Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

e-ISSN:2320-1215
p-ISSN: 2322-0112

Validation and Determination of Macrolide Antibiotic Clarithromycin Tablets By HPLC Method as Per ICH Guidelines Q2 (R1)

Rajesh Kumar Nayak*, Dillip Kumar Mohapatra, Manjit Singh

Institute of Pharmaceutical Science and Research, Sardar Patel University, Balaghat, Madhya Pradesh, India

Research Article

Received: 01/10/2021
Accepted: 15/10/2021
Published: 22/10/2021

***For Correspondence:**
Rajesh Kumar Nayak, Institute of Pharmaceutical Science and Research, Sardar Patel University, Balaghat, Madhya Pradesh, India

E-mail:
RajeshKumarNayak34 @gmail.com

ABSTRACT

Our aim of study is to develop an efficient least time consuming and simple and sensitive method for the determination of clarithromycin tablets USP 250 mg and Clarithromycin tablets USP 500 mg are dose weight proportionate. The chromatographic analysis was performed in an isocratic separation mode by a Capcell Pak C18 column (150 mm × 4.6 mmi.d, 5 µm particle size). The mobile phase was a homogenous mixture of acetonitrile and potassium dihydrogen phosphate (0.035 M) in the ratio of (55: 45, v/v) at pH 4.4 ± 0.017, pumped at a flow rate of 0.6 ml/min and the effluent was monitored at wavelength 210 nm. The injection volume was 20 µl and the run time was about 6 min as the retention time of clarithromycin was found about 4.1 min. Hence entire validation shall be carried out on highest strength i.e. 500 mg. The developed method was validated as per ICH guidelines and its updated international convention. The linearity of response, precision, ruggedness and robustness of the described method has been checked. The system suitability parameters met the acceptance criteria, which were commenced during study of each individual validation characteristics.

INTRODUCTION

Macrolide antibiotics have been used for the treatment of bacterial infections caused by Gram-positive organismus [1]. Clarithromycin has a tertiary amino group which is reactive for electrochemical oxidation, making electrochemical detection (ECD) a potentially useful tool for ensuring the accurate determination of CLA in dosage forms [2]. Like erythromycin, it has no conjugated double bond in the lactone ring, hence significant UV absorbance is only obtained at wavelengths below 210 nm [3] ·Detection at these wavelengths is suitable for most *in vitro* samples but lack the necessary sensitivity for the quantitation of low concentrations of CLA, such as those observed in biological matrices and nanoparticles [4-7]. CAM has a 14-membered macrocyclic lactone ring attached to two sugar moieties a neutral sugar cladinose and amino sugar desosamine and has

e-ISSN:2320-1215
p-ISSN: 2322-0112

substituted an O-methyl group at position C6 with resultant acid stability and improved antimicrobial and pharmacokinetic properties [8]. CAM is first metabolized to 14-OH CAM, which is active and works synergistically with its parent compound. Like other macrolides, it then penetrates bacteria cell wall and reversibly binds to domain V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome. Binding inhibits peptidyl transferase activity and interferes with amino acid translocation during the translation and protein assembly process CAM is well-absorbed from GIT (50% ± 50), acid stable and may be taken with food [9]. After a 250 mg tablet every 12 hours, approximately 20% of the dose is excreted in the urine as CAM, while after a 500 mg tablet every 12 hours, the urinary excretion of CAM is slightly increased, approximately 30%. Half-life is approximately 3 h to 4 h (250 mg) and 5 h to 7 h (500 mg) [10]. CAM is metabolized in the liver by CYP3A4 and has an active metabolite, 14-hydroxyclarithromycin which works synergistically with its parent compound [11]. It may interact with tiniposide, tamsulosin, sildenafil, warfarin, rifampicin, and vinblastine Clearance of clarithromycin decreases with increasing dose, probably because of saturable hepatic metabolism[12-14]. Analytical methods development of validation plays important roles in the discovery, development and manufacture of Pharmaceutical [15]. The official test methods that result from those processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug product. The aim of the present work was to propose a rapid, simple and sensitive method for the determination of clarithromycin tablets USP 250 mg and Clarithromycin tablets USP 500 mg are dose weight proportionate. Hence entire validation shall be carried out on highest strength i.e. 500 mg. The developed method was validated as per ICH guidelines and its updated international convention. The linearity of response, precision, ruggedness and robustness of the described method has been checked.

