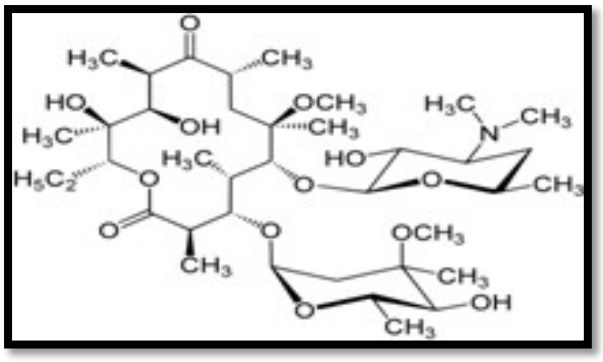


Fig. 1 Structural Representation of clarithromycin

2. MATERIALS AND METHODS

Clarithromycin, RANKEM Chemicals supplied the AR grade chemicals of potassium dihydrogen phosphate, orthophosphoric acid (OPA), methanol and HPLC grade acetonitrile. Milli-Q water purification system produced water was used during analysis (Make & Model: MILLIPORE/Integral 5).

2.1 Instrumentation and Software

Waters HPLC 2 2695 series consisting 4 pump. Auto sampler with 5 racks, each has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature. Waters (alliance) HPLC System is equipped with Empower software-2 software.

2.2 Methods

Chromatographic conditions			
Column	Inertsil ODS, C18, 150 X 4.6, 5µ.		
Mobile phase	Gradient programming		
	Time	**Buffer (KH_2PO_4)**	**Acetonitrile**
	0	90	10
	17	70	30
	20	70	30
	21	90	10
	25	90	10
Detector	UV detector		
Flow rate	1 ml/min		
Wavelength	210nm		
Injection Volume	10µL		
Temperature	30°C		
Diluent	Methanol		

Table 1: System suitability parameters of Clarithromycin, tinidazole and lansoprazole

S.No.	Property	Clarithromycin	Tinidazole	Lansoprazole	Acceptance criteria
1.	Retention Time (RT)	16.643	5.493	12.940	-
2.	Tailing factor (T)	1.21	1.14	1.32	NMT 2.0
3.	Theoretical plates (N)	4136	5264	3638	NLT 2000

From the data it was found that all the system suitability parameters for developed method were within the limit.

Fig.2 (a) Chromatogram: Blank

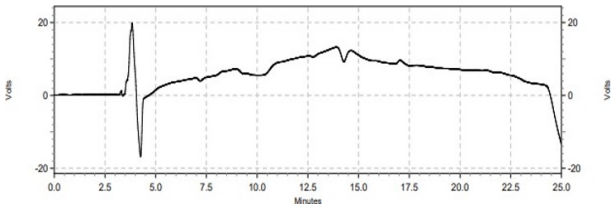
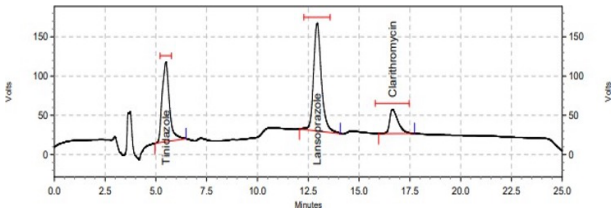


Fig. 2 (b)Chromatogram: Standard



2.8 Linearity and Range

Linearity of the developed method demonstrates the ability of method to produce a result which is directly proportional to concentration of analyte in the sample. The amount of clarithromycin, tinidazole and lansoprazole were prepared for linearity in the range of 80-120%. The amount of clarithromycin, tinidazole and lansoprazole in five different concentrations is 80%, 90%, 100%, 110% and 120% of working strength respectively. The graph was plotted between concentrations versus area of peak. The clarithromycin, tinidazole and lansoprazole shows good correlation coefficients (R^2= 0.9991, 0.9989 and 0.9994) and the proposed method was linear in concentration range 80-120 %.

