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Original Article

# Pharmacokinetic Profile of Bioanalytical Method Development and Validation of Clarithromycin from Human Plasma by Using Liquid Chromatography Tandem Mass Spectrometer

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## ABSTRACT

**Aim:** To develop and validate a sensitive and specific bioanalytical method for the quantification of clarithromycin in human plasma using Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS). Additionally, to characterize the pharmacokinetic profile of clarithromycin, including its Absorption, Distribution, Metabolism, and Excretion (ADME) in humans. This study aims to support the clinical application and therapeutic monitoring of clarithromycin. **Background:** Of this study was to develop and validate a bio-analytical method for the quantification of clarithromycin in human plasma utilizing Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Clarithromycin, a widely used macrolide antibiotic, requires accurate and sensitive measurement in biological matrices to ensure therapeutic efficacy and monitor Pharmacokinetic (PK) profiles. The test preparation is the product being evaluated and the reference preparation is the preparation which is used to compare the test product. Pharmacokinetic or Indirect method There exist a linear relation between the drug level in the biological fluid and therapeutic response; therefore, these methods are also known as indirect methods. Solid Phase Extraction SPE is a technique to clean, separate and concentrate analyte prior to analysis. Involves passing sample through a sorbent bed that preferentially retains the analyte under the right conditions. PPT Precipitation is another method of concentration that is used extensively for biopolymer such as protein, polypeptide, etc. by increasing the concentration of a protein solution some protein can be precipitated. Materials and Methods: Human plasma samples were analyzed using a developed and validated LC-MS/MS method to quantify clarithromycin. The method underwent rigorous validation for accuracy, precision, linearity, and stability, followed by pharmacokinetic analysis to assess clarithromycin's ADME profile. **Conclusion:** Utilizing advanced analytical techniques such as HPLC coupled with MS, the method exhibits excellent performance characteristics, including high sensitivity, specificity, and reproducibility. he successful implementation of this method underscores its value in both clinical settings and research, contributing to better understanding and management of clarithromycin pharmacokinetics. his method's ability to provide precise and reliable data makes it an essential tool for therapeutic drug monitoring and pharmacokinetic studies of clarithromycin, ultimately contributing to improved patient care and optimized treatment outcomes. And determine the suitable parameters followed by ICH guidelines.

**Keywords:** Pk, LCMS System, Clarithromycin, Bioavailability, Solid Phase Extraction, Protein Precipitation.

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## INTRODUCTION

The use of HPLC coupled with Mass Spectrometer (LCMS) has increased dramatically over the past ten years as a result of its unparalleled sensitivity, exceptional selectivity, and quick pace of analysis. The creation of ions from molecules that have been analyzed and separated or filtered based on their mass-to-charge ratio (*m/z*) is the fundamental component of Mass Spectrometry (MS). The majority of applications for quantitative bioanalysis utilize tandem Mass Spectrometers (MS/MS), which use two mass analyzers: one for the precursor ion in the first quadrupole and the other for the product ion in the third quadrupole following the collision activated dissociation of the precursor ion in a collision cell.[1] The development of micro-particulate packing materials of improved efficiency and stability and introduction of bonded

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phases has increased the versatility of technique and have great improved the analysis of multicomponent mixtures by LCMS system. The Aim of our study is development of a highly rapid, sensitive, accurate and reproducible method in Human plasma for estimation of Clarithromycin. The US FDA defines it referred to as "the pace and extent at which the active component is absorbed from a drug product and becomes accessible at the site of action" Measurements that represent the pace and degree to which the active moiety becomes accessible at the site of action can be used to determine bioavailability for pharmaceutical medicines that are not meant to be absorbed into the blood stream. The objective of this work was to develop simple, highly rapid, sensitive and cost-effective method for estimation of Clarithromycin in human plasma by liquid-liquid extraction method with quantitative recovery of both analyte and IS. setting the Tuning Parameter for Mass Spectroscopy. Optimization of mobile phase, column and other chromatographic parameters. Development of method for of recovery study of Clarithromycin in human plasma. Validating the method as per regulatory requirements.[2,3]

