CLINICAL AND PRECLINICAL STUDIES OF CEFEPIME AND GH501 USING LC AND LCMS/MS

by

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(Under the Direction of Michael G. Bartlett)

ABSTRACT

The GH501 study developed a specific LC-MS/MS method to evaluate the preclinical pharmacokinetics in rats. The study found that GH501 had slow absorption and elimination, moderate distribution throughout the body, and a relatively high clearance rate. The AUC value suggested that the total amount of drug exposure in the body was moderate. These parameters can provide vital information for drug dosing and therapeutic monitoring for future studies.

The Cefepime study investigated drug pharmacokinetics in 12 critically ill patients with sepsis or sepsis shock. It found that cefepime quickly reached its maximum concentration in the bloodstream, was well-distributed, and rapidly eliminated in critically ill patients. The study also found no significant difference in pharmacokinetic parameters between critically ill patients and healthy adults.

INDEX WORDS: GH501, Cefepime HCl, Prostate cancer, Clinical studies, Preclinical studies, PKPD study, Mass spectrometry, HPLC, LC-MS, LC- MS/MS

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DEDICATION

This thesis is dedicated to my loving parents as a token of my appreciation for their never-ending love, guidance, prayers, and sacrifices, which have shaped me into who I am today.

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES ix

LIST OF FIGURES x

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW 1

Prostate Cancer 1

Small Molecular for Prostate Cancer Treatment 10

Pharmacokinetic Studies 14

LCMS as Analytical Tool for Drug Analysis 19

Sample Extraction Techniques 26

LCMS in GH501 Analytical Studies 28

CHAPTER 2: PRECLINICAL STUDY OF GH501 USING LC ESI-MS/MS 30

Method Development and Modification 30

Material and Methods 31

Chemicals and Reagents 31

Instrument and Conditions 31

Solution and Standards 32

Spiked Samples 32

Sample Preparation 33

Method Validation 33

GH501 Preclinical Study 35
Results and Discussion 35
Preclinical PK Results 39
Conclusion of GH501 Study 39
CHAPTER 3: LC-UV METHOD FOR EVALUATION OF CEFEPIME IV PUSH DOSAGE FORM IN ICU PATIENTS 49
Introduction 49
Previously Conducted Clinical Studies on cefepime HCl 51
Material and Method 53
Chemicals and Reagents 53
Instrument and Conditions 53
Solutions and Standard 54
Spiked Samples 54
Samples Preparation 55
Method Validation 55
Cefepime ICU Patients Study 57
Results and Discussion 57
Clinical Samples Results 61
Conclusion of Cefepime Clinical Study 62
CHAPTER 4: SUMMARY AND FUTURE PROSPECTS 64
Summary 64
Future Prospect 64
REFERENCES 66

LIST OF TABLES

Page

Table 2.1: MS-ESI conditions for MRM of GH501 and GH503 41

Table 2.2: Calibration curve of GH501 41

Table 2.3: Accuracy and Precision of GH501 in rat plasma 42

Table 2.4: Recovery of GH501 43

Table 2.5: Stability study data of GH501 43

Table 2.6: Summary of preclinical PK study of GH501 44

Table 3.1: Calibration curve of Cefepime HCl 78

Table 3.2: Accuracy and Precision of Cefepime HCl in human plasma 78

Table 3.3: Recovery of Cefepime HCl 79

Table 3.4: Stability study data of Cefepime HCl 79

Table 3.5: Summary of clinical PK study of Cefepime HCl 80

LIST OF FIGURES

Page

Figure 2.1: Chemical structures of GH501 and GH50345 45

Figure 2.2: Chromatogram of GH501 and GH503 45

Figure 2.3: GH501 compare graph with blank plasma 46

Figure 2.4: GH503 compare graph with blank plasma 46

Figure 2.5: Calibration Curve of GH501 47

Figure 2.6: Mean plasma concentration of GH501 versus time graph 47

Figure 2.6: GH501 extracted chromatogram from rat plasma samples 48

Figure 3.1: Chemical structures of Cefepime HCl and Cefotaxime Sodium 81

Figure 3.2: Chromatogram of Cefepime HCl and Cefotaxime Sodium 81

Figure 3.3: Cefepime HCl and Cefotaxime Sodium compare graph with blank plasma . 82

Figure 3.4: Calibration Curve of Cefepime HCl 82

Figure 3.5: Mean plasma concentration of Cefepime HCl graph 83

Figure 3.6: Cefepime HCl extracted chromatogram from ICU patient's samples 83

CHAPTER 1

INTRODUCTION

Prostate Cancer

The prostate gland is an integral part of the male reproductive system. It is situated in front of the rectum, directly below the bladder. Based on age, prostate size is different; it is about the size of a walnut in young, healthy males and larger in older men (Daniyal et al., 2014) (Force, 2018). The main function of this gland is to generate a portion of the ingredients contained in semen. The urethra allows passage for semen and urine; as the prostate enlarges, it produces pressure upon the urethra, causing dysfunction with the release of semen and urine (Roehrborn, 2005). An increase in prostate gland size is called benign prostatic hyperplasia. Although some increase in prostate size is normal, the International Classification of Diseases (ICD) code 600 defines this as a disease but not necessarily causing a problem (Roehrborn, 2005).

Any uncontrolled cell growth in the prostate is associated with prostate cancer (society, 2021). According to GLOBOCAN 2020, globally, prostate cancer is the second most common cancer type and the fifth leading cause of cancer death among men (Sung et al., 2021). The American Cancer Society estimated 268,490 new cases and 34,500 deaths from prostate cancer in 2022. However, California and Florida have the highest number of new cases registered in 2022; estimated numbers are 26,890 and 20,680, respectively. The number of cancer incidents decreased from 1990 to 2013 and stabilizedthroughout 2018 for widespread and localized stage disease but increased by

4% to 6% annually for advanced stage cancers. Since 1993 there have been many advancements in diagnosis and treatment, resulting in a decrease of around 53% in mortality from prostatecancer (Siegel et al., 2022).

As prostate cancer is amongst the leading causes of cancer, there are some recommendations and policies in order to control prostate cancer in the United States. The United States Preventive Services Task Force recommends periodic screening of PSA level in men who are 55 to 69 years old; they should consult their doctors and decide to take a test, and men older than 70 years should not take routine PSA test because of increased false positive results in older men. Although this screening recommendation is controversial, as it is still unknown whether this periodic screening could help in controlling cancer, a substantial benefit of this screening could be earlier diagnosis of cancer, and treatment in the initial stages prevents the spread (Force, 2018) (Lin et al., 2008).

There are various factors for the development of prostate malignancy, but genetic, racial, and dietary factors are the most considerable (Siref, 2022). Prostate cancer develops more often in black men compared to white men. Black men are less aware of PSA screening; therefore, disease identification in black men is mainly in an advanced stage. Due to the lack of resources and late diagnosis of prostate cancer in black men, the mortality rate is higher (Lillard Jr et al., 2022). Consumption of certain nutrients, saturated and trans fatty acids, and food like processed meat products lead to disruption of prostate hormonal regulation, induction of oxidative stress and inflammation, and alteration of growth factor signaling and lipid metabolism, which all contribute to prostate carcinogenesis (Oczkowski et al., 2021). Excessive vitamin

supplements can increase the risk of prostate cancer. Some studies on vitamins and folic acid mentioned that over-dose of any vitamin should be avoided to minimize prostate cancer risk (Mandair et al., 2014).

Based on origin and growth, prostate cancer is defined into several types. Small cell carcinoma, squamous cell carcinoma, prostatic ductal adenocarcinoma, prostatic neuroendocrine carcinoma, and prostatic neuroendocrine carcinoma are rare types of prostate cancer (Grignon, 2004). Adenocarcinoma is the most common type, accounting for over 90% of all prostate cancer cases. Acinar adenocarcinoma and prostatic ductal adenocarcinoma are subtypes of epithelial prostate cancer. Acinar adenocarcinoma originates from the periphery of the prostate near the rectum. It is the most common form of prostate cancer and can often be diagnosed through prostate-specific antigen testing, digital rectal exam, and confirmed through biopsy (Wei et al., 2022). Prostatic ductal adenocarcinoma is a rare form of prostate cancer that originates in the ducts of the prostate gland. These ducts carry semen from the prostate to the urethra. This type of prostate cancer typically grows more slowly than other forms and is often detected in later stages when the cancer has already spread to other body parts (Baig et al., 2015). The risk of cancer-specific mortality and overall mortality is significantly higher for ductal adenocarcinoma than for Acinar adenocarcinoma. Early detection at a lower stage and surgical treatment are beneficial factors for the prognosis of ductal adenocarcinoma (Weiet al., 2022).

Prostate cancer is a complex illness that can manifest from small, docile, low-grade tumors to massive, aggressive, potentially fatal tumors. Traditionally, digital rectal examination and PSA screening were the primary tools for prostate cancer diagnosis; any

abnormality discovered in DRE and PSA test results were further confirmed by transrectal ultrasound-guided biopsy. Although these two effective diagnosis tools have some drawbacks since, along with age, it is common for PSA levels to increase, giving false positive results. Also, more than 60% of prostate cancer cases are asymptomatic and have normal DRE and high PSA levels of more than 4.0 ng/ml (Descotes, 2019). According to the National Comprehensive Cancer Network, approx. 85% of men with PSA levels under 4 ng/ml are found to have non-cancerous biopsy findings, whereas men with PSA levels between 4 and 10 ng/ml have a 30–35% probability of receiving positive biopsy result (White et al., 2018). To overcome this issue, biomarkers or a combination of biomarkers and clinical variables tests have been developed, like the prostate health index, the 4K score, Stockholm 3 circulating biomarkers, the Mi-prostate score, and Exo DX Prostate as a urinary-based biomarker (Ferro et al., 2020). The Prostate health index is a blood test approved by the FDA to determine the probability of prostate cancer; in this test, the levels of three different proteins (prostate-specific antigen, human kallikrein 2, and intact PSA) are tested together. The prostate gland generates these proteins, and the levels can be affected by prostate conditions, including prostate cancer. The PHI test helps to differentiate between benign conditions and prostate cancer. However, the PHI test should not be used as a standalone diagnostic tool for prostate cancer and should always be confirmed using other tools (Loeb & Catalona, 2014). The 4Kscore test is an FDA approved test which measures the levels of four specific proteins in blood: PSA, free PSA, intact PSA, and human kallikrein 2. The test uses a formula to calculate the probability of an aggressive level of prostate cancer and the risk of developing prostate cancer metastases within 20 years. This test is relatively

more accurate and can save unnecessary biopsies. It is like the PHI tool but in an advanced form and the same as PHI. It is recommended to go for a biopsy to confirm the presence of prostate cancer before starting any treatment (Punnen et al., 2015) (Administration, 2022).

Other than blood tests, several urine-based markers have been approved by FDAto identify prostate cancer. Researchers are currently exploring using urinary mRNA as a biomarker for prostate cancer. Some studies have identified specific mRNA molecules present at higher levels in the urine of individuals with prostate cancer. In this test, HOXC6and DLX1 urinary levels determine the stage of prostate cancer. However, more study isrequired to completely validate urine mRNAs as prostate cancer biomarker and evaluatetheir therapeutic usefulness (Narain & Sooriakumaran, 2022) (Visser et al., 2022). After traditional diagnosis, biopsy and MRI tools are used to confirm prostate cancer. In addition, targeted biopsies are potential diagnosis tools as it is more accurate due to real-time visualization (Dias et al., 2022). Among the advanced diagnosis tools, multiparametric MRI has been revolutionary. Recent novel developments in ultrasound have also been made to offer a simple and affordable method for prostate cancer detection; it includes elastography, contrast-enhanced ultrasound, and improved B-mode and Doppler techniques. Pre-biopsy magnetic resonance imaging is also a helpful tool to confirm cancer cells in the prostate gland in men with suspicion of prostate cancer (Israëlet al., 2022) (Dias et al., 2022).

Globally, prostate cancer is a significant cause of cancer-related morbidity and mortality. Prostate cancer prognosis evaluation and treatment planning depend on accurate staging (Cheng et al., 2012). Clinical and pathological staging are important

methods to assess tumor extent and treatment planning. Clinical staging is done by non-invasive tests, such as digital rectal examination, prostate-specific antigen blood test, transrectal ultrasound, biopsy, and imaging studies, like CT scan and MRI, to determine the size and location of a tumor (Cheng et al., 2012). The most popular way to describe the clinical stage of prostate cancer is the TNM system suggested by American Joint Committee on Cancers. Where T (tumor): represents the tumor size and spreading of theprimary tumor in the prostate. N (lymph nodes): refers to the involvement of nearby lymph nodes. M (metastasis) indicates whether cancer has spread to distant organs (Cheng et al., 2012) (Paner et al., 2018). On the other hand, pathological staging is done after a radical prostatectomy or prostate removal. It involves a thorough examination of the prostate tissue removed during the surgery by a pathologist to determine the exact size, location, and spread of cancer. In general, pathological staging provides more accurate information about the extent of cancer (Cosma et al., 2016).

Pathological staging is performed through prostate tissue collected in surgery, and without knowing the extent of the tumor, it is not always possible to get prostate tissue; therefore, clinical staging (TNM system) is still more feasible and widely used. This TNM system, combined with the Gleason score, is used to determine a patient's exact stage of prostate cancer. Gleason's score is based on the appearance of the cancer cells under a microscope and is used to help predict how likely the tumor will spread, how aggressively, and what level of treatment is needed. The Gleason score ranges from 2 to 10, with higher scores indicating more aggressive cancer. It is typically reported as a number (Swanson et al., 2021) (Borley & Feneley, 2009).

Stage 0, prostate cancer, also known as carcinoma in situ, is the earliest stage of prostate cancer. At this stage, the cancer cells are confined to a small area within the prostate gland and have not spread to other body parts (UK, 2021). Stage 0 prostate cancer is not considered life-threatening, but it still needs to be treated to prevent it from progressing to more advanced stages. Traditional treatment options for stage 0 prostate cancer included active surveillance, radiation therapy, radical prostatectomy, or surgery to remove the prostate gland (Keyes et al., 2013).

Stage I, prostate cancer is the initial stage in which cancer cells are present on only one side of the prostate gland. In this stage, the tumor size is minimal and restricted to the prostate gland only (Cheng et al., 2012). Sometimes the cancers identified in a DRE test may still be considered stage 1 if the PSA level is less than 10 and the Gleason score is 6 or below (Society, 2019) (Rodrigues et al., 2012). Stage 1 prostate cancer is usually treated with surgery to remove the prostate gland or with external beam radiation therapy. In some cases, active surveillance is recommended, especially if the cancer is small and slow growing. Active surveillance involves monitoring the cancer closely with regular prostate-specific antigen tests and biopsies to ensure the cancer is not progressing (Keyes et al., 2013) (Schatten, 2018). In some recent reports, cryotherapy (using extreme cold to destroy cancer cells) and high-intensity focused ultrasound (using high-frequency sound waves to destroy cancer cells) suggested good management options for selected cases of clinically localized prostate cancer (Nguyen et al., 2013) (Napoli et al., 2020).