MATERIALS AND METHODS

All chemicals were of analytical grade Glacial acetic Acid ACS-ISO was from Panreac. Methylene chloride 99.9% HPLC grade (Labscan), Monobasic potassium phosphate and Ortho phosphoric acid were from Merck, and sodium acetate trihydrate were all from Labscan. Water of HPLC grade was used throughout the experimental section where water is mentioned. The analysed pharmaceutical formulations were obtained from commercial sources. Clarithromycin reference standard (98% purity) was provided as generous gift from Sigma Aldrich.

HPLC instrumentation and conditions

Electric balance (Type AUY 120 Shimadzu).Dissolution USP type II (Paddle), Electrolab. The chromatographic system used for the investigation was Shimadzu LC-20AT (Kyoto, Japan), equipped with UV/visible detector (Shimadzu SPD-20A), degasser (Shimadzu DGU-20A3), manual injector (Rheodyne, USA) and software (LC solution). The chromatographic analysis was performed in an isocratic separation mode by a Capcell Pak C18 column (150 mm × 4.6 mmi.d, 5 µm particle size). The mobile phase was a homogenous mixture of acetonitrile and potassium dihydrogen phosphate (0.035 M) in the ratio of (55: 45, v/v) at pH 4.4 ± 0.017, pumped at a flow rate of 0.6 ml/min and the effluent was monitored at wavelength 210 nm. The injection volume was 20 µl and the run time was about 6 min as the retention time of clarithromycin was found about 4.1 min.

e-ISSN:2320-1215
p-ISSN: 2322-0112

Methodology

Dissolution conditions:

Apparatus	:	USP type II (Paddle)
Medium	:	0.1 M Sodium acetate buffer
Volume	:	900 mL
Speed	:	50 rpm
Temperature	:	37°C ± 0.5°C
Time	:	30 minutes

Dissolution medium: (0.1 M Sodium acetate buffer)

Mix 9500 mL of the solution I and 500 mL of solution II which will result Buffer solution of pH 5.0.

Preparation of Solution I:

Weigh 13.61 g of Sodium acetate trihydrate in 1000 mL of water and dissolve. (i.e. 13.61 mg/mL)

Preparation of solution II:

Dilute 5.7 mL of glacial acetic acid to 1000 mL of water.

Mobile phase:

Prepare a degassed mixture of methanol and 0.067 M monobasic potassium phosphate in the proportion of 65:35 v/v. Adjust to pH 4.0 with ortho phosphoric acid.

Preparation of 0.067 M monobasic potassium phosphate:

Dissolve 9.11 g of monobasic potassium phosphate in 1000 mL of water.

Diluent :

Use mobile phase as diluent.

Blank solution:

Dilute 2 mL of Methanol to 10 mL with mobile phase

System suitability stock solution:

Weigh accurately about 2.5 mg of Clarithromycin Related compound A USPRS in 10 mL volumetric flask. Add 7 mL of methanol. Sonicate to dissolve and dilute upto the mark with methanol.

System suitability solution:

Dilute 2 mL of Standard stock solution and 5 mL of System suitability stock solution to 10 mL with mobile phase.

Standard stock solution:

Weigh accurately about 12.5 mg of Clarithromycin working standard in 20 mL volumetric flask. Add 15 mL of methanol. Sonicate to dissolve and dilute up to the mark with methanol. (625 ppm)

Standard solution:

Dilute 2 mL of Standard stock solution to 10 mL with mobile phase. (125 ppm)

Sample solution:

Introduce one tablet of Clarithromycin Tablets USP 500 mg in individual vessel containing dissolution medium previously maintained at temperature 37°C ± 0.5°C and immediately operate the instrument as per the methodology.

e-ISSN:2320-1215
p-ISSN: 2322-0112

Immediately start the apparatus and run for 30 minutes. After specified time interval withdraw 10 mL of aliquot from a zone midway between the surface of the dissolution medium and the top of the blade not less than 1 cm from the vessel wall. Filter the aliquot through 0.45 μ nylon membrane syringe filter discarding first five mL of the filtrate.

For 250 mg strength:

Dilute 4 mL of sample solution to 10 mL with mobile phase. (111.11 ppm)

For 500 mg strength:

Dilute 2 mL of sample solution to 10 mL with mobile phase. (111.11 ppm)

Procedure:

Equilibrate the HPLC system with mobile phase. Inject Blank solution in single.

Inject System suitability solution in single and check resolution between peak due to Clarithromycin and Clarithromycin related compound A.

Inject Standard solution in five replicates and calculate %RSD for area of peak due to Clarithromycin.

Inject Test solution and calculate amount of Clarithromycin dissolved.

Note: The relative retention times for Clarithromycin and Clarithromycin related compound A are 0.75 and 1.0 respectively.