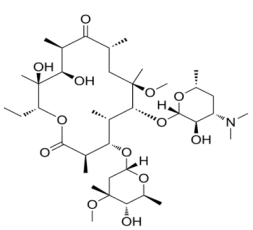
The PI drugs Clarithromycin drugs in real patient samples were analyzed in a 25-µL sample volume, which was only diluted with 200 µL of $H_2O$ (containing 500 gm/mL of the internal standard reserpine) to minimize the matrix concentration and to add the internal standard. No additional tedious and time-consuming for sample clean-up, procedures including protein precipitation, centrifugation, and pipetting were carried out. Results: The API MDS Sciex Method, which was developed, allowed the simultaneous analysis of two samples (the first analysis took 6.6 min, and the subsequent analyses took 3.3 min between injections), and it has been validated in terms of the Lower Limit of Quantification (LLOQ), which ranges from 78 to 156 ng/mL for LOD. As compared to an IV dosage form, the bioavailability data for a specific formulation offer an indication of the relative percentage of the oral dose that is absorbed into the systemic circulation. There are two ways to determine bioavailability: absolute bioavailability by contrasting the differential bioavailability following an oral and intravenous bolus injection, one may determine the absolute bioavailability of a pharmacological product. Relative bioavailability: Relative bioavailability is defined as the extent of absorption from the test preparation relative to the extent of absorption from the reference preparation. The test preparation is the product being evaluated and the reference preparation is the preparation which is used to compare the test product.[4,5]

## MATERIALS AND METHODS

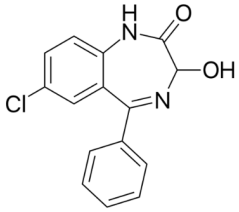
### Chemicals and Reagents

Clarithromycin, Human Plasma, Oxazepam, Acetonitrile HPLC grade, Milli-Q water, Methyl tert-Butyl Ether (TBME) (HPLC Grade), Methanol HPLC grade, Glacial Acetic acid (AR Grade), Ammonium Acetate (AR Grade), Sodium Hydroxide (NaOH) (AR Grade), Ammonia Solution (AR Grade).

### Chemical structure



Clarithromycin



Oxazepam

### Preparation of Reagents

#### *Preparation of Mobile Phase (Buffer: Methanol 10:90 v/v)*

100 mL of 10 mM Ammonium acetate buffer pH 4.50±0.05 with 900 mL of $CH_3OH$ were added in a reagent bottle. Then it was mixed well and sonicated. This solution was used within one week of its preparation.

### Stock Solution of Internal Standard

Stock Interior Standard Solution (Oxazepam) Percentage Purity is 99.04%, the water name 0.96%, actual weight taken on Ultra Micro Balance: 4.98745 and Final Concentration is 415786.88 gm/Ml Weighed the accurately about 2.0957 mg. Of Oxazepam transfer it into 5.0 mL Volumetric Flask. Dissolved the contents in about 2.0 mL Diluent and made up to the volume with the same up to the mark. Stored the stock in the Refrigerator at 2-8°C.

### Pharmacokinetic or Indirect Method

There exist These approaches are sometimes referred to as indirect methods since there is a linear relationship between the drug level in the biological fluid and the treatment response and the plasma data followed on the time of peak plasma concentration ($T_{max}$), The peak plasma concentration ($C_{max}$), The Area Under the plasma concentration Curve (AUC).[6,7]

### Solid Phase Extraction

Prior to analysis, analyte is cleaned, separated, and concentrated using SPE. involves putting a sample through a sorbent bed where, under the correct circumstances, the analyte is retained preferentially.[8,9]

### Instruments and Conditions

Applied Biosystems MDS brand API 3000 model LC-MS/MS were used to create pharmacokinetic profile, Thermo Hypurity C18, 4.6×100 mm column was used to produce chromatographic separation using an isocratic technique at room temperature. Methanol: 2 mM Ammonium Acetate buffer (pH 5) (90:10) (v/v). flowed through the mobile phase at a rate of 1.2 mL/min. 3.5 min of total runtime and a 4.000 L injection volume were used. Mass

spectrometry was carried out in Multiple Reaction Monitoring (MRM) mode.