Stage II, prostate cancer is much larger and more advanced than stage 1, but it does not spread beyond the prostate gland, is still localized (Carlsson et al., 2020), and

has three substages. In Stage 2A, the tumor is in half of 1 side of the prostate gland or even less. PSA levels are between 10 and 19, and the Gleason score is 7. In stage 2B, the tumor can easily be felt in a DRE and presents only inside the prostate gland. The PSA level is at least 10 and less than 20, and the Gleason score is 7. Stage 2C is a more advanced second-stage cancer. The PSA level is 20, and Gleason scores 7 to 8 (Society, 2019) (Borley & Feneley, 2009). Possible treatment in this stage is active surveillance, radiation therapy, and hormonal therapy (Carlsson et al., 2020). By reducing the levels of male hormones in the body, antiandrogen treatment can slow the growth of prostate cancer cells. (Desai et al., 2021). Cyproterone acetate is a steroidal antiandrogen, and bicalutamide, flutamide, and nilutamide are nonsteroidal antiandrogens that block the effects of testosterone on cancer cells (Wirth et al., 2007). It is commonly given in combination with other medications like chemotherapy and radiotherapy.

Stage-III, cancer is a locally advanced type. In this stage, cancer has spread outside the prostate into local tissue and may have spread into seminal vesicles (Wirth et al., 2007). The PSA and Gleason scores are higher at this stage and based on these scores; it is substaged into three parts. In stage III A**,** cancer cells have spread outside the prostate to nearby tissues and to the seminal vesicles. The PSA level is 20, and the Gleason score is 8 or less. In stage III B, the tumor spread faster and reached the bladderand rectum. The PSA level is more than 20, and the Gleason score is 8 or less. In stage III C, the cancer cells take over almost all healthy prostate tissues, and this is a severe localized cancer stage. The PSA level could be anything, and the Gleason score is 9 or 10 (Wirth et al., 2007) (Society, 2019). Although at this stage, cancer is localized, it

is very close to spreading, and reaching the metastasis stage, therefore, correct treatment is critical at this point. There is no single best treatment to avoid local complications to ensure optimal oncological survival for locally advanced prostate cancer patients. Radical prostatectomy, external beam radiotherapy, and hormonal therapies are options, but most experts recommend combination therapies, like androgen deprivation therapy with radiation therapy or surgery plus radiation therapy (Evans, 2018). As long-term safety data are unavailable for high-dose-rate brachytherapy, some reports still show acceptable toxicity data and promising short-term disease-control rates in patients with locally advanced prostate cancer (Amiya et al., 2017). Hormone therapy, immunotherapy and radiotherapy are used after removing a patient's pelvic lymph nodes (Janiczek et al., 2017). In one study, 224 stage-III prostate cancer patients were selected between 2001 and 2010 and treated with prostatectomy, external beam radiation therapy with/without androgen deprivation, or hormone alone. The overall survival rates were 91, 88, and 67% in surgery, radiation therapy, and hormone therapy groups, respectively, at 5 years (Amiya et al., 2017).

Stage-IV, prostate cancer has spread to distant body parts, and it could be in nearby lymph nodes or bones, most often of the pelvis or spine, and other organs such as the bladder, rectum, lymph nodes, organs, or bones. Cases of stage IV are rarely cured. Still, treatments can extend a patient’s life and ease a patient's pain. Stage IV has any PSA and Gleason scores. Stage IVA cancer has spread to nearby lymph nodes but may or may not have spread to other nearby tissues. Stage IVB cancer has spread to other body areas, such as the bones or distant lymph nodes. This stage is called metastatic prostate cancer. In this stage, various possible treatments have been

discovered, including surgery, chemotherapy, and drugs that will shrink cancer cells and slow their growth (Society, 2019) (Schatten, 2018).

Small-Molecule for Prostate Cancer Treatment (GH501)

The relationship between persistent inflammation and cancer is well recognized. Chronic inflammation can cause DNA damage, mutations, and changes to the way cells divide, (Atlihan, 2022) leading to the formation of cancerous cells. Additionally, inflammatory cells and molecules can stimulate the growth and spread of cancer cells. Therefore, it is believed that anti-inflammatory drugs may be helpful in the prevention or management of cancer (Singh et al., 2019). Arachidonic acid is present in cell membranes and is critical for mammalian cells to produce prostaglandins, which are known mediators of inflammation. The cyclooxygenase enzymes COX-1 and COX-2 catalyze the synthesis pathway of various forms of prostaglandin. Non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit the COX enzymes and the synthesis of COX products. Inhibition of prostaglandin synthesis involved in inflammation can lead to a reduction in inflammation.The COX-2 enzyme is over-expressed in some cancers, including prostate cancer; however, it is not known how COX-2 overexpression affects individuals with prostate cancer who have had radiation treatment (Khor et al., 2007) (James, 2007). Flurbiprofen is one of the non-selective COX inhibitors within the NSAID class. Studies have reported that flurbiprofen and its structural derivatives exhibit anticancer activity. COX inhibitors have been studied in the treatment of prostate cancer. Inhibiting the activity of COX-2 with COX inhibitors may reduce the growth and spread of prostate cancer cells. COX inhibitors have been studied in combination with other treatments for prostate cancer, such as radiation therapy and androgen deprivation therapy (Atlihan,

2022). Flurbiprofen-thioether derivatives have been studied for their potential anticancer activity against prostate cancer cell lines. Some studies have shown that these derivatives can inhibit prostate cancer cell growth and induce apoptosis (programmed cell death) in these cells. One study found that a flurbiprofen-thioether derivative called S-flurbiprofen- thiocarbamate was particularly effective at inhibiting the growth of prostate cancer cells and inducing apoptosis. Another study showed that a derivative called S-(2,3-difluoro benzyl) flurbiprofen-thiocarbamate induced apoptosis in prostate cancer cells and suppressed the activity of a protein called AKT known to promote the growth of cancer cells (Yılmaz et al., 2020).

GH501 is a flurbiprofen analog compound derived using "molecular hybridization" approach. It combines pharmacophoric components "A-B-C" to create new and highly potent small molecules, where each pharmacophore is known to have anticancer properties on its own. These multi-component "A-B-C" Compound are expected to simultaneously target two or more molecular mechanisms in a single entity, reducing the likelihood of drug resistance (Lajos Gera, 2015). A flurbiprofen analog means a compound having a flurbiprofen parent structure that may contain halide substitutions at one or both phenyl rings, alkyl substitutions at its carbon alkanoic acid group, and which is derivatized at its carboxyl alkanoic acid group to an amino acid that itself terminates at an ester or amide group (Lajos Gera, 2013). GH501 is derived after making changes in the A component of GH101. Component A in GH501 is Flurbiprofen, component B is 4- Biphenylalanyl, and C is 4-Amino, 2,2,6,6- tetramethyl piperidinyl refer to Figure 2.1. GH501 differs from GH101 by a methyl group on the α-carbon and a fluorine atom on the phenyl group. These subtle changes dramatically increased the

activity of GH501 and GH503 compared to the starting prototype, GH101, by 3 to 5-fold in 60 human tumor cell lines involving 9 cancer types tested by the NCI-60 tumor cell line screen and 5-10-fold against cell lines in each of the 9 cancer cell types. The average GI50 value for all 60 cell lines from the 9 cancer types was 498 nanomolar for GH501 (Lajos Gera, 2015). Flurbiprofen analogs derivatives contain molecules with very lipophilic structures. GH501 is a highly lipophilic compound with experimental partition coefficient (log P) value of 6.84 (Hooshfar et al., 2018).

GH501 is a potent anticancer compound with distinctive drug-like properties. GH501 has a nanomolar potency against mCRPC and many other human cancer cell lines. In preclinical models of CRPC, GH501 was examined for its anti-cancer effectiveness and mechanism of action. GH501 efficiently produces cell cycle arrest and apoptosis in these cells in the nanomolar range, regardless of resistance to enzalutamide. RNA-seq analysis of GH501 combined with western blotting analysis identified key targets implicated in CRPC progression. A modest dose of GH501 effectively inhibits the skeletal growth of CRPC in a xenograft model without evidence of in vivo toxicity. It has an excellent preclinical safety profile and it's interesting to note thatGH501 seems to be easily distributed to bone tissues.

Mechanism of action- S-phase kinase-associated protein 1 (SKP1) is a protein that plays a vital role in regulating the cell cycle. It functions as a component of a complex called the SCF (Skp1-Cullin-F-box protein) ubiquitin ligase, which is responsible for the targeted degradation of specific proteins (Bai et al., 1996). In cancer, SKP1 is overexpressed in several types of tumors and has been implicated in the progression of cancer by promoting cell proliferation and inhibiting cell apoptosis. In addition, SKP1

hasbeen shown to interact with and regulate the activity of several oncogenic proteins, including p27 and p53, which are important for maintaining cell cycle control and preventing the development of cancer (Hussain et al., 2022) (Tsvetkov et al., 1999). In cancer, ubiquitination plays a role in the disease's development and progression. The loss of normal ubiquitination can lead to the accumulation of oncogenic proteins, which can promote the growth of cancer cells. The overexpression of ubiquitin enzymes can lead to the degradation of tumor suppressor proteins, which can stop the growth of cancer cells. F-box protein is essential for the ubiquitination process. It serves as the substrate recognition component of the SCF complex, binding to specific target proteins and marking them for degradation by the proteasome (Sakamoto et al., 2003) (Tsvetkov et al., 1999). Skp1 stabilizes FBPs and enhances their substrate identification, thereby playing a central role in the formation and function of SCF complexes (Bai et al., 1996). Interruption of protein-protein interaction between Skp1 and FBPs dismantles the SCF machinery, thereby simultaneously affecting the expression and activity of multiple SCF signaling components. Moreover, current drug mechanisms target individual SCF components; in contrast, blocking Skp1-FBP interactions could provide a general, flexible, and more effective approach to inhibit SCF oncogenic signaling and maximize therapeutic efficacy against tumors with high heterogeneity (Skaar et al., 2014). GH501 is a compound that targets these oncogenic factors for the treatment of mCRPC and specifically binds Skp1 and inhibits Skp1-FBPs protein-protein interaction.

GH501 is a first-in-class synthetic molecule that binds to Skp1 with high specificity and prevents Skp1-FBPs from interacting with other proteins in SCF complexes. GH501 can potentially interrupt Skp1-dependent oncogenic processes and achieve maximal

inhibition of metastatic prostate cancer, which is highly heterogeneous at the molecular and cellular levels. GH501 can potentially interrupt Skp1-dependent oncogenic processes and accomplish maximal inhibition of metastatic prostate cancer.

Pharmacokinetic Studies

Pharmacokinetic studies show how a drug substance is absorbed, distributed, metabolized, and eliminated from the body. It is a very important field in pharmacology that helps predict a drug's appropriate dosage and timing for a given patient based on the drug's properties and characteristics (Grogan & Preuss, 2022). Pharmacokinetic studies are important to know about the drugs' movement in the body. Before introducing any new molecule into the human body, it is necessary to perform a preclinical pharmacokinetic study (Starkey & Sammons, 2015). Preclinical pharmacokinetic studies are conducted in the laboratory or with animals before a drug is tested in humans (Administration, 2018). The main goal of preclinical PK studies is to gather information about a drug's absorption, distribution, metabolism, elimination, and potential toxicity in a living organism (Polson & Fuji, 2012). Preclinical pharmacokinetic studies can help identify potential issues that need to be addressed before testing in humans (Leblanc et al., 2018). The results of preclinical pharmacokinetic studies can also inform the design of clinical pharmacokinetic studies. Preclinical pharmacokinetic studies are an important part of the drug development process and are used to ensure that a drug is safe and effective enough to be administered to humans (Li, 2001).

Several different types of preclinical PK studies can be conducted, including,

- *In vitro PK studies* are conducted in the laboratory using cell cultures, biological fluids, permeability models, co-culture models, and isolated organs and

tissues to evaluate a drug's ADME properties. In vitro PK studies are the earliest stage of drug development (Vaddady et al., 2010). In vitro studies are cost-effective compared to in vivo studies because they are typically less expensive, require fewer resources, and are less time-consuming. In vitro studies can be performed using small amounts of drug and require less specialized equipment, making them less expensive than in vivo studies. In vitro PK models identify promising drug molecules and improve chances of success in clinical trials (Polli, 2008). However, it is essential to note that in vitro PK models have limitations and may not fully predict in vivo drug behavior.

- *Single-dose PK studies in animals*: In a single-dose PK study, a single dose of the drug candidate is administered in a group of test animals (rat, mice, guinea pig, etc.), and the concentration of the drug over time is measured in the blood or other body fluids by using HPLC, LCMS, and other analytical techniques (Rajpoot et al., 2021) (M. Li et al., 2019). The PK parameters that are typically measured in the study include the maximum concentration of the drug (C_{max}), the time it takes for the drug to reach C_{max} (T_{max}), and the half-life of the drug ($t_{1/2}$) (Hanberg et al., 2019).

- *Multiple-dose PK studies in animals*: In a multiple-dose PK study, the testing animal is administered the drug at regular intervals over a certain period. Then blood samples are collected at specific time points after each dose, and the blood concentration-time curve measures the drug concentration using analytical methods and mathematical models. These observed PK parameters help predict the drug's pharmacological response in the animal (Depenbrock et al., 2021) (M. Li et al., 2019).

- *PK-pharmacodynamic (PD) studies in animals*: These studies involve the simultaneous evaluation of PK and PD parameters in animals and are used to understand

the relationship between the concentration of a drug in the body and its therapeutic effects. PD studies in animals can provide important information about the mechanisms of action of the drug, the dose-response relationship, and the safety and efficacy of the drug. They are often conducted as a precursor to human clinical studies and can help inform the design and conduct of clinical trials (Zhang et al., 2022).

PK-PD Modeling and Simulation

Pharmacokinetic and pharmacodynamic (PK-PD) modeling and simulation involve developing a mathematical model describing the relationship between a drug's PK and PD. The model can be used to predict the PK and PD of the drug based on various factors such as the dose, frequency of administration, and the characteristics of the individual receiving the drug (e.g., age, weight, gender) (Lim, 2019). PK-PD modeling and simulation are often used in drug development to optimize dosing regimens, predict drug-drug interactions, and evaluate the safety and efficacy of new drugs. It can also predict the PK and PD of drugs in special populations, such as children or elderly individuals, for which PK and PD data may be limited. PK-PD modeling and simulation are valuable tools for improving our understanding of the relationships between a drug's PK and PD and can help inform the drug's clinical use (Rajman, 2008).