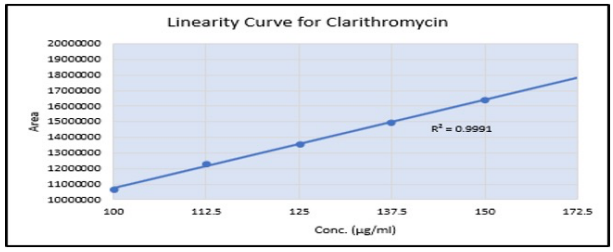
Table 2: Linearity of Clarithromycin, Tinidazole and Lansoprazole

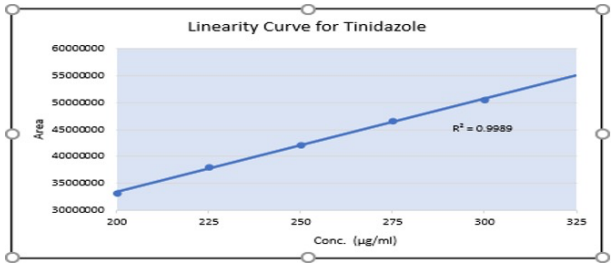
S. No.	Compound	Values of X and Y Variables						Correlation co-efficient
1	Clarithromycin	Variable	1	2	3	4	5	0.9991
		X	100	112.5	125	137.5	150	
		Y	1065284 7	1225856 4	1225856 4	1225856 4	1225856 4	

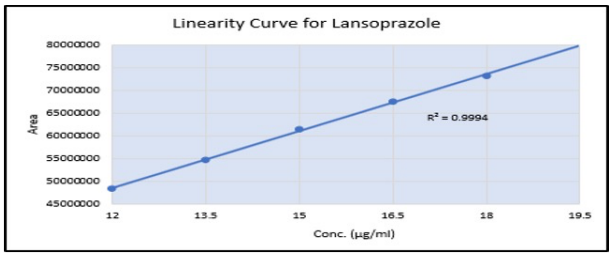
Note: X is the concentration of the respective component in µg/mL. Y is the peak response of the respective component in area counts.

2.9 Linearity Curve

Calibration curve was constructed between concentrations versus peak area. Results were recorded for equation of line and correlation co-efficient were determined.







2.10 Precision

It reveals the data regarding closeness between the series of measurements. The precision of the developed method was verified by system precision and method precision. A homogenous sample concentration of 125 µg/mL for clarithromycin, 250 µg/mL for tinidazole and 15 µg/mL for lansoprazole respectively were prepared under prescribed conditions and estimation was carried out. The results are expressed in the form of standard deviation and RSD value. Table 3 and 4 shows the result of system precision and method precision respectively and the developed method is highly precise as % RSD is less than 2%.

Table 3: Calculation of %RSD for Clarithromycin

S. No.	Compound	No. of Injections						Mean	S.D.	% RSD
		1	2	3	4	5	6			
1	Reference Standard Clarithromycin	1485 2634	1487 4629	1481 2463	1488 4614	1490 1663	1486 3265	14864878	3078 1.7396	0.207

Table 4: **Calculation of %RSD for Clarithromycin**

S. No.	Compound	No. of Injections						Mean	S.D.	% RSD
		1	2	3	4	5	6			
1	Sample Clarithromycin	1377 1885	1374 0332	1373 2656	1376 3211	1378 4155	1378 0817	13762176	2132 8.3378	0.155

Mean represents the average values of six replicates analysis. **SD** is the standard deviation calculated on the six replicates. **RSD** is the relative standard deviation.

Table 5: System Precision and Method precision

Precision	Drug	% RSD
System precision	Clarithromycin	0.207
Method precision	Clarithromycin	0.155

2.11 Accuracy

It is also termed as trueness or recovery. This method was determined using 80%, 100% and 120% of working strength of Clarithromycin, tinidazole and lansoprazole. Each level solution was prepared in duplicate and analysed as per the method given. This is usually demonstrated in the form of SD and RSD. The results reveal that the value of % RSD is less than 2%. The percent recovery results are in Table: 6.

Table 6: Summary of assay of Clarithromycin, tinidazole and lansoprazole

S. No.	Level	Compound	% Average Assay	%RSD
1	80%	Clarithromycin	98.49	0.26
2	100%	Clarithromycin	98.96	0.23
3	120%	Clarithromycin	98.94	0.24

The percentage of assay values of clarithromycin were in the range of 99.49-99.96 %, tinidazole in the range of 99.20-99.71 % and lansoprazole in the range of 99.53-99.88%. The % RSD of assay values of clarithromycin were in the range of 0.21-0.26 %, tinidazole in the range of 0.16-0.26 % and lansoprazole in the range of 0.13-0.18%. The study proves that the method is accurate for the estimation of amoxicillin, tinidazole and omeprazole assay over the range of 80-120% of target concentration.