System suitability:

1) Resolution between peak due to Clarithromycin and Clarithromycin related compound A from System suitability solution should not be less than 2.0.
2) RSD for the area for the peak due to Clarithromycin in five replicate injections of Standard solution should not be more than 2.0%.
3) Tailing factor for the peak due to Clarithromycin in five replicate injections of Standard solution should be between 0.9 to 1.5.

Column efficiency for the peak due to Clarithromycin in five replicate injections of Standard solution should not be less than 750.

Calculations: %Dissolution: (For 250 mg) $Dn = (AT \times WS \times 2 \times 900 \times 10 \times P) / (AS \times 20 \times 10 \times 1\text{-tablet} \times 4 \times LC)$

% Dissolution: (For 500 mg) $Dn = (AT \times WS \times 2 \times 900 \times 10 \times P) / AS \times 20 \times 10 \times 1\text{-tablet} \times 2 \times LC$

Where, AT=Peak response of Clarithromycin from Test solution, AS=Mean peak response of Clarithromycin from Standard solution, WS=Weight of Clarithromycin working standard in mg, P=Potency of Clarithromycin working standard, LC=Label claim of Clarithromycin in mg/tablet.

Validation of the test procedure

Method validation study was performed based on the current pharmaceutical regulatory guideline (Tables 1-21).

Specificity:

Observations:

Table 1. Resolution between peak due to Clarithromycin and Clarithromycin related compound.

Sr. No.		Renal replacement therapy	Resolution	Limit

e-ISSN:2320-1215
p-ISSN: 2322-0112

1	Clarithromycin	0.67	5.66	NLT 2.0
2	Clarithromycin Related compound A	1.00		

Table 2. RSD for the area for the peak due to Clarithromycin in five replicate injections of standard solution.

Sr. No.	Retention time	Area	Theoretical plates	Asymmetry
1	4.20	2.377	4413	1.20
2	4.20	2.358	4448	1.18
3	4.20	2.359	4442	1.20
4	4.20	2.358	4431	1.20
5	4.20	2.353	4425	1.21
Mean	4.20	2.361	4432	1.20
Std. Dev.	0.00	0.01	---	---
%Relative standard deviation	0.00	0.42	---	---

Table 3. Tailing factor for the peak due to Clarithromycin in five replicate injections of standard solution.

Sample	Retention time	Peak purity
Blank solution	NA	NA
Placebo solution	NA	NA
Standard solution	4.20	1000
Test solution	4.22	1000

Acceptance criteria:

1) There should not be any interference from blank and placebo at the retention time of main peak.

2) Peak purity for the peak due to Clarithromycin in standard solution and test solution should not be less than 0.99 or 990 depending upon the software of HPLC used.

Conclusion:

From the above data it is concluded that the proposed analytical method is specific.

e-ISSN:2320-1215
p-ISSN: 2322-0112

Table 4. Resolution for System precision of HPLC apparatus.

Sr. No	Name	Renal replacement therapy	Resolution	Limit
1	Clarithromycin	0.67	5.86	LNT 2.0
2	Clarithromycin related compound A	1.00		

Table 5. Clarithromycin standard.

Sr. No.	Retention time	Area	Theoretical plates	Asymmetry
1	4.04	2.243	4715	1.18
2	4.04	2.232	4722	1.19
3	4.04	2.235	4709	1.19
4	4.04	2.235	4715	1.19
5	4.04	2.233	4729	1.18
6	4.04	2.239	4709	1.20
Mean	4.04	2.237	4717	1.19
Std. Dev.	0.00	0.00	---	---
% Relative standard deviation	0.00	0.00	---	---

Table 6. %Assay for system precision of dissolution test apparatus and observations.

Sample. No.	%Assay
1	99.9
2	100.1
3	101.2
4	100.1
5	100.1
6	100.2
Mean	100.3

e-ISSN:2320-1215
p-ISSN: 2322-0112

Stad. Dev.	0.47
%Relative standard deviation	0.47

Table 7. Resolution of renal replacement therapy

Sr. No	Name	RRT	Resolution	Limit
1	Clarithromycin	0.67	5.80	LNT 2.0
2	Clarithromycin Related compound A	1.00		
Note: RRT: Renal Replacement Therapy				

Table 8. Clarithromycin standard.

Sr. No.	Retention time	Area	Theoretical plates	Asymmetry
1	4.04	2.223	4702	1.18
2	4.04	2.226	4695	1.17
3	4.04	2.219	4702	1.16
4	4.04	2.219	4702	1.16
5	4.04	2.218	4715	1.17
Mean	4.04	2.221	4703	1.17
Std. Dev.	0.00	0.00	---	---
%Relative standard deviation	0.00	0.00	---	---

Table 9. Percent dissolution area.