## Method of Analysis

### *Extraction Processing*

Spiking Processing process was followed the sample from Deep Freeze and thaw the sample in normal water After thawing the sample take out 200 µL sample from Ria Vial. And add to the prelabelled Ria via, then add 50 µL ISTD spiking solution to the ria vial Add 50 µL Sodium Carbonate buffer to it and vortex for 15 sec. then add 1.0 mL of TBME, and again vortex for 15 sec. Centrifuge the sample at 3500 rpm for 10.0 min. at 10°C. then take out the sample from Centrifuge and Flash fridge the sample with Alcohol bath Flash fridge causes separation the plasma & ether (which containing Analyte) A then evaporate ether under $N_2$ gas at 40°C at 15 psi pressure. After the evaporation add the RS to ria vial vortex for 15 sec. then fill in HPLC vial.[10,11]

# RESULTS

## System Suitability

Filled the standard samples in the Autosampler vial. Set the chromatogram conditions run the standard solution of Clarithromycin. Made the sequence to run the standard solution. Here standard samples 8 injected for 6 time. Then calculated % CV in analyte 0.1% and the ISTD 0.3% & % SD of analyte is 0.0041% and ISTD 0.0041% (Figure 1). The acceptance criteria for % CV of Analyte & ISTD area should not more than 5% and also Standard Deviation for both peak area as well as for Rt should not more than 2.0.[12]

## Spiking Solution Check (SSC)

Filled the standard & Sample in the Auto sampler vial. The vial was placed in vial tray in HPLC. Set the chromatogram conditions, The acceptance criteria for SSC experiment are the standard conc. Should be within 20% of nominal value. (It is 96.6%) i.e., within