PK study or ADME profile is an essential key factor in selecting potential drug candidates. Since many potential drugs are eliminated from further development due to poor PK properties, drug discovery procedures increasingly include early ADME screening for potential "drug-like" properties to maximize the selection of successful candidates. These predictive ADME approaches include *in silico* models, physiochemical parameters, *in vitro* assays, and drug metabolism to better evaluate the medicinal

properties of potential drug compounds and narrow down and concentrate efforts on only the most promising compounds. Based on this preliminary data, a molecule that has efficient PK properties will be further evaluated including measuring the drug's concentration in the plasma, blood, or any pertinent biological matrix for a selected period. Future animal and clinical research can use the knowledge from PK studies to set dosagelevels and dosing frequency. IND applications require preclinical PK studies and safety evaluation from at least two different animal species (one rodent and one nonrodent). These investigations often use multiple dosage levels to assess PK dose dependence. If an oral route is anticipated for the clinic, oral and intravenous administrations are contrasted to evaluate the oral bioavailability of the medicine. Since the initial clinical investigation often focuses on the pharmacokinetics and metabolism of the medication inhumans, it is optional to outline all ADME features of a molecule at the time of IND application (Kumar & Suttle, 2011) (Negus & Banks, 2018) (Wang et al., 2015).

Bioavailability of Drugs- Drug bioavailability refers to the amount or fraction of a drug that enters systemic circulation and is available to exert its pharmacological effect after administration (Price & Patel, 2022). The bioavailability of a drug depends on several factors, such as the route of administration, the drug's chemical properties, and the patient's physiological characteristics. The route of administration plays a crucial role in drug bioavailability. For instance, drugs administered intravenously (IV) have 100% bioavailability, as the drug is directly infused into the bloodstream. In contrast, oral drugs undergo several processes that can affect their bioavailability. Another important factor isdrug biopharmaceutical classification. Some drugs have high solubility

and permeability, allowing them to cross biological membranes and be easily absorbed into the bloodstream. In contrast, others have low solubility or are poorly absorbed, leading to low bioavailability (Song et al., 2004) (Price & Patel, 2022). Bioavailability can be calculated as a ratio between non-IV routes of drug administration (such as oral, intramuscular, or subcutaneous) to the dose of the IV reference dose (Divvela Hema, 2019).

T_{max} and C_{max}- T_{max} refers to the time it takes the drug to reach its maximum concentration in the bloodstream after administration, and it is also known as the time to peak concentration. C_{max} refers to the maximum drug concentration in the bloodstream after administration. It is an important pharmacokinetic parameter used to determine the dose of a drug, the duration of its effect, and its potential side effects (Han et al., 2018). Each participant's experimental data can be used to directly determine the peak time (T_{max}) and concentration (C_{max}). These two measures following an intravenous bolus dose, rely on experimental technique since the concentrations constantly drop after the injection. On the other hand, if the medication is administered intravenously at a steady rate, the peak time coincides with the infusion time. On the other hand, in oral administration, C_{max} and T_{max} rely on the drug amount, rate, and disposition profile; as a result, they can describe the characteristics of several formulations in the same patient (Urso et al., 2002).

Terminal Half-life- In pharmacology, terminal half-life is a term used to mention the time taken by the drug to eliminate 50% from the body after the drug has reached a steady state or equilibrium in the body. After administering a drug, it goes through several phases of elimination from the body. The terminal phase is the final phase of

elimination and occurs when the drug has been metabolized and is being eliminated from the body at a constant rate (Toutain & Bousquet-Mélou, 2004). The terminal half-life is essential in determining the dosing interval and the duration of treatment for a particular drug. It alsoestimates how long a drug takes to be eliminated from the body.

LCMS as Analytical Tool for Drug Analysis

Chromatography arose in 1901 by a Russian botanist Mikhail Tsvet. He separated plant pigments using a glass column packed with a solid adsorbent material, calcium carbonate. This process allowed him to separate and identify the individual components of the pigments. Since then, chromatography has been widely adopted as a powerful analytical tool in analytical chemistry, biology, and many other fields (Altova & Hargittai, 2022). The modernization of chromatography has involved the development of new techniques, instrumentation, and applications for separating and analyzing complex mixtures. Modern chromatography techniques improve with new column materials, advancements in detectors, automation, and multidimensional chromatography. Overall, the modernization of chromatography has dramatically expanded its capabilities and made it an essential tool in a wide range of scientific and industrial applications.

One significant advancement was the introduction of gas chromatography (GC) inthe 1950s, which used a gas as the mobile phase and a packed or capillary column as the stationary phase. Martin and Synge did significant development work on paper chromatography and received the Nobel Prize in Chemistry in 1952 for their pioneering work on separating amino acids by partition chromatography. They used paper as the stationary phase to separate and analyze various compounds (Leslie & John, 2008). Thenext developments were thin-layer chromatography in 1938 (Ettre & Kalasz, 2001)

and high-performance liquid chromatography (HPLC) in 1967; it took around 40 years to advance from LC to HPLC (Surgeons, 2019).

High performance liquid chromatography (HPLC) is the most widely used technique to separate individual chemical components from the mixture of samples based on their column affinity and mobile phase interaction. To run a sample mixture through a column and reach the detector, HPLC has the following elements, a mobile phase reservoir, a degasser unit, a high-pressure pump, an autosampler injector, a column and detector, and a data acquisition system (Ghanjaoui, 2020). The solvent reservoir is typically made of glass and is designed to hold the solvent or mixture of solvents used for the HPLC separation. The solvent reservoir is usually equipped with a level sensor or a sight glass to indicate the amount of solvent in the reservoir (Ali, 2022). In addition, HPLC systems have a degassing unit, which is a device that removes dissolved gases, such as oxygen and nitrogen, from the mobile phase to prevent bubble formation and ensure stable flow rates during the separation. The mobile phase delivered by the HPLC pump can either be isocratic, supplying the same composition, or a gradient, delivering a changing composition. Gradient HPLC pumps are either quaternary (which mix the various mobile phase components in a low-pressure mixing design where one pump draws each component via a four-port proportioning valve) or binary (which mix the mobile phase components in a high-pressure mixing design downstream from two pumpsheads). A pump aspirates the mobile phase from the mobile phase reservoir and forces it through the column and detector. Based on column dimension, stationary phase particle size, and mobile phase composition, the pump can operate at pressures up to 42000 kPa (about 6000 psi) (Ali, 2022). Normal flow rates in HPLC can be anywhere

from 0.1 to 10 ml/min. The upgraded version of this pump system is called ultrahigh performance liquid chromatography (UPLC) and it can operate between 0.01-5 ml/min with pressure range >15000psi (Ken Broeckhoven, 2019). Pumps provide a constant flow of mobile phase to the column under constant pressure. After the pump system, the autosampler injector introduces a precise aliquot of a sample solution from a sample vial to the column under high-pressure flow conditions. It is a sophisticated automation device capable of great precision and long-term reliability (Ali, 2022). The column has a stationary phase, and after injecting the sample into the column through the injector, the column is responsible for separating the compound from the mixture and reaching the detector; the time taken by the compound to travel from column to detector is called the retention time, and this retention time is based on column's interaction affinity towards the compound. The column oven controls the column temperature.

HPLC columns are made up of 316-grade stainless steel, resistant to high pressure, and are relatively chemically inert. The usual HPLC column length is between 50- 300mm and has an internal diameter between 2-5 mm (Ali, 2022). HPLC columns are packed with stationary phases made from organic polymers or silica, including porous, nonporous, and core-shell particles. Theoretical plates are measures of column efficiency, and microparticles (3-10μm) stationary phase is used to achieve large numbers of theoretical plates (Žuvela et al., 2019). The most widely used columns are silica columns, consisting of silicon atoms bridges with -OH groups. Among the silica column, C_{18} is commonly used in reversed-phase HPLC (Klimek-Turek et al., 2020). The C_{18} column is an alkyl-bonded silica (octadecylsilane) that has 18 carbon atoms in the alkyl chain. The C_{18} column is known for its high efficiency, reproducibility, and

excellent peak shape. C_4 and C_8 columns are similar to C_{18} but have a shorter alkyl chain with 4 and 8 carbon atoms, respectively. The C_8 column is used when a slightly less hydrophobic interaction is desired than C_{18} (Žuvela et al., 2019) (Barth et al., 1988). After the compound elutes from the column and reaches the detector, a signal of the substance is captured by the detector and reflected as a chromatographic peak on the data acquisition system. Various columns are coupled with HPLC, such as UV-VIS, FLD, RID, PDA, and electrochemical detectors (Ali, 2022).

While all these types of detectors are helpful in detecting lots of molecules, they each still have some limitations, like their ability to detect very low concentrations and they often are unable to distinguish between compounds of similar structures. It is under these circumstances where HPLC coupled with mass spectrometry becomes very beneficial for analyzing complex and biological substances. liquid chromatography-mass spectrometry (LCMS) can provide additional information about the analyte's molecular weight and structure, including its fragmentation pattern. MS is a highly sensitive and selective technique that can detect low levels of analytes, often down to the parts-per- billion to parts-per-trillion level. For PK analysis, LCMS provides more accurate detection of drugs and their metabolites, clearance, half-life, potential drug interactions, or adverse effects (Jannetto, 2015). The region where these two sets of instruments are connected is called the interface and enables the transfer of ions from the liquid phase to the gas phase. The interface typically includes components, such as the ionization source, the ion optics, and the inlet capillary.

Mass Spectrometry

The basic components of a mass spectrometer include a sample inlet system,

ionization source, mass analyzer, and ion detector. After separating analytes through the LC system, the sample inlet introduces the analyte into the mass spectrometer. Here analytes get ionized creating charged particles. These charged ions travel to the mass analyzer and are separated according to their mass-to-charge (m/z) ratio. The separated ions are then detected and recorded by a detector in the form of a current (Nikalje Anna Pratima, 2018) (Banerjee & Mazumdar, 2012). To ionize the analyte there are two types of techniques used, hard and soft ionization. These categories refer to the amount of energy transferred to the sample molecules during ionization, which affects the extent to which the sample molecules are fragmented during the ionization process. High internal energy in molecules results in extensive fragmentation of the sample molecules. This produces a complex mass spectrum with numerous peaks typically well-separated, which can provide valuable structural information about the sample. However, extensive fragmentation can also result in losing important structural information since you may only see the basic elements of the molecule. This is particularly problematic for large complex molecules. Hard ionization techniques include electron ionization (EI) and chemical ionization (CI). On the other hand, soft ionization techniques provide lower internal energy resulting in less fragmentation of the sample molecules. Soft ionization is particularly useful for analyzing large and complex molecules, such as proteins and polymers, where preserving the molecular structure is important. The most commonly used soft ionization techniques are ESI and MALDI (Wang et al., 2018) (Vikse & Scott McIndoe, 2018).

In ESI the analyte solution is infused into a metal capillary held at an electric potential of several kV. This allows a positive or negative charge to form on the drops of

sample. The solution at the capillary tip is distorted into a Taylor cone that emits a fine mist of droplets. Each droplet is charged due to the presence of excess ions. The drops created begin to shrink due to the solvent evaporating from around the analytes present in the drop of solution. As the drops get smaller, they reach the Rayleigh limit, where an explosion happens, breaking the drop into multiple tiny drops where the analyte is now in its ionized form. The droplets are then attracted toward the mass spectrometer by a metal plate that is charged with the opposite charge of the ionized analytes. This charge directs the droplets into the mass spectrometer, which is then analyzed by the type of mass analyzer attached to the ESI source (Pitt, 2009) (Nikalje Anna Pratima, 2018). ESI is popular due to several reasons. The first advantage is that this process induces very minimal fragmentation of in analyte, allowing most of the compounds to stay in their intact state. ESI also can ionize compounds over a large mass range. Theoretically, ESI can ionize any size of compound if the settings on the ESI source are adjusted to the appropriate range (Winger et al., 1993). ESI is also suitable for very different types of compounds; nonvolatile and polar compounds can be ionized easily using this source. Being able to ionize a wide range of compounds allows a researcher to use the same source for all samples being analyzed, regardless of the type of compound being targeted. Finally, it is excellent for quantitative analysis and has high sensitivity and resolution (Breitbach et al., 2010) (Konermann et al., 2013). One major drawback of ESI is its susceptibility to ion suppression and enhancement (Jessome & Volmer, 2006). After ionization, the ions are transferred into a mass analyzer, where the ions are separated according to their mass-to-charge (m/z) ratio. The ESI ionization source can be attached to many mass analyzers. The most popular mass analyzer types are

quadrupole, time- of-flight (TOF), orbitrap, and ion trap mass analyzers.

The quadrupole mass analyzer is comprised of four cylindrical rods placed parallel in a radial array. These rods are connected to radiofrequency (V) and direct currents (U). The combination of DC and RF is applied to the rods and set radiofrequency in a manner that all the ions collected from the source will make one beam of ions; now this ion beam passes through the Mathieu stability region tip and at a one-time point only one m/z ratio passes through the tip and rest of the ions hit the rods and are discharged. When this U/Vratio becomes constant, it controls the oscillation of ions in a stable region (Douglas, 2009) and each successive ion follows the same path until they reach the detector. The RF values are generally in the range of 1-2MHZ. The DC Voltage may be 1000V, and the maximum RF voltage is 6000V. Quadrupole ion filters can be arranged in series to improve sensitivity and selectivity. This type of arrangement is called a triple quadrupole mass analyzer; it consists of three sets of quadrupole ion filters. The first quadrupole selects m/z values. The second quadrupole works as a collision-induced cell and fragments the ions selected by the first quadrupole using a collision gas. The third quadrupole acts as a mass filter and separates the resulting fragment ions according to their mass-to-charge ratios (m/z) (Ho et al., 2003). The triple quadrupole mass analyzer operates in multiple reaction monitoring (MRM) modes, which allows for highly selective and sensitive detection of specific analytes in a complex mixture. In MRM mode, the first and third quadrupoles are used as mass filters, while the second quadrupoles is used to generate fragment ions. The resulting fragment ions are then filtered and detected by the third quadrupole, allowing for the precise quantification of specific analytes (Fu et al.,2016).