2.12 LOD and LOQ (Limit of Detection and Limit of Quantification)

Limit of detection (LOD) and Limit of Quantification (LOQ) reveal information regarding concentration of analyte that yields signal-to-noise around 1 to 10. Serial dilutions are made from solution of clarithromycin for determination of LOQ and LOD. The samples were injected in HPLC and compare the signals of sample and blank sample of LOD and LOQ. According to earlier mentioned parameters, LOD and LOQ were estimated for clarithromycin is 2.5 µg/ml and 7.5 µg/ml respectively.

2.13 Robustness

The robustness of the developed HPLC method was carried out by making small deliberate changes in the HPLC process parameters. These parameters include variation in wavelength, flow rate of mobile phase and changes in proportion of buffer and acetonitrile. The method was performed on single concentrations of clarithromycin, tinidazole and lansoprazole. The alteration of parameters may leads to some significant changes in the peak area and RSD. Robustness studies concludes that the method is robust under ± 2 wavelength, ± 10% flow rate and ± 10% increase and decrease in mobile phase and at the different column (Zorbax CN column (250mmx4.6mm), 5 micron. There is no significant change in recovery of amoxicillin, tinidazole and omeprazole. The % RSD shown in Table:7 negligible changes were observed during robust condition. So, we can say that the developed method is robust.

Table 7: Robustness Data

Drug	Parameters	% RSD
Clarithromycin	Wavelength minus	0.004
	Wavelength plus	0.003
	Flow minus	0.002
	Flow plus	0.004
	Mobile phase ratio change	0.002
	Column Change	0.001
	Temperature minus	0.005
	Temperature plus	0.004

3. RESULT AND DISCUSSION

After a number of trials with different, mobile phases were tested but the adequate separation of Clarithromycin, tinidazole and lansoprazole was found in Potassium dihydrogen phosphate: Acetonitrile (Gradient programming). The best results were obtained with flow rate gradient programming of selected mobile phase for the purpose of rapid analysis. Mobile phase was started at a flow rate of 1.0 ml/min which was continued for 1.0 min to 25.00 min.

The validation of the developed and the optimized RP-HPLC method was carried out with respect to the parameters such as specificity, linearity, accuracy, precision, limit of quantification (LOQ) and limit of detection (LOD) in the light of internationally accepted ICH guidelines.

4. CONCLUSION

The HPLC method was successfully developed and validated on an Agilent 1220 LC for simultaneous determination of clarithromycin, tinidazole and lansoprazole in PYLOKIT combination. This present method is simple and accurate for the determination of drug at a single wavelength, 10 µL injection capacity and Ultisil XB C18 5µm 4.6*150 mm column. It was found that the method is sufficiently simple, rapid and sensitive as well as precise, accurate, linear, robust which compiles the ICH guidelines. The entire experimentation was proved that the developed HPLC method shows good resolution, linearity and RSD values (less than 2%) which indicates that method is suitable for the estimation of clarithromycin.

5. REFERENCES

[1]. Morgan, D.P., Cugier, B., Marello, C., Sarocka, A.C. and Plasz, A.C. (1990),

"Impurity profiling of clarithromycin using high performance liquid chromatography with ultraviolet detection", ***J. Chromatagr***, 502, 351-358.

[2]. Srinivasu, T., Rao, B.N., Mathrusri, A., Ashutosh, S. and Chandrashekhar, T.G. (2012), "Development and validation of high performance liquid chromatography method for quantification of related substances in clarithromycin powder for an oral suspension dosages form", ***Int. J. Anal. Pharm. Biomed. Sci.,*** 1, 1-12

[3]. O' Neil, M.J. (2006), "The Merck Index- An Encyclopedia of Chemicals, Drugs and biologicals",***Merck and Co.***, Inc., N. J., Whitehouse Station., 392.

[4]. Hedenmo, M. and Ericksson, B.M. (1995), "Liquid chromatographic determination of macrolide antibiotics roxithromycin and clarithromycin in plasma by automated solid phase extraction and electrochemical detection", ***J. Chromatagr.***, 692,161-166.