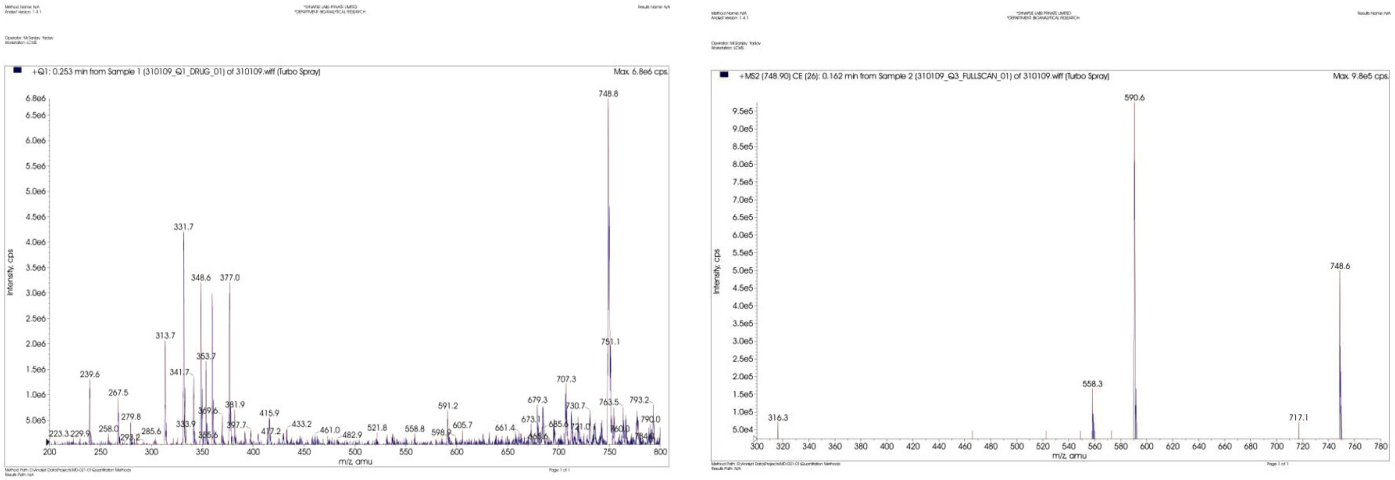



**Figure 1:** Spectra of Clarithromycin.

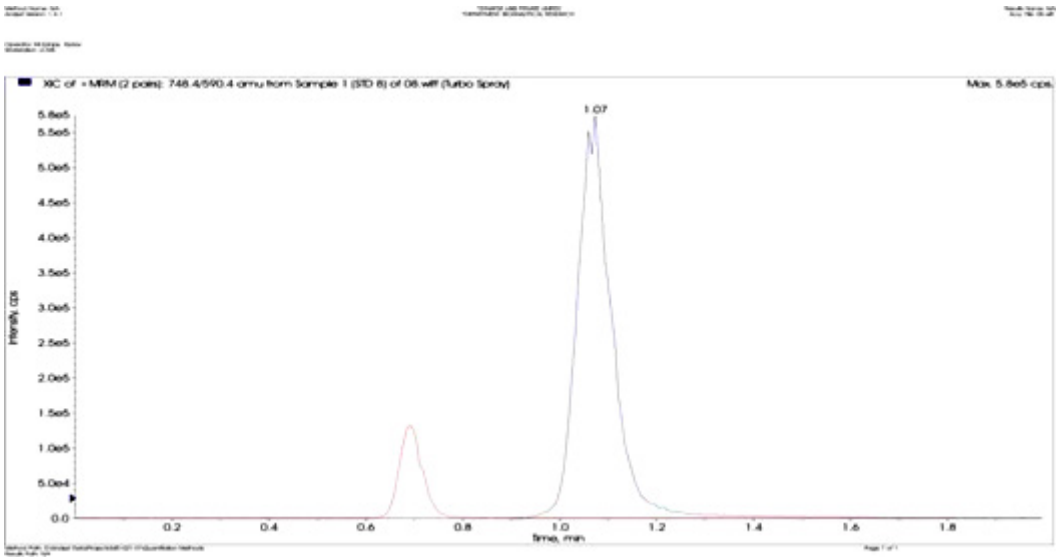



**Figure 2:** Clarithromycin (Decentroid).

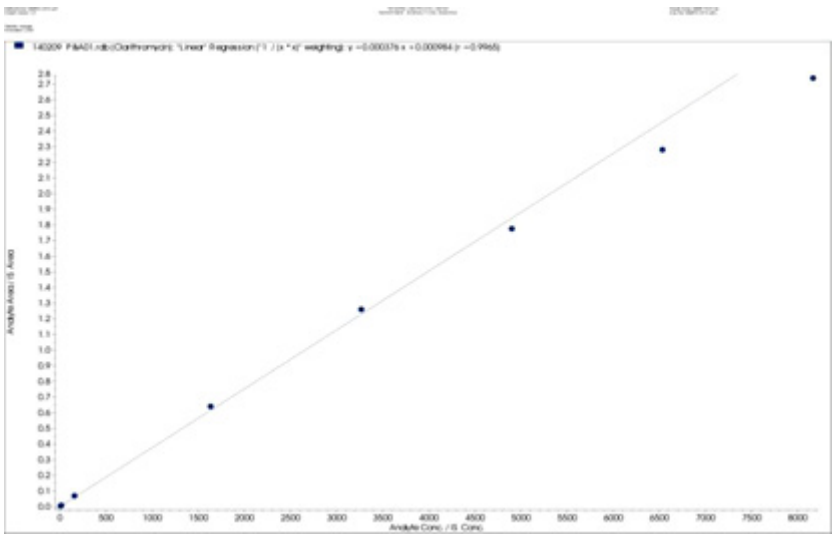


**Figure 3:** Calibration Curve for Precision & Accuracy.

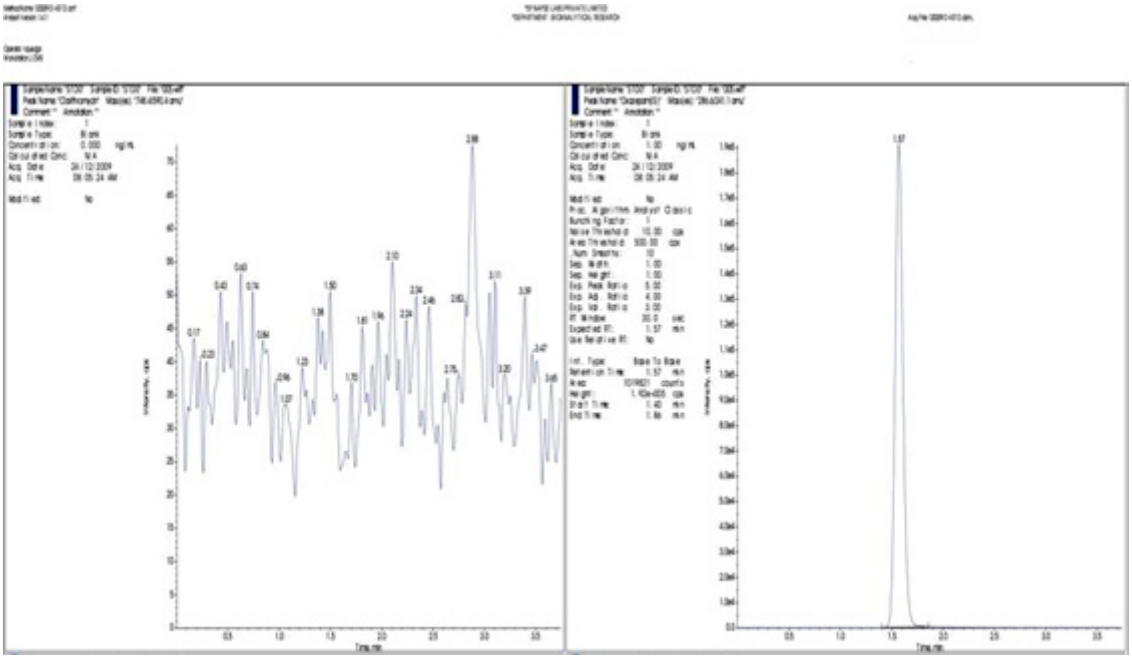


**Figure 4:** Spectra of Specificity.

the acceptance criteria (Figure 2), The regression coefficient value of C.C. should be more than 0.99 we found 0. 9971.So, the SSC experiment was passed.[13]

## Precision & Accuracy

Filled the standard & Sample in the Auto sampler vial. The vial was placed in vial tray in HPLC. Set the chromatogram conditions. Figure 3 and Table 1 showed the LOQ QC, LQC and MQC1 values are less than 5%. Accuracy for LOQQC should be within ± 20% of nominal value. Accuracy of LQC, LMQC, MQC and HQC should be within 15% of nominal value.

## Specificity

In Specificity experiment different lots of plasma were used to determine whether these lots are within the acceptance criteria or not. So, spike the Spiking solution of Standard & Quality Control solution to the various plasma lot.[14] Filled the standard & Quality Control Sample in the Autosampler vial (Figure 4). The acceptance criteria for specificity experiment are there should not be any interfering peak at the Rt of Analyte/ISTD and if any interfering peak found at Rt then that peak should contain area less than 20% of standard.[15,16]