Sample Extraction Techniques

Before injecting samples into mass spectrometry, human or animal samples, whether blood, urine, serum, plasma, or other fluids, typically needs a cleanup step or an extraction process. The most commonly used extraction methods are protein precipitation (PPT), solid-phase extraction (SPE), and liquid-liquid extraction (LLE) (French, 2017). Every sample preparation method has advantages and disadvantages, so choosing the correct method is critical in bioanalysis.

Protein precipitation (PPT) is a common sample preparation technique to remove proteins from biological samples. PPT involves the addition of organic solvents. This organic solvent causes the proteins in the sample to denature and precipitate out of the solution (Polson et al., 2003). This denaturation occurs due to the disruption of hydrogen bonds and hydrophobic interactions between the protein molecules, which are crucial for maintaining protein structure and stability (Morteza Khabiri, 2013). The mixture is then centrifuged at high speed to separate the precipitated proteins from the supernatant. The centrifugal force causes the heavy protein sediments to settle to the tube's bottom, and smaller molecules remain in the supernatant. The supernatant is carefully removed, leaving behind the protein precipitate. The supernatant can then be dried down, reconstituted, or directly injected into the LC system for MS analysis (Wawrzyniak et al., 2018) (French, 2017).

Liquid-liquid extraction (LLE) is another common sample preparation technique widely used in bioanalysis. The method involves the extraction of the analyte of interest or unwanted interference components from one liquid phase to another immiscible liquid phase to get a clean sample (Kole et al., 2011). The mechanism of LLE relies primarily

on partition coefficient and solubility, a simple physical chemistry term “like dissolves like” (A solute dissolves better in a solvent with similar polarity) (Li & Bartlett, 2014). A nonpolar substance dissolves better in organic and polar substances dissolve better in water. When two immiscible solvents are present in one tube, the solute currently being dissolved in the solvent with less solubility will diffuse across the liquid-liquid interphase of the two immiscible solvents to enter the one in which the solute solubility is higher. When analyte molecules are extracted from the aqueous phase into the immiscible organic phase or vice versa in LLE, interactions occur between the analyte and solvent molecules. This interaction involves hydrophobic, dispersion, dipole, or hydrogen bonding interactions (W. Li et al., 2019). Common solvents used for LLE is methyl tert-butyl ether (MTBE), dichloromethane, ethyl acetate, diethyl ether (Li & Bartlett, 2014).

The LLE technique involves several steps, including sample preparation with a pair of immiscible solvents with different densities. The organic solvent should be able to dissolve the target analyte, while the aqueous solvent should dissolve the other components of the sample (Reddy & Yachawad, 2016). A pH is adjusted some-times for better extraction of a drug (Medvedovici et al., 2018). After that, the samples are shaken vigorously to allow layer separation; the supernatant will be collected in another tube (Reddy & Yachawad, 2016) Finally, the supernatant can be dried down, reconstituted, orinjected into the LC system for MS analysis.

Solid-phase extraction (SPE) is a sample preparation technique used in bioanalysis to extract, purify, and concentrate target analytes from a complex biological sample matrix. The method passes the liquid sample through a solid phase cartridge that

selectively retains the target analytes while allowing unwanted matrix components to pass through. The retained analytes are then eluted from the solid phase with a suitable solvent or mobile phase, and the collected sample can be analyzed using chromatography (Li & Bartlett, 2014).

The solid-phase material used in this technique can be made of various materials, such as silica, polymeric or carbon-based materials, depending on the analyte's chemical nature and the complexity of the sample matrix (Ścigalski & Kosobucki, 2020). The choice of the solid-phase material is critical to ensure that the analytes of interest are retained selectively and efficiently. Solid-phase extraction-based sample preparation has several advantages over other sample preparation techniques. For example, it allows for the selective extraction of target analytes from a complex sample matrix, provides high sample throughput, and is amenable to automation. Additionally, it can be used to remove interfering substances that may affect the accuracy and precision of the analysis, such as proteins, lipids, and salts (Bojko, 2022). Overall, solid-phase extraction-based sample preparation is a powerful technique for extracting and purifying target analytes from complex sample matrices, making it an essential tool in bioanalysis.

LCMS in GH501 Analytical Studies

GH501 is a recently synthesized patented drug molecule (Gera et al., 2013) and as a result, limited research has been conducted on it. Currently, only one published analytical method for determining GH501 was developed using liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in rat plasma and tissues (liver, kidney, and femur bones). The method involves extracting

GH501 from rat plasma and tissue homogenates using liquid-liquid extraction (Hooshfar et al., 2018).

CHAPTER 2

PRECLINICAL STUDY OF GH501 USING LC ESI-MS/MS

Method Development and Modification

There is already a GH501 analytical method available, but it was developed for two different anticancer compounds along with their internal standard (Hooshfar et al., 2018). In this new GH501 drug method, the internal standard differs from the available method, and hence a few suitable changes have been made to get better results and responses. For GH501, two precursors to product ion transitions were selected for analysis. The precursor ion confirmation and product ion determination were optimized via direct infusion of standard solution (flow rate 10 µL/min with 10 µg/mL standard concentration). The most abundant fragment ion was used for quantification, and the second one was used only for confirmation. Final GH501 drug concentration optimized with ESI (+) source operated in multiple reaction monitoring. The collision energy and cone voltage used in the previous method, 55eV, and 30 volts, respectively. The collision energy changed to 30eV, and cone voltage to 35 volts in the current method.

In the next step, the chromatographic conditions were modified as the previous method had a long run time. The method was modified to cut the run time from 15 to 10 minutes. Initially, a method was tried on the same mobile phase and column. A few experiments were run by adding formic acid to mobile phase B, achieving better signal intensity and optimal peak shape, as formic acid is a weak organic acid and acts as a

proton donor, making it useful for ionizing analytes in the mass spectrometer (Nshanian et al., 2018). Additionally, formic acid can help to promote efficient desolvation, which can improve the sensitivity of the analysis. Therefore, final mobile phases A 10 mM ammonium format–0.1% formic acid in the water, and mobile phase B was acetonitrile with 0.1% formic acid.

Besides chromatography, the sample preparation method was also changed fromliquid-liquid extraction to protein precipitation. With the LLE method, GH501 signal sensitivity and reproducibility issues were faced, so protein precipitation was tried and compared to LLE, theresults showed less variability and better consistency.

Materials and Methods Chemicals and Reagents

GH501 drug and GH503 internal standard were synthesized according to the procedure mentioned by Gera et al USA patent (Lajos Gera, 2013) chemical structure of GH501 and internal standard are shown in Figure 2.1. LC-MS grade ammonium format was purchased from Millipore Sigma (MILWAUKEE, WI). LC-MS grade formic acid purchasedfrom Fischer chemicals (NJ, USA) water and acetonitrile were also obtained from Millipore Sigma (Allentown, PA). Sprague–Dawley rat plasma (with K2EDTA anticoagulant) was purchased from BioIVT (Westbury USA).

Instrument and Conditions

An Agilent 1100 binary pump HPLC system (Santa Clara, CA) was equipped with aWaters Atlantis dC18 (2.1 × 30 mm, 3 µm) column coupled with a Phenomenex C18 guard column (4.0 × 3.0 mm) for LC separation. The HPLC system was interfaced to a Waters Quattro Micro triple quadrupole mass spectrometer with an electrospray ionization (ESI) source (Milford, MA, USA). Mass lynx 4.1 software by Waters (Beverly,

MA, USA) was used for instrument control and data processing. The column was maintained at 30 °C and autosampler is at room temperature. Mobile phase A was 10 mM ammonium formate–0.1% formic acid in water and mobile phase B was ACN with 0.1% formic acid. The injection volume was 50 µL and the flow rate was 0.3 mL/min. The gradient condition for GH501 was (time/minute, % mobile phase B): (0, 30), (6, 80), (7, 30), (10, 30). Samples were analyzed by mass spectrometryin positive ion ESI mode. Nitrogen was used as the desolvation gas at a flow rate of 500L/h and the cone gas flow was set to 30 L/h. The desolvation temperature was 500°C and the source temperature was 120°C. Argon was used as the collision gas and the collision cell pressure was 2.5 × 10^{-3}. A summary of the cone voltages, collision energies, and precursor and product ions of the analytes is presented in Table 2.1.

Solutions and Standards

Initial stock solutions of drug GH501 and internal standard GH503 were prepared in acetonitrile with a final 1 mg/mL concentration. Both secondary stock solutions were prepared in acetonitrile with a final 10 µg/mL concentration and stored at -80 °C. GH501 secondary stock solution was used to prepare standard calibration solution in acetonitrile toobtain the concentrations 60, 120, 200, 400, 800, 1600, 2000, 4000, and 6000ng/mL. The quality control solution was prepared in acetonitrile at concentrations of 60, 180, 1800, and4800 ng/mL. Internal standard (GH503) solution was prepared in acetonitrile up to the final concentration of 7.5ng/mL. These working solutions were used to make calibration curvesand plasma QC samples.

Spiked Samples

10 µL aliquot of calibration standard solutions or QC working solutions were

spiked into 190 µL of blank Sprague Dawley rat plasma to generate the final concentration of 3, 6, 10, 20, 40, 80, 100, 200 and 300 ng/mL and QC samples with 3, 9, 90 and 240ng/mL of GH501 in plasma. The samples were set aside for 20 minutes at room temperature prior to sample preparation.

Sample Preparation

For the sample preparation a simple protein precipitation method was used. 100 µL of spiked plasma sample was precipitated with 900 µL of acetonitrile containing 7.5ng/mL concentration of internal standard. Acetonitrile was used as a precipitating solvent. The samples were vortexed for 15 minutes and centrifuged at 18,213 g at 4 °C for 15 minutes. Collect 800 µL of supernatant and evaporated to complete dryness using vacuum concentrator. Then the sample was reconstituted with 100 µL of 60:40 mobile phase A–mobile phase B and vortexed for 5 minutes, sonicated for 5 minutes and then centrifuged at 18,213g at 4°C for 5min. Finally, 90 µL of supernatant was transferred for injection.

Method Validation

The method was validated as per the U.S. FDA guidance on bioanalytical method validation (USFDA, 2018). The validation parameters included selectivity, linearity, precision and accuracy inter and intraday, recovery and stability.

In bioanalytical method validation, selectivity refers to the method's ability to measure drug substances in the presence of other plasma components. In a biological sample, many endogenous substances are present that could interfere with the measurement of the analyte. This information is crucial for ensuring that the method is suitable for its intended purpose, like quantifying drug concentration in blood plasma. and

that the results obtained are reliable and can be used to make decisions about the pharmacokinetics of a drug. Selectivity was tested by comparing the GH501 chromatogram at the LLOQ level blank plasma with the same method.

The linearity test was performed by evaluating a 9-concentration point range over 3-300 ng/mL in plasma and plotting a graph against nominal concentration. The linearity calibration curves were built by using 1/x weighted linear regression model.

Accuracy and precision for intra-day (n=5) and inter-day (n=15) were assessed by analyzing QC samples at 3 (LLOQ), 9 (LQC), 90 (MQC) and 240 (HQC) ng/mL for the plasma. Method validation for inter-day accuracy and precision had to be done with three different complete sets of five replicates at each QC level long with calibration curve. Accuracy and precision were calculated as relative error and relative standard deviation,respectively.

Recovery was performed on three replicates at LQC, MQC and HQC concentration (n=3), and compare with post-extraction GH501 spiked samples, post-extraction GH501 and internal standard spiked samples and standard solutions of GH501. Recovery was expressed as absolute recovery (peak area ratio between spiked sample and average of standard at same concentration), relative recovery (peak area ratio between spiked sample and average of post spiked samples at same concentration) and matrix effect (peak area ratio between post spiked sample (GH501 and IS) and average of standards at the same concentration).

Stability studies were performed on LQC and HQC samples of GH501. These included autosampler stability study where, three replicates of LQC and HQC samples were analyzed after 12 hours inside the autosampler at room temperature. Bench top

study performed on three replicates of LQC and HQC spiked samples which were set on a bench top at room temperature for 4 hours and then extracted and analyzed by LCMS. The third stability study parameter was freeze-thaw stability study, the LQC and HQC spiked samples wereanalyzed up to three free-thaw cycles after freezing samples at -80 °C.

GH501 Preclinical Studies

For the GH501 preclinical studies, a total of 15 adult male Sprague Dawley rats withan average body weight of 200-220 g and free access to food and water were used. Therats were orally dosed at a single dose of 10, 20, and 40 mg/kg, with 5 rats in each dose group. Blood samples were taken from the retro-orbital sinus of each rat at pre-dose and at 0.25, 0.5, 1, 2, 4, 8, 16, 24, 36, and 48 hours after dosing. The blood samples were collected in EDTA tubes and centrifuged immediately at 5000RPM for 4 minutes at 4°C, and the plasma obtained was stored at −80 °C until LCMS analysis.

Results and Discussion Selectivity and Specificity

The specificity and selectivity of this method were assessed by comparing the chromatograms of the double blank plasma samples (neither analytes nor IS), Internal standard blank samples (no drug, only IS), and a lower limit of quantification (LLOQ), and comparing if there was any interference of endogenous plasma substances with the retention time of drug or internal standard. The internal standard was also evaluated as a potential source of interference. The interfering peak area of the blank samples should remain within 20 % of the peak area for the analytes atthe LLOQ and be <5 % for the IS. In this method, no interference is found at the retention time of GH501 (6.89 minutes) and internal standard (7.16 minutes). Chromatograms from MRM mode are referred to

in Figure 2.2. Both the peaks are well separated and when compared to chromatograms of a blank plasma run within the same retention time range. Given that no significant peaks were present in blank plasma runs where the analytes are supposedto elute, the method was selective.

Linearity and Sensitivity

A series of known analyte concentrations were prepared and measured using the analytical method. The response of the analytical method for each concentration was plotted on the y-axis against the concentration of the analyte on the x-axis. The resulting curve was then used to determine the concentration of an unknown plasma sample based on its response to the analytical method, Calibration curves were built using 1/x weighted linear regression from the concentration of 3 ng/ml to 300 ng/ml based on peak area ratios between analytes and the IS. The calibration curves for each day of validation had $R2$ values>0.99, refer to Table 2.2 demonstrating the linearity of the method when determining GH501 in plasma (Figure 2.3).

The sensitivity was defined by the LLOQ, which was considered to be the lowest concentration that could be measured by analytical method within 20% precision and accuracy. The LLOQ for GH501 was 3 ng/ml. A highly sensitive analytical method can detect and quantify very low concentrations of the analyte, which can be critical for pharmacokinetic and toxicokinetic studies.

Precision and Accuracy

Accuracy and precision are crucial components of any bioanalytical method validation because they ensure that the results obtained from the method are reliable and reproducible. Accuracy measures how close the estimated value is to the actual value.