[5]. Li, W., Retting, J., Jiang, X., Francisco, D.T. and Naidong, W. (2006), "Liquid chromatographic electrospray tandem mass spectrometric determination of clarithromycin in human plasma", ***Biomed. Chromatogr***., 20, 597-604.

[6]. Erah, P.O., Barrett, D.A. and Shaw, P.N. (1996), "Ion pair high performance liquid chromatographic assay method for the assessment of clarithromycin stability in aqueous solution and in gastric juice", ***J. Chromatagr.*** 682, 73-78.

[7]. Gangishetty, S. and Verma, S. (2013)), "RP-HPLC method development and validation for simultaneous estimation of clarithromycin and paracetamol", ***ISRN Anal. Chem.*,** 948547.

[8]. Sweetman, SC (2011), "Martindale-The Complete Drug Reference", 37th edition., ***J Acta Pharm***.

[9]. Abou-Taleb NH, El-Sherbiny DT, El-Wasseef DR, Abu El-Enin MA, El-Ashry SM. (2011),"Simultaneous determination of norfloxacin and tinidazole binary mixture by difference spectroscopy", ***Int J of Biom Sci***, 7(2,137-44.

[10]. Alhemiary NA, Saleh MH. (2012), "Spectrophotometric determination of tinidazole using promethazine and ethyl vanillin reagents in pharmaceutical preparations", ***Der Pharma Chemica.J Acta Pharm*** ,4(6),2152-60.

[11]. Zheltvai OI, Zheltvai, II, Spinul VV, Antonovich VP, J.(2013), "Spectrophotometry determination of metronidazole and tinidazole by their complexation with copper(II)", ***Chem. J Acta Pharm*** 68(7),600-5.

[12]. Pasha K, Ali A, Bana S, Humair S, J.(2010), "Reverse phase-HPLC method for the analysis of tinidazole in pharmaceutical dosage form & bulk drug", ***J Int Pharm Sci Acta Pharm***,2(2),46-7.

[13]. Sneha JK, Nirav PB, Parag PR, Nikita PN, Hemant DT, Iosr(2012), "Development and validation of stability indicating method for simultaneous estimation of ciprofloxacin HCl and tinidazole using RP-UPLC method, ***J Acta Pharm***,2(5),12-9.

[14]. Kasnia V, Kumar MS, Mahadevan N, Int J. (2012), "Simultaneous estimation of amoxicillin, tinidazole and omeprazole in microsphere formulation by RP-HPLC", ***Recent Adv Pharm Res. J Acta Pharm***,2(2),78-83.

[15]. Brummer RJ; Geerling BJ; Stockbrugger RW(1997), ***Dig Dis Sci.***,42, 2132–2137.

[16]. Threlkeld DS, ed.(1998), "Gastrointestinal Drugs, Proton Pump Inhibitors. In Facts and Comparisons Drug Information", ***St. Louis, MO: Facts and Comparisons***, 305r.

[17]. Tolman KG; Sanders SW; Buchi KN; Karol MD; Jennings DE; Ringham GL.(1997), ***J Clin Gastroenterol***, 24(2), 65-70.

[18]. Tsukasa Uno; Norio Yasui-Furukori; Takenori Takahata; Kazunobu Sugawara; Tomonori Tateishi (2005), ***Journal of Chromatography B***, 816(1-2), 309-314.

[19]. M. D. Karol; G. R. Granneman; K. Alexander (1995), ***Journal of Chromatography B: Biomedical Sciences and Applications***, 668(1), 182-186.

[20]. B. Delhotal Landes; G. Miscoria; B. Flouvat. (1992), "***Journal of Chromatography B:***

Biomedical Sciences and Applications, 577(1) , 117-122.

[21]. Prasanna Kumar Reddy. B; Ramanjaneya Reddy Y; Ramachandran D. (2009), ***E-Journal of chemistry***, 6(2) , 489-494.

[22]. Dadhich Bharat; Goyal Rakesh; Agarwal Dilip.(2020), "Simultaneous Determination Tinidazole, Clarithromycin and Lansoprazole of in Tablets By HPLC", ***International Journal of All Research Writings***, 2020, 2(1), 9-14.