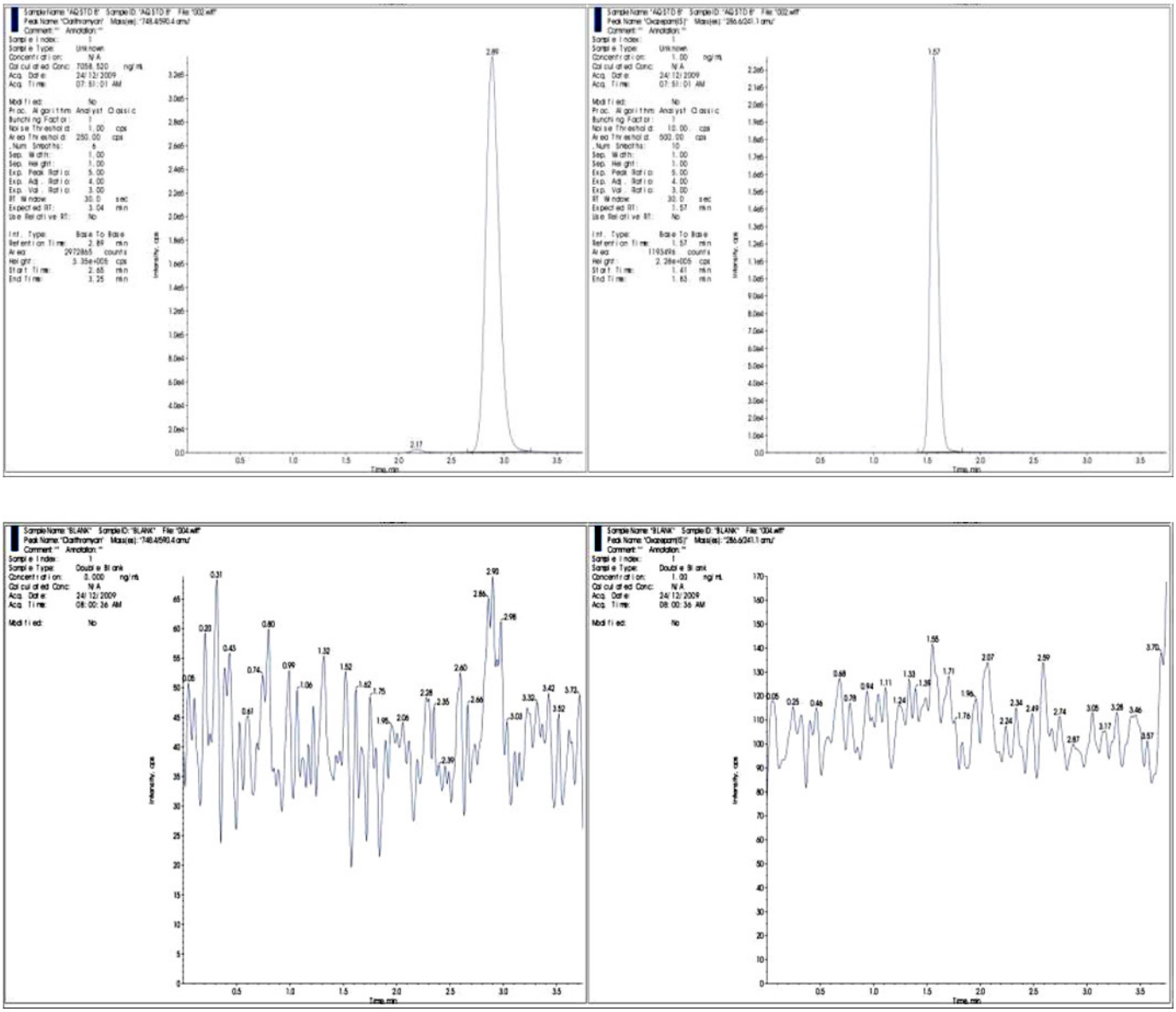


**Figure 5:** Spectra of Matrix Effect.

## Matrix Effect

Matrix effect is nullified if accuracy is within ± 15% and precision is ≤ 15% at the low and high QC concentration. Figure 5 showed the evaluation of matrix effects plays a crucial role in the validation of quantitative LC-MS-MS techniques used to assist pharmacokinetics research. It was completed by processing duplicate samples of six separate plasma batches. After carrying out extraction, LQC and HQC functioning solutions were spiked the LQC & HQC spiking Solution to the different plasma The % CV was found to be less than 5%. And also, regression coefficient was found to be 0.9973 which is more than 0.99.[17,18]

## Short Term Stock Solution Stability

The STSSS mens Stability of the Short-Term Stock Solution, that experiment was performed to check the Stock Solution's Short-Term Stability. Here stock solution was placed on working platform for 15 hr,[19] The Rt of Analyte of stability sample was found to be 2.98/2.97 min. and that of ISTD was found to be 1.56/1.57 min i.e., both values are same as that of 0.0 hr. That is there was no considerable change was found in to Rt of Analyte or ISTD. And also, the Area ratio of Analyte/ISTD was shown reproducibility So STSSS experiment was found to be passed.[20]

## DISCUSSION

Clarithromycin is a macrolide antibiotic widely used for various infections. Its pharmacokinetics involve distribution, metabolism, and elimination. It is extensively. distributed, metabolized by CYP3A4, and excreted primarily through bile. Its half-life is 2-4 hr. A sensitive and specific LC-MS/MS method is developed for clarithromycin quantification in plasma. Sample preparation

**Table 1: Precision & Accuracy Result.**

| Precision & Accuracy | Clarithromycin in human plasma (ng / mL) | | | | |
|---|---|---|---|---|---|
| | Quality control samples ID | | | | |
| | LOQ QC | LQC | MQC1 | MQC | HQC |
| P&A | 5.169 | 22.958 | 932.169 | 4014.499 | 5726.325 |
| | 6.738 | 21.596 | 887.747 | 4072.229 | 5618.989 |
| | 6.759 | 21.033 | 901.702 | 4456.905 | 5478.896 |
| | 7.570 | 19.194 | 945.387 | 4133.219 | 5435.320 |
| | 7.249 | 21.306 | 917.180 | 4124.248 | 5520.038 |
| Mean | 6.6970 | 21.2174 | 916.8370 | 4160.2200 | 5555.9136 |
| SD ± | 0.92292 | 1.35176 | 23.05204 | 172.49787 | 117.02578 |
| Precision (%CV) | 13.8 | 6.4 | 2.5 | 4.1 | 2.1 |
| Nominal value (ng/mL) | 8.249 | 22.600 | 903.982 | 4109.011 | 6321.555 |
| % Accuracy | 81.2 | 93.9 | 101.4 | 101.2 | 87.9 |
| n | 5 | 5 | 5 | 5 | 5 |