On the other hand, precision refers to the consistency or reproducibility of the measurements obtained from the method. The inter-day (n=15) and intra day (n=5) precision and accuracy in rat plasma were validated at the LLOQ, LQC, MQC, and HQC levels for GH501 analytes on three different days using freshly prepared neat standards and were conducted in quintuplicate. The intra-day and inter-day run coefficient of variation (%CV) and relative error (%RE) did not exceed 15 % (20 % for LLOQ) when compared to nominal values, respectively refer to Table 2.3.

Extraction Recovery and Matrix Effect

The extraction recovery was determined by quantifying the detector response in different QC samples containing a known amount of analyte added to the plasma. Absolute and relative recovery was calculated by comparing the area ratio of the extracted and unextracted GH501 compounds at the same concentration. It was determined at three different QC levels, LQC, MQC, and HQC corresponding to 9, 90, and 240 ng/mL concentrations. All QC samples were prepared in triplicate to obtain a precise result in the recovery study. The values of absolute and relative recovery for both analytes in plasma were consistent, precise, and reproducible (Table 2.4).

For the determination of the matrix effect, similar QC samples used in the recovery experiment were taken in triplicate and calculated by comparing the area response of post-extracted with the neat standard at same concentration. A ratio > 100% demonstrates an enhancing ME, a ratio < 100% demonstrates a suppressing ME, and the percentage of enhancement or suppression was calculated as the absolute difference from 100%. In GH501, sample ion enhancement was observed at each recovery experiment. Plasma matrices can contain a wide range of endogenous

compounds that can contribute to ion enhancement in LC-MS/MS. These compounds can interact with the analyte of interest, leading to increased ionization efficiency and signal intensity. These biological residues can also compete with the analytes during ESI and cause variablesuppression or enhancement in the MS response of analytes.

Stability

Stability studies in method validation evaluate expected conditions in sample analysis, including storage and handling. Validation covers autosampler stability, benchtop stability, and freeze-thaw cycle stability.

Two sets of spiked plasma samples were prepared at LQC and HQC levels in triplicate for the autosampler stability study. One set was run immediately after preparation, and the second set was placed on an LC autosampler for 12 hours at room temperature and run. The measured concentrations were compared between these two sets of data. Two additional sets were prepared for the benchtop study. One set was extracted right after spiking the standards into plasma, and the second set was kept on the benchtop for 4 hours at room temperature and then processed. The measured concentrations of GH501 were compared. The accuracy at each level should be less than 15%; for both stability studies, the accuracy and RSD were below 15%. GH501 samples were found to be stable for up to 12 hours on the autosampler, and plasma samples were stable for up to 4 hours on the benchtop (refer to Table 2.5). We prepared four sets of LQC and HQC samples in triplicate to evaluate freeze-thaw stability. One set was extracted and run immediately as a zero-time sample, and the remaining three sets were stored at -80°C.The next day all these sets are taken out, thawed and then one set processed and analyzed. The remaining two sets were again

stored at -80°C. The same process was repeated until three freeze-thaw cycles were generated over an additional two days. The response of the freeze-thaw cycle samples with zero-time samples were compared. The observed results (sown in Table 2.4) demonstrate that GH501 plasma samples were stable up to three freeze-thaw cycles without any significant change in analyte concentration.

Preclinical PK Results

The above-validated LC-ESI-MS/MS method was wholly and successfully applied to evaluate the preclinical pharmacokinetics of GH501 in rats. The pharmacokinetic parameter data obtained for one-compartment modeling and mean plasma concentration-time profiles for GH501 are shown in Table 2.6 and Figures 2.4 and 2.5, respectively. This pharmacokinetic data (Table 2.6) indicated the maximum plasma concentration (Cmax) as 37.36 ng/mL, and the time (Tmax) needed to reach the Cmax was calculated as 8 hours at at 10mg/kg oral dose. The area under the curve was 116 ng.h/mL, and the volume of distribution was calculated as 3.58 liters. The clearance of GH501 from the body was found to be 14.37 liters/hr, and its elimination half-life was 9.6 hours. All the calculated pharmacokinetic parameters revealed that GH501 had slow absorption in the bloodstream.

Conclusion

A specific LC–MS/MS method was developed to identify and quantify the novel anticancer compound GH501 in rat plasma using protein precipitation as a sample cleanup procedure. The method was specific, sensitive, precise, and accurate over a 3-300 ng/mL concentration range. Furthermore, the developed method was successfully applied to pharmacokinetic studies of GH501 following oral administration to rats at

10mg/kg doses. The observed pharmacokinetic parameters demonstrate slow absorption and elimination with a half-life of 9.6 hours. The volume of distribution indicated that the drug was distributed moderately throughout the body. The drug has a relatively high clearance rate of 14.37 liters/hour, so it is effectively removed from the body. The AUC value suggests that the total amount of drug exposure in the body was moderate. These parameters provided important information for drug dosing and therapeutic monitoring. Further study is required to better understand drugs' pharmacokinetic and pharmacodynamic behavior.

TABLES

Table 2.1 MS-ESI conditions for multiple reaction monitoring (MRM) of GH501 and GH503 (IS).

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Cone voltage (V)
GH501	606.56	83.56	15	30
GH503	568.05	84	15	30

Table 2.2 Calibration curves of GH501 in rat plasma (n = 4).

Analyte	Slope ± SD	Intercept ± SD	R2 ± SD
GH501	0.03398 ± 0.0095	0.04358 ± 0.0096	0.9957 ± 0.0021

Table 2.3 Accuracy and precision of GH501 in rat plasma

Analyte	Nominal concentration (ng/ml)	Intra-day n=5			Inter-day n=15		
		Measured concentration ± SD (ng/ml)	Precision RSD (%)	Accuracy (RE %)	Measured concentration ± SD (ng/ml)	Precision RSD (%)	Accuracy (RE %)
GH501	3 (LLOQ)	2.5±0.21	8.3	16.55	2.5±0.19	7.66	18.17
	9 (LQC)	9.58±0.25	2.59	6.41	9.6±0.41	4.29	6.98
	90 (MQC)	87.48±1.76	2.01	2.8	95.1±7.60	7.99	5.67
	240 (HQC)	239.65±7.16	2.99	4.14	243.0±8.91	3.67	2.81

Table 2.4 Recovery (n = 3) of GH501.

Matrix	Concentration (ng/ml)	Absolute recovery (%)	Relative recovery-01 (%)	Relative recovery-02 (%)	Matrix effect (%)	Type of effect
Rat plasma	9.0 (LQC)	142.18±3.56	96.44±2.42	106.11±2.66	134.00±1.94	Enhancement
	90.0 (MQC)	160.50±0.21	96.74±0.13	101.71±0.14	157.81±1.93	Enhancement
	240.0 (HQC)	181.77±2.75	95.91±1.45	98.97±1.50	183.66±6.69	Enhancement

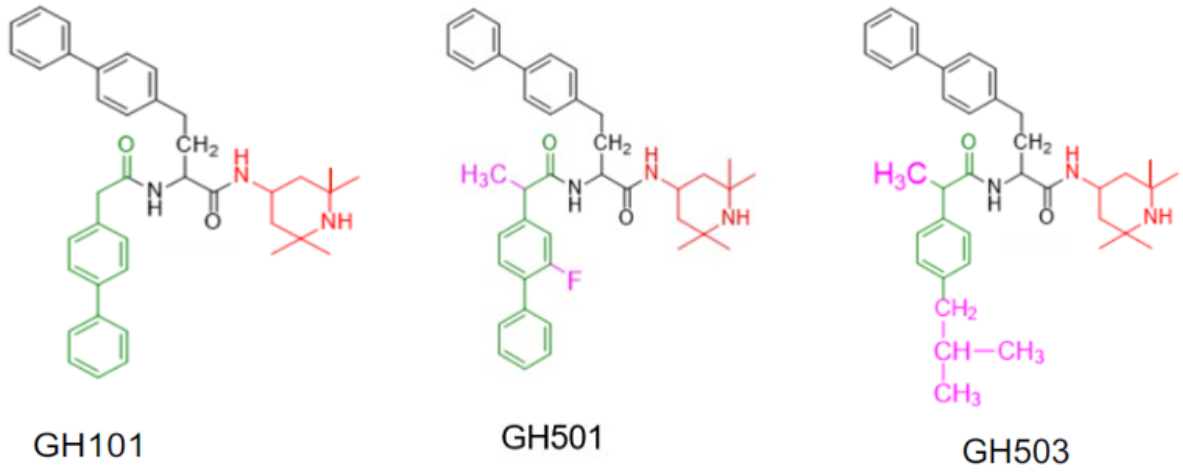
Table 2.5 Stability studies of GH501: autosampler stability (n = 3), bench top stability (n = 3), and freeze-thaw cycle stability (n = 3).

Matrix	Stability	Nominal conc. (ng/ml)	Measured conc. ± SD (ng/ml)	RSD (%)	RE (%)
Rat Plasma	Autosampler (12 hours)	9	9.34±0.14	1.49	3.76
		240	228.23±2.61	1.14	4.9
	Bench-top (4 hours)	9	8.66±0.89	10.25	3.77
		240	245.58±9.36	3.81	2.32
	Freeze-thaw	9	8.37±0.10	1.15	6.96
		240	233.36±8.49	3.64	2.77

Table 2.6 Summary of preclinical pharmacokinetic parameters of GH501 at 10mg/kg dose (n=5).

Parameters	**Pk values**
Cmax (ng/mL)	8.31
Tmax (hour)	8
AUC (ng.h/ml)	116
Elimination rate constant (per hour)	0.072
Volume of distribution (Liter)	3.58
Elimination half-life (hour)	9.6
Clearance (Liter/hour)	14.37

FIGURES



A- Flurbiprofen and Ibuprofen

B- 4-Biphenylalanyl

C- 4-Amino, 2,2,6,6- tetramethyl piperidinyl

Figure 2.1 Chemical Structures of parent compound GH101 and modified GH501 (MW = 605.78 g/mol), and GH503 (MW = 568.05 g/mol.

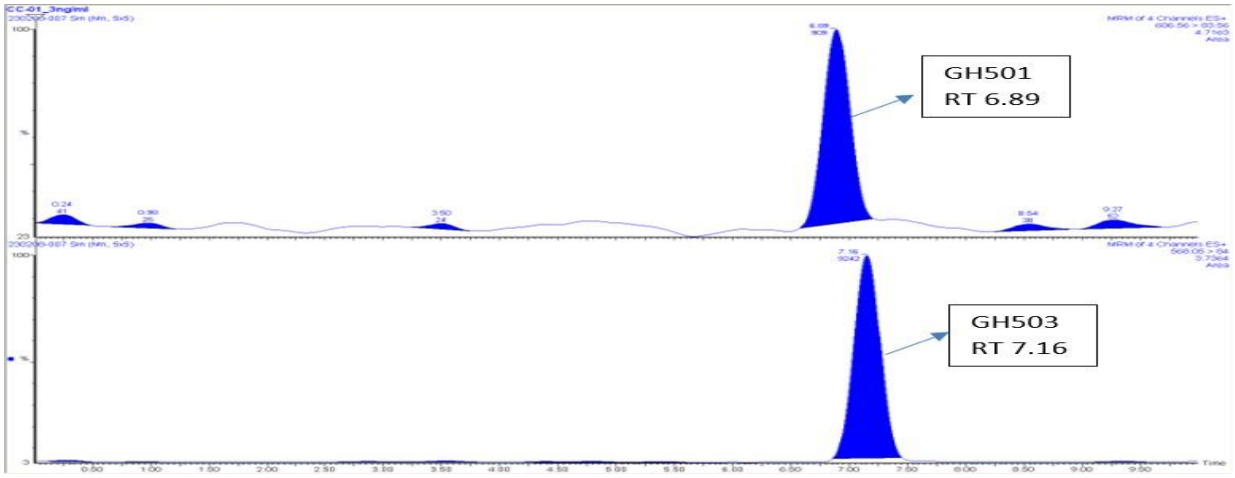


Figure 2.2 Representative chromatograms of GH501 and GH503(IS) in plasma at LLOQlevel (3.0ng/mL) on MRM mode, Retention times for analytes are shown in minutes.

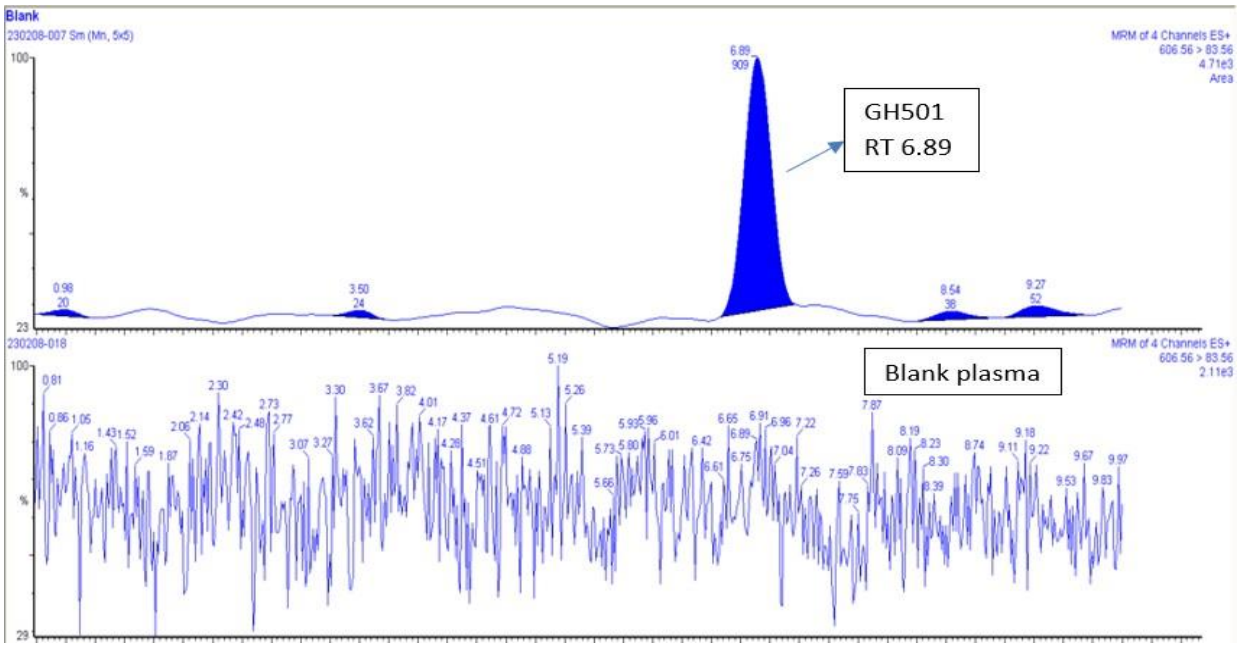


Figure 2.3 Representative chromatograms of GH501 in blank plasma, and plasma atLLOQ of method (3.0 ng/mL) Retention times for analytes are shown in minutes.