Sample No.	Sample name	Area	% dissolution
1	Test preparation-1	2.045	103.1
2	Test preparation-2	2.050	103.4
3	Test preparation-3	2.046	103.2
4	Test preparation-4	2.045	103.1
5	Test preparation-5	2.069	104.3
6	Test preparation-6	2.050	103.4
Mean			103.4
Std. Dev.			0.45

e-ISSN:2320-1215
p-ISSN: 2322-0112

%Relative standard deviation	0.44

Acceptance criteria:

1) RSD for %dissolution should not be more than 2.0%.

Intermediate precision (Ruggedness):

Table 10. Resolution for renal replacement therapy.

Sr. No	Name	Renal replacement therapy	Resolution	Limit
1	Clarithromycin	0.67	5.72	LNT 2.0
2	Clarithromycin Related compound A	1.00		

Table 11. Clarithromycin standard.

Sr. No.	Retention time	Area	Theoretical plates	Assymetry
1	4.20	2.358	4407	1.22
2	4.20	2.343	4443	1.18
3	4.20	2.341	4431	1.18
4	4.20	2.338	4431	1.18
5	4.20	2.337	4431	1.18
Mean	4.20	2.343	4429	1.19
Std. Dev.	0.00	0.01	---	---
%Relative standard deviation	0.00	0.43	---	---

Table 12. Percent dissolution for test samples.

Sample. No.	Sample name	Area	%Dissolution
1	Test preparation-1	2.210	103.8
2	Test preparation-2	2.232	104.8
3	Test preparation-3	2.202	103.4
4	Test preparation-4	2.171	101.9
5	Test preparation-5	2.207	103.6
6	Test preparation-6	2.216	104.1

e-ISSN:2320-1215
p-ISSN: 2322-0112

Mean	103.6
Std. Dev.	0.97
% Relative standard deviation	0.94

Table 13. Resolution for solution stability: Observations.

Sr. No	Name	Renal replacement therapy	Resolution	Limit
1	Clarithromycin	0.67	5.99	LNT 2.0
2	Clarithromycin Related compound A	1.00		

Table 14. Clarithromycin standard Initial.

Sr. No.	Retention time	Area	Theoretical plates	Assymetry
1	4.04	2.219	4682	1.16
2	4.04	2.216	4669	1.16
3	4.04	2.212	4682	1.16
4	4.04	2.210	4689	1.17
5	4.04	2.210	4682	1.17
Mean	4.04	2.213	4681	1.16
Std. Dev.	0.00	0.00	---	---
%Relative standard deviation	0.00	0.00	---	---

Table 15. Stability of standard preparation.

Sample	Area	%Dissolution	%Difference
Initial	2.215	100.3	---
After 24 Hours	2.220	100.2	0.1
After 48 Hours	2.231	100.6	0.3

Table 16. Stability of test preparation.

Sample	Area	%Dissolution	%Difference
Initial	2.058	100.7	---
After 24 Hours	2.067	103.8	0.1
After 48 Hours	2.052	103.1	0.6

e-ISSN:2320-1215
p-ISSN: 2322-0112

Table 17.Observations for Clarithromycin standard and filter paper Interference:

Sr. No	Name	Renal replacement therapy	Resolution	Limit
1	Clarithromycin	0.67	5.40	LNT 2.0
2	Clarithromycin Related compound A	1.00		

Table 18. Clarithromycin standard.

Sr. No.	Retention time	Area	Theoretical plates	Assymetry
1	4.04	2.220	4676	1.15
2	4.04	2.215	4669	1.17
3	4.04	2.215	4676	1.17
4	4.04	2.218	4676	1.17
5	4.04	2.216	4682	1.17
Mean	4.04	2.217	4675	1.17
Std. Dev.	0.00	0.00	---	---
% Relative standard deviation	0.00	0.00	---	---

Table 19. %Variation of standard preparation.

Filter paper	Area	%Variation
Unfiltered standard preparation	2.215	NA
Standard Filtered through Whatman No.1	2.207	0.36
Standard Filtered through Whatman No.41	2.211	0.18
Standard Filtered through Whatman No.42	2.252	1.67
Standard Filtered through Whatman No.0.45 µ nylon membrane syringe filter	2.217	0.09

Table 20. %Variation of test preparation.