Precision & Accuracy is less than 5%. Accuracy for LOQQC should be within ± 20% of nominal value. Accuracy of LQC, LMQC, MQC and HQC should be within 15% of nominal value.

involves protein precipitation or liquid-liquid extraction. Chromatographic separation is achieved using a suitable stationary phase and mobile phase. Mass spectrometry parameters are optimized for sensitivity and selectivity. The method is validated according to ICH guidelines. Key parameters assessed include linearity, accuracy, precision, specificity, and robustness. Linearity is evaluated over a relevant concentration range. Accuracy is determined by comparing measured concentrations to known values. Precision is assessed through replicate analysis. The Precision & Accuracy is less than 5% and LOQQC with in the limit, Specificity is ensured by evaluating matrix effects and interference. Robustness is evaluated by assessing the method's performance under varied conditions, The % CV was found to be less than 5%. And also, regression coefficient was found to be 0.9973 which is more than 0.99. All the parameters accepted in Regulatory guidelines such as ICH Q2 (R1).

## CONCLUSION

The proposed method's high throughput is suggested by the 3.5 min run time per sample analysis. at low sample volume 5 µL. Due to the low run time and sample volume which helps to extending the life time and efficiency of the Column. From the results of all the validation parameters, the method can be useful for the estimation and interpretation of pharmacokinetic parameters of the Clarithromycin. And analysis of routine Samples of single dose or multiple dose pharmacokinetics and also for the clinical trial samples with desired precision and accuracy. Clarithromycin in human plasma may be detected using a technique that has been devised. The approach has been found to be accurate, precise, and selective, and the limit of quantification was enough for a study's objectives.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTION STATMENT

Jayaprakash J, UmaMaheshwari D designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript, and managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

## ABBREVIATIONS

**PK:** Pharmacokinetics; **LCMS:** Liquid chromatography-mass spectrometry; **HPLC:** High Performance Liquid Chromatography; **AUC:** Area under the Curve; **$C_{max}$:** Maximum concentration; **$T_{max}$:** Time to peak concentration; **ISTD:** Internal standard; **SSC:** Spiking Solution Check.

## SUMMARY

Method Development: Chromatographic Optimization This includes selecting appropriate mobile phases, column types, and gradient programs to ensure efficient separation of Clarithromycin from endogenous plasma components. Spectrometric Settings: Parameters such as ionization mode selection of precursor ions, and optimization of fragment ions are fine-tuned for maximum sensitivity and specificity. sample preparation A critical step

where techniques like protein precipitation, liquid-liquid extraction, or solid-phase extraction are employed to isolate Clarithromycin from plasma, ensuring clean samples for analysis. Method validation Linearity and Range: Establishing the linearity of the method across the required concentration range of Clarithromycin. Accuracy and Precision: Assessing the method's ability to produce consistent and accurate results within specified limits. Recovery and Matrix Effect: Evaluating the efficiency of the extraction process and ensuring that the plasma matrix does not interfere with the quantification of Clarithromycin. Stability: Determining the stability of Clarithromycin in plasma under different storage conditions and through various stages of sample processing, Kinetic analysis Pharmacokinetic Parameters: The validated method is applied to clinical samples to determine pharmacokinetic parameters such as $C_{max,}$ $T_{max,}$ half-life, and AUC (Area Under the Curve) for Clarithromycin. Application in Bioequivalence Studies: The method is used to support bioequivalence studies by comparing the pharmacokinetic profiles of different formulations of Clarithromycin. This process ensures that the method is robust, reliable, and suitable for use in clinical studies to monitor Clarithromycin levels in human plasma.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was Approved by the Institutional Ethical Committee, Vinayaka Mission's College of Pharmacy Approval Number: P.co/66/2022/IEC/VMCP.