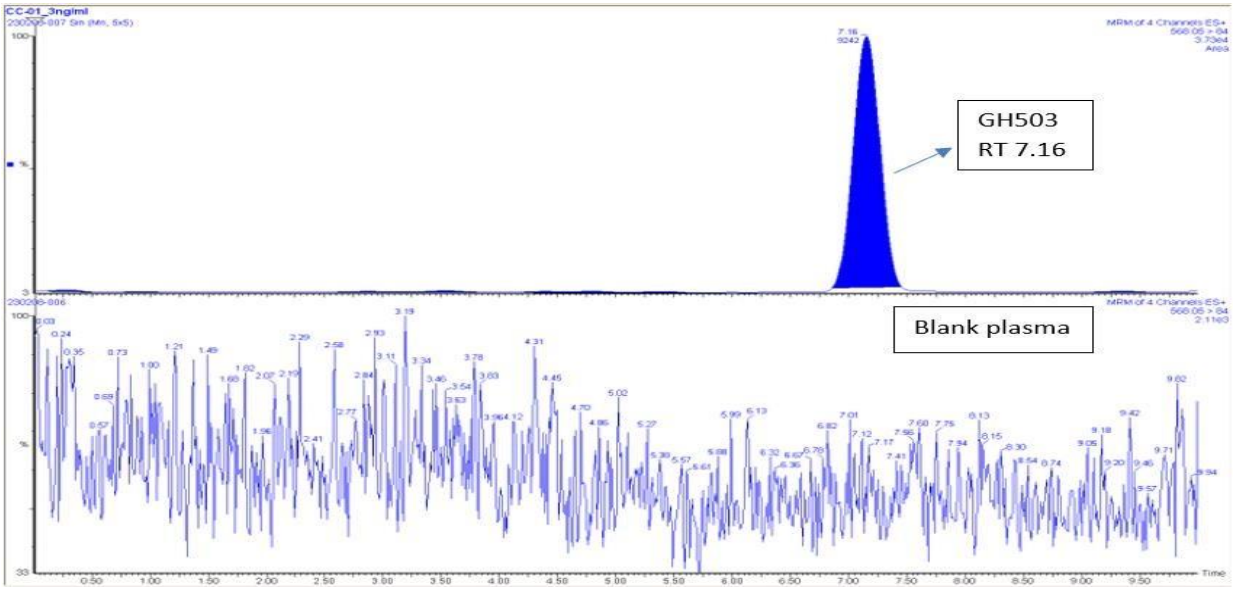


Figure 2.4 Representative chromatograms of internal standard (GH503) selectivity in blank plasma, and spiked plasma. Retention times for analytes are shown in minutes.

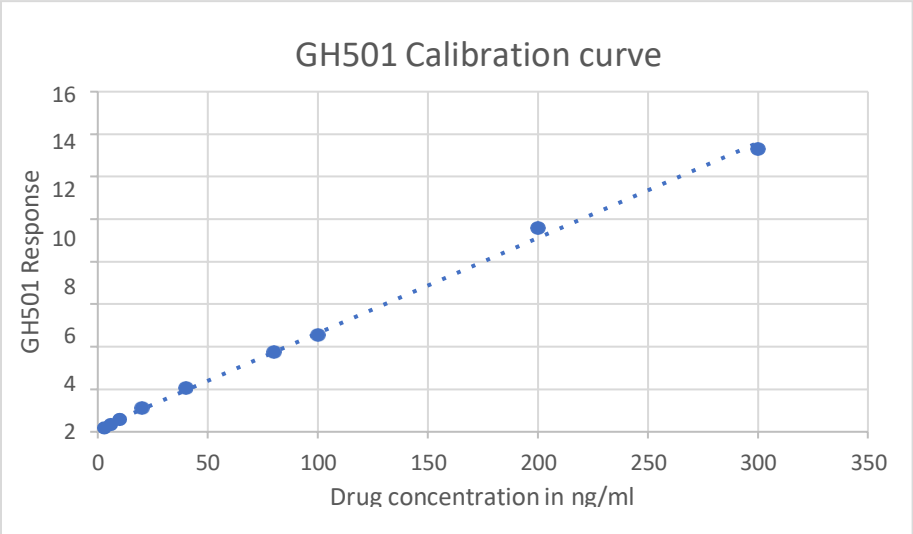


Figure 2.5 Calibration curve graph of GH5 01 using 1/ x weighted linear regression model.

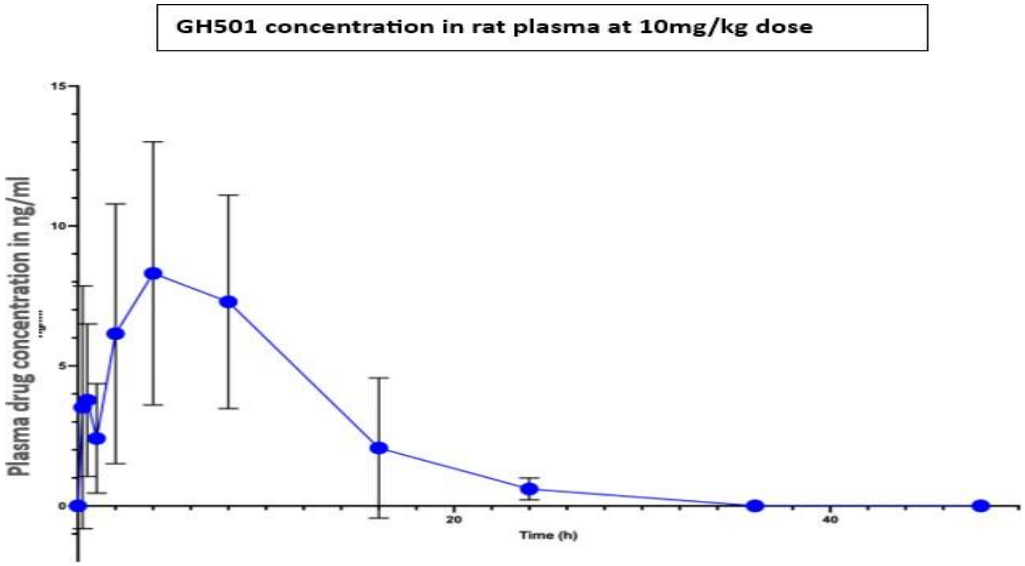


Figure 2.6 Mean plasma concentration-time curves of GH501 in rats after administrationof single oral 10mg/kg dose over 48-hour period.

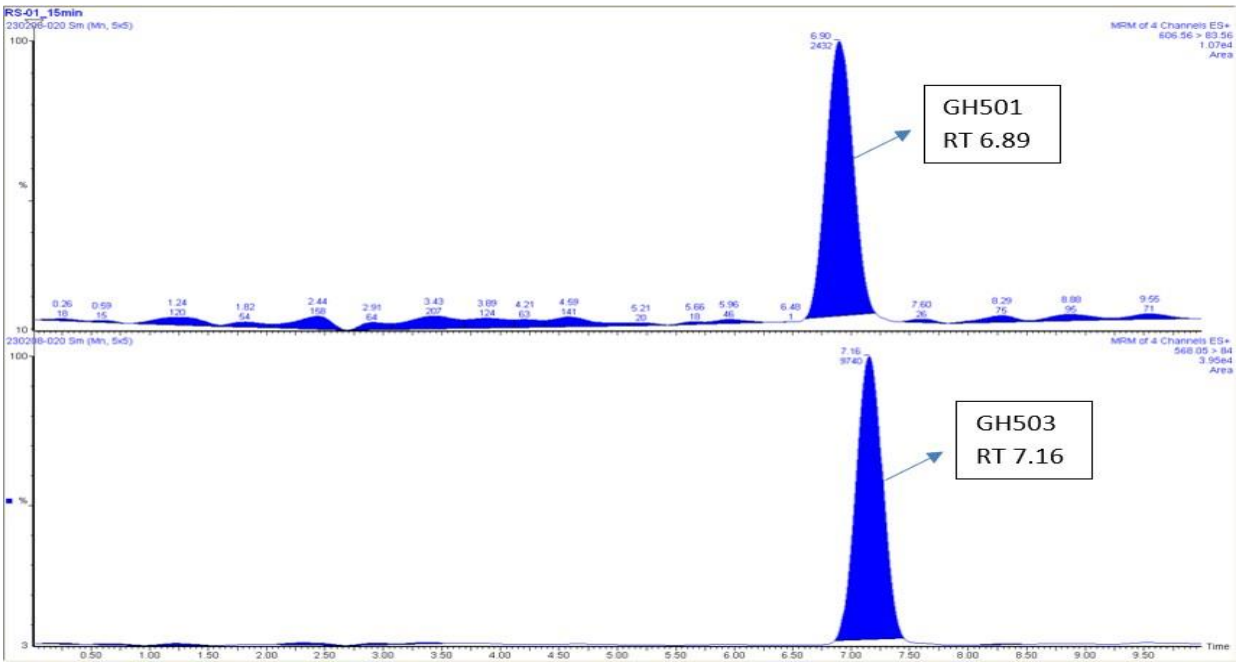


Figure 2.7 Representative chromatograms of GH501 extracted from rat plasma samples.

CHAPTER 3

LC-UV METHOD FOR EVALUATION OF CEFEPIME IV PUSH DOSAGE FORM IN ICU PATIENTS

Introduction

Cefepime is a broad-spectrum cephalosporin used in the treatment of a variety of infections, including pneumonia, complicated urinary tract infections, and kidney infections (Gwendolyn M. Pais et al., 2022). It is a popular agent in the hospital setting, being the sixth most commonly used antibiotic for the treatment of active infections (Gwendolyn M. Pais et al., 2022). Due to its enhanced coverage against Gram-positive and Gram-negative bacteria, cefepime is commonly chosen for treatment of sepsis. Therapeutic agents such as cefepime require frequent dosing to support the time-dependent bacterial activity associated to these compounds. However, national shortages of IV fluid products pose a threat to frequent IV administration (Patiño et al., 2018). These shortages have forced hospitals to explore alternative dosing regimens such as IV push administration, since it requires the use of less consumables. As a response to shortages of IV fluid products, hospitals tend to transition administration of several intravenous piggyback (IVPG) antibiotics to IV push (IVP).

As previously mentioned, cefepime demonstrates time-dependent bacterial activity that may be susceptible to changes when implementing push IV doses over traditional high frequency doses. In IVP, the therapeutic agent is administered in

smaller, more concentrated, volumes and over shorter intervals when compared to IVPG. The concentrated IV push infusion may increase the risks of adverse events and decrease bactericidal activity due to a shorter time of free drug concentration exceeding the minimum inhibitory concentration (MIC) (Gwendolyn M. Pais et al., 2022). Disadvantages of IVP are especially concerning to the critically ill population which is known to exhibit an increased volume of distribution, hypoalbuminemia, augmented renal clearance, and/or multi-organ failure (Mahmoud & Shen, 2017) (Masich et al., 2018). While some studies have investigated the impact of beta-lactams IV push in clinical patients, the pharmacokinetic effects of this type of administration remains largely unknown in ICU patients, which are known to have altered pharmacokinetics and pharmacodynamics (Liuet al., 2020) (Masich et al., 2018).

Previous research considers the transition of IVPG to IVP for several beta-lactam antibiotics to be safe and have similar adverse events to IVPB administration (Marsh et al., 2020). However, there are additional concerns for cefepime administration. A meta-analysis of 57 trials, taking all-cause mortality as the primary outcome, suggests that cefepime treatment is associated with higher all-cause mortality when compared to other □-lactams (RR 1.26, 95% CI 1.08-1.49) (Yahav et al., 2007). Potential factors for higher risks include unrecognized adverse events and inadequate antimicrobial efficacy.

This study investigates the impact of IV push dosing on cefepime pharmacokinetics in critically ill patients with sepsis. Plasma cefepime concentrations were determined through a validated LC-UV analytical method and allowed for steady-state cefepime concentration calculations in plasma of critically ill adults with sepsis. The developed method can be used to better understand the relationship between plasma

cefepime concentrations and clinical outcomes.

Previously Conducted Clinical studies on Cefepime HCl

The first clinical trials on cefepime were likely conducted in the early 1990s, and after that, cefepime was approved by the US FDA in 1994 (Endimiani et al., 2008). Since then, numerous clinical trials have evaluated its efficacy and safety for treating pneumonia, urinary tract infections, sepsis, and other bacterial infections. The first study concluded that cefepime's Pk parameters were dose-independent, the drug did not show significant differences between single and multiple-dose administration, and the elimination half-life was approximately 2 to 2.5 h in patients. Patients tolerated cefepime well; no meaningful drug interactions were reported until 1993 (Okamoto et al., 1993).

Cefepime is administered via several routes, including intravenous (IV) injection or infusion, intramuscular (IM) injection, and, less commonly, subcutaneous (SC) injection. The appropriate mode of administration and dosage of cefepime will depend on the condition being treated and the severity of the infection and after 1993 there were several studies conducted on cefepime to assess variety of factors. Some of these studies are mentioned below.

Study-01, Cefepime is a broad-spectrum antibiotic used for various sepsis conditions, and it is effective alone and in combination with some other antibiotics. Separate clinical trials have been conducted on combination drug therapies for specific disease conditions and populations to evaluate the efficacy and safety of the drug. In 2019, a clinical study was conducted to evaluate the effectiveness of Cefepime-Taniborbactam in treating complicated urinary tract infections and concluded cefepime was safe and well-tolerated (McGovern et al., 2022).

Study-02, A study was conducted on 118 patients to evaluate the clinical outcome of Beta-lactam drugs on IV push versus slow IV infusion and find out that there is no measurable difference in clinical effect and no statistically significant difference in the incidence of phlebitis between these two groups (Baize et al., 2019).

Study-03, Patients with sepsis in the emergency department need a quick shot of antibiotics during the first hour of care. IV piggyback takes 30 minutes to dose 1-2g of cefepime; in contrast, IV push needs only 5 minutes to administer. Especially for emergency care patients, IV push is a viable option for antibiotic administration (Tran et al., 2017). On the other hand, this fast drug administration questions the safety and adverse drug reaction. In 2019 a double-blind study was conducted on Beta-lactam drug safety. The results stated that there was no significant difference in the number of specific adverse reactions or the total number of adverse reactions between the two groups, piggyback and push, at 90 minutes after administering antibiotics. Out of all the patients, 11.8% of patients in the piggyback group had an adverse drug reaction (ADR), whereas 18.9% of patients in the push group had an ADR. Statically, no significant difference in ADR were observed and study concluded the mode of administration does not impact the drug's safety (Rahbar et al., 2021).

