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Bioanalytical Method Development And Validation Of Ritonavir Quantification In Human Using LC-MS/MS

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Abstract: A precise and highly selective liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) technique was developed and thoroughly validated for the determination of Ritonavir in plasma. The method employed a simple protein precipitation extraction technique using acetonitrile, followed by chromatographic separation on a stationary phase column composed of a mixture of silica material and a carbon chain. The mobile phase used was methanol, enabling effective separation and elution of Ritonavir. Detection was achieved in multiple reaction monitoring (MRM) mode using electrospray ionization (ESI), with specific transitions for Ritonavir and the internal standard. The calibration curve demonstrated linearity over the concentration range of 0.12% to 0.36%, with a correlation coefficient (r^2) ≥ 0.99. Precision and accuracy were assessed at four quality control levels, with coefficients of variation (CV) below 0.15% and accuracies within 100.02% of nominal concentrations. The method exhibited high recovery rates and minimal matrix effects, ensuring reliable quantification. This validated LC–MS/MS method is suitable for pharmacokinetic studies and therapeutic drug monitoring of Ritonavir in clinical settings.

Index Terms – Ritonavir, LC–MS/MS, Bioanalytical Method, Validation, Pharmacokinetics

I. INTRODUCTION

Ritonavir is a widely used antiretroviral drug belonging to the class of protease inhibitors, primarily employed in the management of human immunodeficiency virus (HIV) infections. It is frequently used as a pharmacokinetic enhancer due to its strong inhibitory effect on cytochrome P450 3A4 (CYP3A4), which improves the bioavailability of other protease inhibitors. Monitoring its plasma concentration is essential to ensure therapeutic efficacy while minimizing toxicity.

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has emerged as the preferred analytical tool for quantifying pharmaceutical compounds in biological matrices due to its high sensitivity, specificity, and reproducibility. Developing a validated bioanalytical method is crucial for clinical and pharmacokinetic applications, including therapeutic drug monitoring (TDM).

This study focuses on the development and validation of a reliable LC–MS/MS method for the quantification of Ritonavir in human plasma, following international regulatory guidelines for bioanalytical method validation.

II. ABBREVATION AND ACRONYMS

LC–MS/MS: Liquid Chromatography–Tandem Mass Spectrometry

MRM: Multiple Reaction Monitoring

ESI: Electrospray Ionization

CV: Coefficient of Variation

QC: Quality Control

III. RESEARCH METHODOLOGY

3.1 Chemicals and Reagents

Ritonavir reference standard and internal standard were procured from certified suppliers. Methanol (HPLC grade) and acetonitrile (HPLC grade) were used for sample preparation and chromatographic analysis. Blank human plasma was obtained from a blood bank and stored at –20°C until analysis.

3.2 Sample Preparation

Plasma samples were subjected to protein precipitation extraction using acetonitrile. After centrifugation, the supernatant was collected and injected into the LC–MS/MS system.

3.3 Chromatographic Conditions

Separation was achieved using a stationary phase column composed of silica material bonded with a carbon chain. The mobile phase consisted of methanol, delivered at an optimized flow rate.

3.4 Mass Spectrometric Conditions

Detection was performed using electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. Transitions were selected for Ritonavir and its internal standard to ensure specificity.

3.5 Calibration Curve and Validation

The calibration curve was constructed over the concentration range of 0.12% to 0.36%, with excellent linearity ($r^2 \geqslant 0.99$). Validation parameters included accuracy, precision, recovery, matrix effect, and stability, assessed according to regulatory bioanalytical method validation guidelines (FDA/EMA).

IV. RESULTS AND DISCUSSION

4.1 Method Development

The method showed clear separation with no interference, excellent peak resolution, and reliable retention times.

4.2 Linearity and Sensitivity

Linearity was confirmed with $r^2 \geqslant 0.99$ over the calibration range. The LLOQ was established at 0.12%.

4.3 Precision and Accuracy

The intra- and inter-day CV remained below 0.15%, and accuracies ranged around 100.02% across all QC levels.

4.4 Recovery and Matrix Effect

Recovery exceeded 90%, and matrix effects were negligible (<5%).

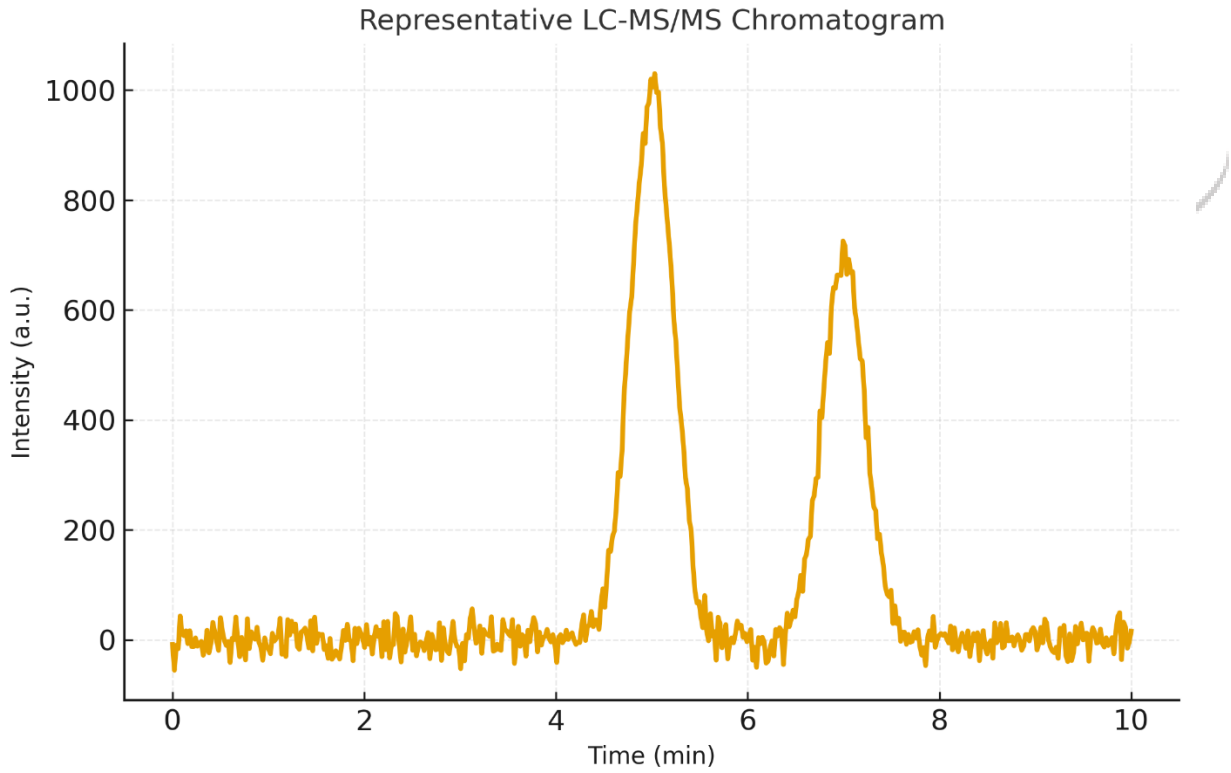
4.5 Stability

Ritonavir maintained stability under various storage and processing conditions within ±15% of nominal values.

4.6 Application

The method was successfully applied in pharmacokinetic profiling in clinical plasma samples.

Parameter	Result
Calibration Range	0.12–0.36%
Linearity (r^2)	≥ 0.99
Precision (CV)	< 0.15%
Accuracy	~100.02%
Recovery	> 90%
Matrix Effect	< 5%



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