## REFERENCES


1. Salem II, Brittain HG. Clarithromycin. Anal Proc⩾le of Drug Substances and Excipients. 1996;24:45-85.
2. Rodvold KA. Clinical pharmacokinetics of clarithromycin. Clin Pharmacokinet. 1999;37(5):385-98. doi: 10.2165/00003088-199937050-00003, PMID 10589373.
3. Fraschini F, Scaglione F, Demartini G. Clarithromycin clinical pharmacokinetics. Clin Pharmacokinet. 1993;25(3):189-204. doi: 10.2165/00003088-199325030-00003, PMID 8222460.
4. Amini H, Ahmadiani A. Sensitive determination of clarithromycin in human plasma by high-performance liquid chromatography with spectrometric detection. J Chromatogr B. 2005;817(2):193-7. doi: 10.1016/j.jchromb.2004.12.003.
5. Zhaq J, Jiang Y, Li J, Ren j, Liu F, *et al.* Determination of clarithromycin in human plasma by RP-HPLC. West China J Pharm Sci. 2007:189-90.
6. Li W, Jia H, Zhao K. Determination of clarithromycin in rat plasma by HPLC-UV method with pre-column derivatization. Talanta. 2007;71(1):385-90. doi: 10.1016/j.talanta.2006.04.015, PMID 19071316.
7. Bahrami G, Mohammadi B. Determination of clarithromycin in human serum by high-performance liquid chromatography after pre-column derivatization with 9-uorenylmethyl chloroformate: application to a bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;850(1-2):417-22. doi: 10.1016/j.jchromb.2006.12.027, PMID 17223392.
8. Torano JS, Guchelaar HJ. Quantitative determination of the macrolide antibiotics erythromycin, Roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-uorenylmethyloxycarbonyl chloride and uorescence detection. J Chromatogr B. 1998;720:89-97.
9. Rotsch TD, Spanton M, Cugier P, Plasz AC. Determination of clarithromycin as contaminant on surfaces by high-performance liquid chromatography using electrochemical detection. Pharm Res. 1991;8(8):989-91. doi: 10.1023/a:1015844705331, PMID 1833731.
10. Niopas I, Daftsios AC. Determination of clarithromycin in human plasma by HPLC with electrochemical detection: validation and application in pharmacokinetic study. Biomed Chromatogr. 2001;15(8):507-8. doi: 10.1002/bmc.111, PMID 11748684.
11. Zaater MF, Tahboub YR, Ghanem E. Determination of stability assessment of clarithromycin in human plasma using RP-HPLC with electrochemical detection. J Chromatogr Sci Pp. 2012:1-6.
12. Chu SY, Sennello LT, Bunnell ST, Varga LL, Wilson DS, Sonders RC. Pharmacokinetics of clarithromycin, a new macrolide, after single ascending oral doses. Antimicrob Agent Chemother. 1992;36(11):2447-53. doi: 10.1128/AAC.36.11.2447, PMID 1489187.
13. Choi SJ, Kim SB, Lee HY, Na DH, Yoon YS, Lee SS, *et al.* Column-switching high-performance liquid chromatographic determination of clarithromycin in human plasma with electrochemical detection. Talanta. 2001;54(2):377-82. doi: 10.1016/s0039-9140(00)00676-7, PMID 18968261.
14. Lu X, Chen L, Wang D, Liu J, Wang Y, Li F. Quantification of clarithromycin in human plasma by UPLC-MS-MS. Chromatographia. 2008;68(7-8):617-22. doi: 10.1365/s10337-008-0748-6.
15. Li W, Rettig J, Jiang X, Francisco DT, Naidong W. Liquid chromatographic-electrospray tandem mass spectrometric determination of clarithromycin in human plasma. Biomed Chromatogr. 2006;20(11):1242-51. doi: 10.1002/bmc.691, PMID 16838265.
16. Chaudhary DV, Patel DP, Shah JV, Shah PA, Sanyal M, *et al.* New improved UPLC-MS-MS method for reliable determination of clarithromycin in human plasma to support a bioequivalence study. J Adv Med Life Sci. 2015;2:1-8.
17. Guidance for industry 2001. Bioanalytical method validation. US Department of Health Services, Food and Drug Administration, CDER, CVM.
18. Bahrami G, Mohammadi B. Determination of clarithromycin in human serum by high-performance liquid chromatography after pre-column derivatization with 9-uorenylmethyl chloroformate: application to a bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;850(1-2):417-22. doi: 10.1016/j.jchromb.2006.12.027, PMID 17223392.
19. Smith RM. Understanding mass spectra: a basic approach. John Wiley & Sons; 2004 Oct 20. doi: 10.1002/0471479357.index.
20. Mass spectrometry A foundation course. UK: K Downard, Royal Society of Chemistry; 2004.


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