Cefepime is primarily eliminated from the body unchanged, meaning that the liver or other organs do not metabolize it. Instead, the kidneys eliminate it from the body through glomerular filtration and tubular secretion. In healthy individuals with normal kidney function, approximately 85% of a dose of cefepime is excreted unchanged in the urine with an elimination half-life of 2–2.5 h. However, in patients with impaired renal function, the elimination of cefepime is reduced, and the drug may accumulate in the

body, leading to higher serum concentrations and an increased risk of toxicity (G. M. Paiset al., 2022).

Study-04, To address high cefepime plasma concentration, a study was conducted on febrile neutropenic patients with mild impairment of renal function. This study shows high cefepime plasma concentrations are associated with neurological toxicity in febrile neutropenic patients with mild renal dysfunction. Adherence to normalized dosing per 100 ml/min glomerular filtration rate (GFR) prevents cefepime-related neurological toxicity. This study's findings suggest that monitoring plasma concentrations can be valuable in preventing neurological toxicity in high dose cefepime therapy for febrile neutropenia. Considering the risks and benefits of cefepime therapy in febrile neutropenic patients is essential. Further research is needed to determine the optimal dosing strategies to minimize the risk of neurological toxicity (Lamoth et al., 2010) (Kim et al., 2012).

Materials and Methods Chemical and Reagents

Cefepime HCl hydrochloride drug was obtained from Merck and Cefotaxime sodium (Internal standard) was obtained from USP reference standard refer to Figure 3.1. ACS reagent grade sodium phosphate, dibasic anhydrous purchased from J.T. Baker (Center Valley,PA, USA), LC-MS grade water and acetonitrile were also obtained from Sigma Aldrich. Human Plasma 3.2% NaCit Gender Pooled Not Filtered get from BioIVT (Westbury, NY, USA) DNA Lo-bind microcentrifuge tubes were obtained from Eppendorf® (Hauppauge,NY, USA).

Instrument and Conditions

An Agilent 1200 series HPLC system (Santa Clara, CA) was equipped with an AgilentEclipse Plus C18 column (4.6 mm x 100 mm, I.D., 3.5 µm) (PN 959961-902) for

chromatographic separation. This instrument was operated using ChemStation software. The column and autosampler temperature were maintained at 40 °C and 4 °C respectively. Mobile phase A consisted of 0.68mg/ml solution of sodium phosphate, Dibasic in LC-MS grade water while mobile phase B consisted of 30% mobile phase A and 70% acetonitrile. The final pH was adjusted for both mobile phases up to 5.0. The flow ratewas left constant at 0.8 mL/min and the gradient elution program was as follows: 0-4 min,7% B; 4-5 min, 35% B, 5-7.5 min, 35% B; 7.5-8.5 min, 90% B; 8.5-11.5 min, 90% B, 11.5-11.6 7% B and maintained up to 15 minutes. Aliquots of 25µl of the standard and sample solutions were injected for analysis and the absorbance was monitored using a UV detector at 245 nm.

Solutions and Standards

Initial stock solutions of Cefepime HCl drug and internal standard cefotaxime sodium were prepared in water with 7 mg/mL and 1mg/ml concentrations respectively. Internal standard solutions were prepared in acetonitrile with a final concentration of 7.5 µg/mL. Cefepime HCl primary stock solution was further diluted with waterto get standard calibration solutions of 200, 400, 600, 800, 1600, 2000, 3000, 4000, and 5000 µg/mL concentration. The quality control solutions were prepared in water at concentrations of 200, 500, 1500, and 3800 µg/mL. These working solutions were used to make calibration curves and QC plasma samples.

Spiked Samples

10 µL aliquot of calibration standard solutions and QC working solutions were spiked into 190 µL of blank human plasma to generate final concentration of 10, 20, 30, 40, 80, 100, 150, 200 and 250 µg/mL and QC samples with 10, 25, 75 and 190 µg/mL of

Cefepime HCl in plasma.

Sample Preparation

For sample preparation a simple protein precipitation method was used. 100 µL of spiked plasma was precipitated with 900 µL of acetonitrile contain 7.5 µg/mL of internal standard. Acetonitrile was used as the precipitating solvent. Samples were vortexsamples for 15 minutes and then centrifuged at 8000 g at 8 °C for 15 minutes. Approximately 800 µL of supernatant was removed and evaporated to complete dryness under vacuum a concentrator. Then the sample was reconstituted with 50 µL of 90:10 water and acetonitrile and vortexed for 5 minutes, sonicate for 5 minutes and then centrifuged at 8000g at 8°C for 5 min. Finally, the sample was transferred to an autosampler vial for injection.

Method Validation

The method was validated as per the U.S. FDA guidance for bioanalytical method validation (USFDA, 2018). The validation parameters include selectivity, linearity, precision, and accuracy at inter and intraday, recovery, and stability.

In bioanalytical method validation, selectivity refers to the method's ability to measure drug substances in the presence of other plasma components. In a biological sample, many endogenous substances are present that could interfere with the measurement of the analyte. This information is crucial for ensuring the method is suitable for its intended purpose, like quantifying drug concentration in blood plasma. And that the results obtained are reliable and can be used to make decisions about the pharmacokinetics of a drug. Selectivity was tested by comparing the Cefepime HCl chromatogram at the LLOQ level in blank plasma.

The linearity test evaluated 9 concentration points ranging from 10-250 µg/mL in plasma and plotted a graph against nominal concentration. The linearity calibration curves are built using a 1/x weighted linear regression model.

Accuracy and precision for intra day (n=5) and inter day (n=15) were assessed by analyzing QC samples at 10 (LLOQ), 25 (LQC), 75 (MQC), and 190 (HQC) µg/mL for plasma. Method validation for inter-day accuracy and precision had to be done with three complete sets of five replicates at each QC level along with the calibration curve. Accuracy and precision were calculated as relative error and relative standard deviation, respectively.

Recovery was performed on three replicates at the LQC, MQC, and HQC concentration (n=3) and compared with post-extraction Cefepime HCl spiked samples, post-extraction Cefepime HCl and internal standard spiked samples and standard solutions of Cefepime HCl. Recovery was expressed in absolute recovery (peak area ratio between the spiked sample and the average of the standard at same concentration),relative recovery (peak area ratio between spiked sample and an average of post-spiked samples at same concentration), and matrix effect (peak area ratio between post spiked sample (Cefepime HCl and IS) and an average of the standard at same concentration).

Stability studies were performed on LQC and HQC samples of Cefepime HCl, Including an autosampler stability study. Three replicates of LQC and HQC samples were analyzed after 12 hours inside the autosampler at room temperature. Benchtop stability was performed using three replicates of LQC and HQC spiked samples set on a bench top for 4 hours at room temperature and then extracted and analyzed on LC-

UV. The third stability parameter was freeze-thaw stability; LQC and HQC spiked samples were analyzed up to three free-thaw cycles after freezing samples at -80 °C.

Cefepime ICU Patient Study

Twenty volunteer ICU patients with sepsis were given a 1-2 g dose of cefepime via IV push for over 5 minutes. The institutional review board, Atlanta, Georgia, approved the study protocols. All subjects were screened against the study's inclusion/exclusion criteria and gave written informed consent. Blood samples were collected after the 5th consecutive dose at 0, 5, 15, 30, 60, 120, and 240 minutes after drug administration. Initial 3 ml blood samples were discarded. The remaining blood samples were collected in sodium citrate tubes and centrifuged immediately at 5000 rpm for 4 minutes at 18°C, and the plasma obtained was stored at −80 °C until HPLC analysis.

Results and Discussion

Method Development

Method development for drug plasma analysis on HPLC involves several steps, including selecting a suitable stationary phase, mobile phase, detection method, and sample extraction method. The stationary phase is the material that is packed into the HPLC column and used to separate the drug molecules. The mobile phase is the solvent system used to elute the drug molecules from the stationary phase; the sample extraction method is to extract the drug from a biological fluid; and the detection method.

Chromatographic parameters were initially taken from the United States and European pharmacopeia monographs. However, since the monograph methods were not for drug analysis in biological fluids, a few necessary modifications were made to

achieve better selectivity and separation. The monograph recommended running the LC in gradient mode with mobile phase A, a mixture of 10 volumes of acetonitrile and 90 volumes of a 0.68 mg/mL solution of potassium dihydrogen phosphate adjusted to pH 5.0. Mobile phase B consisted of a 50:50 mixture of acetonitrile and a 0.68 mg/mL solution of potassium dihydrogen phosphate adjusted pH of 5.0. However, the method resulted in peak splitting of the internal standard and overlapping of a blank matrix peak with the cefepimepeak retention time. Next, different combinations of gradient and mobile phase strength were explored resulting in two distinct and separate peaks for cefepime and the internal standard without interference.

After configuring all chromatography parameters using a neat standard solution, the next step is to determine a suitable drug extraction method from the biological sample. To keep the method simple, protein precipitation was selected. After several adjustments to the plasma and organic solvent volume, a final method setup on 100µl of human plasma crashed with 900µl of acetonitrile, followed using the same procedure mentioned in the sample preparation section.

Selectivity and Specificity

The specificity and selectivity of this method were assessed by comparing the chromatograms of the double blank plasma samples (neither analytes nor IS), internal standard blank samples (No drug, only IS), and a lower limit of quantification (LLOQ), It is important to note any interference of endogenous plasma substance with the retention time of the drug. Also, there is a need to evaluate potential interferences from the IS. The interfering peak area of the blank samples should be less than 20 % of the peak area for the analytes at the LLOQ and<5 % for the IS. In this method, no interference

was found at the retention time of Cefepime HCl (5.3 minutes) and internal standard (7.5 minutes). A typical HPLC chromatogram is shown in Figure 3.2 Both the peaks are well separated and compared to chromatograms of blank plasma run within the same retention time range. The method was selective because no significant peaks were present in blank plasma runs when the analytes were supposed to elute.

Linearity and Sensitivity

A series of known analyte concentrations are prepared and measured using the analytical method. The response of the analytical method for each concentration was plotted on the y-axis against the concentration of the analyte on the x-axis. The resulting curve is then used to determine the concentration of unknown plasma sample based on their response to the analytical method, Calibration curves were generated using 1/x weighted linear regression from the concentration of 10 µg/mL to 240 µg/mL based on peak area ratios between analytes and the IS. The calibration curves for each day of validation had $R2$ values>0.999, demonstrating good linearity of the method when determining Cefepime HCl in plasma shown in Table 3.1 and Figure 3.3.

The sensitivity was defined by the LLOQ, which was considered to be the lowest concentration that could be measured by the analytical method within 20% precision and accuracy. The LLOQ for Cefepime HCl was 10 µg/mL. A highly sensitive analytical method can detect and quantify very low concentrations of the analyte, which can be critical for pharmacokinetic and toxicokinetic studies.

Precision and Accuracy

Accuracy and precision are crucial components of any bioanalytical method validation because they ensure that the results obtained from the method are reliable and

reproducible. Accuracy measures how close the estimated value is to the actual value. On the other hand, precision refers to the consistency or reproducibility of the measurements obtained from the method. The inter day (n=15) and intra day (n=5) precision and accuracy in human plasma were validated at the LLOQ, LQC, MQC, and HQC levels for Cefepime HCl analytes on three different days using freshly prepared neat standards and were conducted in quintuplicate. The intra-day and inter-day run coefficient of variation (%CV) and relative error (%RE) did not exceed 15 % (20 % for LLOQ) when compared to nominal values, respectively refer Table 3.2 for summary.

Extraction Recovery

The extraction recovery was determined by quantifying the detector response in different QC samples containing a known amount of analyte added to the plasma. Absolute and relative recovery was calculated by comparing the area ratio of the extracted and unextracted Cefepime HCl compounds at the same concentration. It was determined at three different QC levels, LQC, MQC, and HQC, corresponding to 25, 75, and 190 µg/mL concentrations. All the QC samples were prepared in triplicate to obtain a precise result for recovery study. The absolute and relative recovery values for both analytes in plasma were consistent, precise, and reproducible summary is shown in Table 3.3.

Stability

Stability studies in method validation evaluate expected conditions in sample analysis, including storage and handling. Validation covers autosampler stability, benchtop stability, and freeze-thaw cycle stability.Two sets of spiked plasma samples were prepared at LQC and HQC levels in triplicate to evaluate autosampler stability.

One set was run immediately after preparation, and the second set was placed on the LC autosampler for 12 hours at room temperature and then analyzed. The measured concentrations between those two sets were compared. Two sets of samples were prepared for the benchtop study. One set was extracted right after spiking standards into plasma, and the second set was kept on the benchtop for 4 hours at room temperature and then processed. The measured concentration of Cefepime HCl between these two sets of samples were compared. The accuracy and precision at each level was found to be less than 15%, for both stability studies. Cefepime HCl samples were stable for up to 12 hours on the autosampler, and plasma samples are stable for up to 4 hours on the benchtop. We prepared four sets of LQC and HQC samples in triplicate to evaluate freeze-thaw stability.We immediately extracted and ran one set which was considered as time zero and the remaining samples were stored at -80°C. The next day all the remaining sets of samples were removed from the freezer, thawed and one set was processed. The remaining two sets again stored at -80°C. The same process was repeated through three freeze- thaw cycles over an additional two days. The response from the freeze-thaw cycle samples were compared to the time zero samples (Shown in Table 3.4). The observed results demonstrated that the Cefepime HCl plasma samples were stable up to three freeze-thaw cycles without any significantchange in analyte concentration.

Clinical Samples Results

The pharmacokinetic study was conducted on ICU patients with sepsis or sepsis shock. These patients were administered cefepime for injection, USP manufactured by APOTEX Pharmaceutical Corporation by IV push injection of 1-2g of

cefepime every 8-12 hours. The cefepime amount was determined between 0- 4 hours in human plasma. The above-validated HPLC-UV method was wholly and successfully applied to evaluate the clinical pharmacokinetics of cefepime HCl. The pharmacokinetic parameters data obtained for one-compartment modeling and mean plasma concentration-time profiles for cefepime are shown in Table 3.5 and Figures 3.4 and 3.5, respectively. This pharmacokinetic data (Table 3.5) indicated that cefepime HCl reached its maximum concentration in blood after 5 minutes of IV push administration. It begins to decline slowly due to drug distribution to the tissue and organs. The maximum plasma concentration (Cmax) was determined as 136.8 µg/mL, and the time (Tmax) needed to reach the Cmax was calculated as 5 minutes. The area under the curve was 459.8 µg.h/mL, and the volume of distribution was calculated as 18.8 ± 2.9 liters. The clearance of cefepime HCl from the body was found to be 4.8 ± 1.6 liters/hr, and its elimination half-life was 2.9 ± 0.9 hours. Cefepime is typically administered intravenously, as it has poor oral bioavailability and is not absorbed well through the gastrointestinal tract. When given by an intravenous push, cefepime was rapidly distributed throughout the body, reaching therapeutic concentrations in the blood and tissues within 5 minutes. Cefepime was well-absorbed and distributed in the body following IV push administration, demonstrating that this is an effective means of administrating this medication.