Filter paper	Area	%Variation
Centrifuged test preparation	2.051	NA
Test Filtered through Whatman No.1	2.054	0.15
Test Filtered through Whatman No.41	2.058	0.34
Test Filtered through Whatman No.42	2.048	0.15
Test Filtered through Whatman No.0.45 µ nylon membrane syringe filter	2.040	0.54

e-ISSN:2320-1215
p-ISSN: 2322-0112

From the above data, it is concluded that filtered through Whatman No.1, Whatman No.41, Whatman No.42 and 0.45 µ nylon membrane syringe filter shows variation less than 2.0%. Hence the above filters are recommended for the filtration of sample.

Table 21. System suitability for clarithromycin.

Sr. No.	Parameter	Resolution	Retention time	%Relative standard deviation of area	Theoretical plates	Asymmetry
1	Specificity	4.74	4.12	0.00	4698	1.09
2	System precision	4.86	3.99	0.00	4408	1.22
3	Method precision	4.96	3.99	0.00	4406	1.18
4	Intermediate precision	4.96	4.19	0.41	4494	1.20
5	Solution stability hnitial	4.92	4.03	0.46	4618	1.19
6	Solution stability 24 hours	5.01	4.07	0.00	4707	1.18
7	Solution stability 48 hours	5.02	4.07	0.00	4704	1.15
8	Filter paper interference	4.88	3.99	0.00	4405	1.21
	Minimum	4.74	3.99	0.00	4405	1.09
	Maximum	5.02	4.19	0.46	4707	1.22
	Mean	4.92	4.06	0.11	4555	1.18
	Limit	Not less than 2.0	-	Not more than 2.0%	Not less than 750	Between 0.9 to 1.5

CONCLUSION

A simple, reproducible analytical method with high resolution and sensitivity was used for quantification of Clarithromycin .The observations and result obtained for each parameter including Specificity, Method Precision (Repeatability), Intermediate precision (Ruggedness), Solution stability and System suitability lies well within the acceptance criteria. Specificity of the method was demonstrated by analyzing blank preparation, placebo preparation, standard preparation, test preparation. Blank preparation and placebo preparation did not show any interference at the retention time of Clarithromycin. Ruggedness of the method was evaluated under intermediate precision and results were found within acceptable limits. The data obtained from individual condition and overall conditions including repeatability was found well within the limit. The Standard preparation and Test preparation is found stable after 24 Hours and 48 Hours kept on bench top at room temperature.

e-ISSN:2320-1215
p-ISSN: 2322-0112

The system suitability parameters met the acceptance criteria, which were commenced during study of each individual validation characteristics.

REFERENCES

1. Pappa-Louisi A, et al. Study on the electrochemical detection of the macrolide antibiotics clarithromycin and roxithromycin in reversed-phase high-performance liquid chromatography. J Biol Chem. 2001;755:57-64.
2. Huber MM, et al. Oxidation of pharmaceuticals during water treatment with chlorine dioxide. Water Res .2005;39:3607-17.
3. Morgan D, et al. Impurity profiling of clarithromycin using high-performance liquid chromatography with ultraviolet detection. J Chromatogr A. 1990;502:351-358.
4. Srinivasu T, et al. 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.
5. Li W, et al. Determination of clarithromycin in rat plasma by HPLC–UV method with pre-column derivatization. Talanta. 2007;71:385-390.
6. Bahrami G, et al. Determination of clarithromycin in human serum by high-performance liquid chromatography after pre-column derivatization with 9-fluorenylmethyl chloroformate: Application to a bioequivalence study. J Chromatogr. 2007; 850, 417–422.
7. Sharma M, et al. Implications of designing clarithromycin loaded solid lipid nanoparticles on their pharmacokinetics, antibacterial activity and safety. RSC advances. 2016;6:76621-76631.
8. Perter DH, et al. Azithromycin a review of its antimicrobial activity, pharmacokinetic properties and clinical efficiency. Drugs. 1992;44:750.
9. Chu SY, et al. Pharmacokinetics of clarithromycin, a new macrolide, after single ascending oral doses. Antimicrob Agents Chemother. 1992;36:2447-2453.
10. A D Rodrigues, et al. Oxidative metabolism of clarithromycin in the presence of human liver microsome: major role for the cytochrome P4503A (CYP3A) subfamily. Drug Metab Dispos.1997;25:623-630.
11. Recker MW, et al. Potential interaction between clarithromycin and warfarin. Ann Pharmaco.1997;9:996-998.
12. Riss J, et al. Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. Acta Neurol Scand.2008;118:69-86.
13. Dresser GK, et al. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P4503A4 inhibition. Clin Pharmacokinet. 2000;38:41-57.
14. Davey PG, et al. The pharmacokinetics of clarithromycin and its 14-OH metabolite. J Hosp Infect. 1991;19:29-37.
15. Chung C, et al.Analytical method validation and instrument performance verification.2004.