Conclusion

The study investigated the safety, tolerance, and pharmacokinetics of a 1-2g IV push dosing of cefepime in 12 critically ill patients with creatinine clearance >60 mL/min. The patients were on five consecutive steady-state doses of cefepime before receiving the single IV push dose. Cefepime plasma concentrations were analyzed using the

validated HPLC-UV method. The (Cmax) in the bloodstream was 136.8 µg/mL, which was reached within 5 minutes after administration (Tmax). This suggests that the drug has a rapid onset of action. Cefepime was well distributed in the body, and there was no difference in the volume of distribution in critically ill patients and healthy adults. However, the clearance rate was slower in ICU patients. Also, the short elimination half-life indicated the drug was rapidly eliminated from the body, and there was no evidence of drug accumulation in the body. Overall, there were no impactful differences in the PK parameters of cefepime observed in critically ill patients.

CHAPTER 4

SUMMARY AND FUTURE PROSPECTS

Summary

The GH501 study developed a specific LC-MS/MS method to evaluate the preclinical pharmacokinetics in rats. The study found that GH501 had slow absorption and elimination, moderate distribution throughout the body, and a relatively high clearance rate. The AUC value suggested that the total amount of drug exposure in the body was moderate. These parameters can provide vital information for drug dosing andtherapeutic monitoring for future studies.

The Cefepime study investigated drug pharmacokinetics in 12 critically ill patients with sepsis or sepsis shock. It found that cefepime quickly reached its maximum concentration in the bloodstream, was well-distributed, and rapidly eliminated in criticallyill patients. The study also found no significant difference in pharmacokinetic parametersbetween critically ill patients and healthy adults.

Future Prospects

GH501 is a newly synthesized compound in the early stages of drug development. The study included in this thesis determined preliminary PK values in rats. There still remain multiple additional preclinical and clinical studies before this drug reaches market approval. Henceforward, every future study advances this compound toward this goal. Further determination of absorption, distribution, metabolism, and excretion (ADME) is needed to better understand the drug action within the body. To evaluate bioavailability, IV dose analysis is required. The next step could be a toxicology study of

GH501, like a single-dose maximum tolerated dose and 7-day or long-term repeat-dose toxicity in rats. After performing all the PKPD and toxicity studies on healthy rodents, a study on the in vivo efficacy of GH501 against metastatic castration-resistant prostate cancer in tumor-bearing rodents would be worthwhile.

The Cefepime study approval is for ICU plus non-ICU patients; this thesis includes ICU patients' study data. After registering non-ICU patients, it would be useful to compare the pharmacokinetic parameters of ICU and non-ICU populations when using cefepime IV push (mode of drug administration). Also, Cefepime is primarily eliminated from the body unchanged via the kidneys through urine. Compared to traditional IV infusion, fast drug administration (IV push) may cause sudden dose dumping into the body. Individuals with impaired renal function are at higher risk for accumulation of Cefepime in the body, which can lead to neurotoxicity, including confusion, delirium, myoclonus, and seizures. Therefore, patients with renal impairment would require dose adjustments to avoid an accumulation of Cefepime. A clinical study of IV push on specific populations would be worthwhile.

REFERENCES

Administration, U. S. F. D. A. (2022). *4Kscore Test – P190022*. Retrieved from https://www.fda.gov/medical-devices/recently-approved-devices/4kscore-test-p190022

Administration, U. S. F. D. A. (2018). *Step 2: Preclinical Research*. Retrieved from https://www.fda.gov/patients/drug-development-process/step-2-preclinical-research

Ali, A. H. (2022). High-Performance Liquid Chromatography (HPLC): A review. *HSPI Openaccess journal*. https://doi.org/https://doi.org/10.29328/journal.aac.1001026

Altova, E. P., & Hargittai, I. (2022). Mikhail S. Tsvet—pioneer of chromatography—150 years from his birth. *Structural Chemistry*, *33*(1), 1-3. https://doi.org/10.1007/s11224-021-01804-z

Amiya, Y., Yamada, Y., Sugiura, M., Sasaki, M., Shima, T., Suzuki, N., Nakatsu, H., Murakami,S., & Shimazaki, J. (2017). Treatment of locally advanced prostate cancer (Stage T3). *Jpn J Clin Oncol*, *47*(3), 257-261. https://doi.org/10.1093/jjco/hyw186

Atlihan, I. (2022). Effect of flurbiprofen derivative (SGK597) on cell proliferation and apoptosis ofbreast cancer cell lines. *Istanbul J Pharm 52 (3)*, 265-270. https://dergipark.org.tr/en/download/article-file/2335375

Bai, C., Sen, P., Hofmann, K., Ma, L., Goebl, M., Harper, J. W., & Elledge, S. J. (1996). SKP1connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, theF-box. *Cell*, *86*(2), 263-274. https://doi.org/10.1016/s0092-8674(00)80098-7

Baig, F. A., Hamid, A., Mirza, T., & Syed, S. (2015). Ductal and Acinar Adenocarcinoma of Prostate: Morphological and Immunohistochemical Characterization. *Oman Med J*, *30*(3), 162-166. https://doi.org/10.5001/omj.2015.36

Baize, P., Smith, T., & Faust, A. (2019). 1831: INTERMITTENT IV INFUSION VERSUS SLOWIV PUSH BETA-LACTAM ADMINISTRATION: EFFECT ON CLINICAL OUTCOMES. *Critical Care Medicine*, *47*(1).https://journals.lww.com/ccmjournal/Fulltext/2019/01001/1831_INTERMITTENT_IV_INFUSION_VERSUS_SLOW_IV_PUSH.1780.aspx

Banerjee, S., & Mazumdar, S. (2012). Electrospray ionization mass spectrometry: a techniqueto access the information beyond the molecular weight of the analyte. *Int J Anal Chem*, *2012*, 282574. https://doi.org/10.1155/2012/282574

Barth, H. G., Barber, W. E., Lochmueller, C. H., Majors, R. E., & Regnier, F. E. (1988). Columnliquid chromatography. *Analytical Chemistry*, *60*(12), 387-435. https://doi.org/10.1021/ac00163a025

Bojko, B. (2022). Solid-phase microextraction: a fit-for-purpose technique in biomedical analysis. *Analytical and Bioanalytical Chemistry*, *414*(24), 7005-7013. https://doi.org/10.1007/s00216-022-04138-9

Borley, N., & Feneley, M. R. (2009). Prostate cancer: diagnosis and staging. *Asian J Androl*,*11*(1), 74-80. https://doi.org/10.1038/aja.2008.19

Breitbach, Z. S., Wanigasekara, E., Dodbiba, E., Schug, K. A., & Armstrong, D. W. (2010). Mechanisms of ESI-MS Selectivity and Sensitivity Enhancements When Detecting Anions in thePositive Mode Using Cationic Pairing Agents. *Analytical Chemistry*, *82*(21), 9066-9073. https://doi.org/10.1021/ac102115w

Carlsson, S., Benfante, N., Alvim, R., Sjoberg, D. D., Vickers, A., Reuter, V. E., Fine, S. W., Vargas, H. A., Wiseman, M., Mamoor, M., Ehdaie, B., Laudone, V., Scardino, P., Eastham, J., &Touijer, K. (2020). Risk of Metastasis in Men with Grade Group 2 Prostate Cancer Managed with Active Surveillance at a Tertiary Cancer Center. *J Urol*, *203*(6), 1117-1121. https://doi.org/10.1097/ju.0000000000000742

Cheng, L., Montironi, R., Bostwick, D. G., Lopez-Beltran, A., & Berney, D. M. (2012). Staging ofprostate cancer. *Histopathology*, *60*(1), 87-117. https://doi.org/https://doi.org/10.1111/j.1365- 2559.2011.04025.x

Cosma, G., Acampora, G., Brown, D., Rees, R. C., Khan, M., & Pockley, A. G. (2016). Prediction of Pathological Stage in Patients with Prostate Cancer: A Neuro-Fuzzy Model. *PLoSOne*, *11*(6), e0155856. https://doi.org/10.1371/journal.pone.0155856

Daniyal, M., Siddiqui, Z. A., Akram, M., Asif, H. M., Sultana, S., & Khan, A. (2014). Epidemiology, etiology, diagnosis and treatment of prostate cancer. *Asian Pac J Cancer Prev*,*15*(22), 9575-9578. https://doi.org/10.7314/apjcp.2014.15.22.9575

Depenbrock, S., Urbano, T., Ziegler, J., Wetzlich, S., Clapham, M. O., & Tell, L. A. (2021). Pharmacokinetic Parameters and Tissue Withdrawal Intervals for Sheep Administered MultipleOral Doses of Meloxicam. *Animals (Basel)*, *11*(10). https://doi.org/10.3390/ani11102797

Desai, K., McManus, J. M., & Sharifi, N. (2021). Hormonal Therapy for Prostate Cancer. *EndocrRev*, *42*(3), 354-373. https://doi.org/10.1210/endrev/bnab002

Descotes, J.-L. (2019). Diagnosis of prostate cancer. *Asian Journal of Urology*, *6*(2), 129-136.https://doi.org/https://doi.org/10.1016/j.ajur.2018.11.007

Dias, A. B., O'Brien, C., Correas, J.-M., & Ghai, S. (2022). Multiparametric ultrasound and micro-ultrasound in prostate cancer: a comprehensive review. *The British Journal of Radiology*,*95*(1131), 20210633. https://doi.org/10.1259/bjr.20210633

Divvela Hema, N. (2019). Bioavailability and Bioequivalence Studies. In A. Usama & A. Juber(Eds.), *Pharmaceutical Formulation Design* (pp. Ch. 4). IntechOpen. https://doi.org/10.5772/intechopen.85145

Douglas, D. J. (2009). Linear quadrupoles in mass spectrometry [https://doi.org/10.1002/mas.20249]. *Mass Spectrometry Reviews*, *28*(6), 937-960.https://doi.org/https://doi.org/10.1002/mas.20249

Endimiani, A., Perez, F., & Bonomo, R. A. (2008). Cefepime: a reappraisal in an era of increasing antimicrobial resistance. *Expert Rev Anti Infect Ther*, *6*(6), 805-824. https://doi.org/10.1586/14787210.6.6.805

Ettre, L. S., & Kalasz, H. (2001). The Story of Thin-Layer Chromatography [Article]. *LC-GC North America*, *19*, 712. https://link.gale.com/apps/doc/A76843728/AONE?u=uga&sid=googleScholar&xid=739db781 Evans, A. J. (2018). Treatment effects in prostate cancer. *Mod Pathol*, *31*(S1), S110-121. https://doi.org/10.1038/modpathol.2017.158

Ferro, M., De Cobelli, O., Lucarelli, G., Porreca, A., Busetto, G. M., Cantiello, F., Damiano, R., Autorino, R., Musi, G., Vartolomei, M. D., Muto, M., & Terracciano, D. (2020). Beyond PSA: TheRole of Prostate Health Index (phi). *Int J Mol Sci*, *21*(4). https://doi.org/10.3390/ijms21041184 Force, U. S. P. S. T. (2018). *Prostate cancer: Screening*. https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/prostate-cancer- screening

French, D. (2017). Chapter Five - Advances in Clinical Mass Spectrometry. In G. S. Makowski(Ed.), *Advances in Clinical Chemistry* (Vol. 79, pp. 153-198). Elsevier. https://doi.org/https://doi.org/10.1016/bs.acc.2016.09.003

Fu, Q., Chen, Z., Zhang, S., Parker, S. J., Fu, Z., Tin, A., Liu, X., & Van Eyk, J. E. (2016).Multiple and Selective Reaction Monitoring Using Triple Quadrupole Mass Spectrometer:Preclinical Large Cohort Analysis. *Methods Mol Biol*, *1410*, 249-264. https://doi.org/10.1007/978-1-4939-3524-6_15

Gera, L., Chan, D. C., Hodges, R. S., & Bunn, P. A. (2013). Flurbiprofen analogs and methodsof use in treating cancer. In: Google Patents.

Ghanjaoui, M. (2020). High performance liquid chromatography quality control. *InternationalJournal of Advanced Chemistry*, 160-169.

Grignon, D. J. (2004). Unusual subtypes of prostate cancer. *Modern Pathology*, *17*(3), 316-327.https://doi.org/10.1038/modpathol.3800052

Grogan, S., & Preuss, C. V. (2022). Pharmacokinetics. In *StatPearls*. StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.

Han, Y. R., Lee, P. I., & Pang, K. S. (2018). Finding T_{max} and C_{max} in Multicompartmental Models. *Drug Metabolism and Disposition*,*46*(11), 1796. https://doi.org/10.1124/dmd.118.082636

Hanberg, P., Lund, A., Søballe, K., & Bue, M. (2019). Single-dose pharmacokinetics of meropenem in porcine cancellous bone determined by microdialysis. *Bone & Joint Research*,*8*(7), 313-322. https://doi.org/10.1302/2046-3758.87.Bjr-2018-0308.R1

Ho, C. S., Lam, C. W., Chan, M. H., Cheung, R. C., Law, L. K., Lit, L. C., Ng, K. F., Suen, M. W.,& Tai, H. L. (2003). Electrospray ionisation mass spectrometry: principles and clinicalapplications. *Clin Biochem Rev*, *24*(1), 3-12.

Hooshfar, S., Linzey, M. R., Wu, D., Gera, L., Mamouni, K., Li, X., Chen, Y., Yang, Y., Olorunyolemi, O., & Bartlett, M. G. (2018). Sensitive liquid chromatography/tandem massspectrometry method for the determination of two novel highly lipophilic anticancer drug candidates in rat plasma and tissues. *Biomed Chromatogr*, *32*(2). https://doi.org/10.1002/bmc.4064

Hussain, M., Lu, Y., Tariq, M., Jiang, H., Shu, Y., Luo, S., Zhu, Q., Zhang, J., & Liu, J. (2022). Asmall-molecule Skp1 inhibitor elicits cell death by p53-dependent mechanism. *iScience*, *25*(7), 104591. https://doi.org/10.1016/j.isci.2022.104591

Israël, B., Immerzeel, J., van der Leest, M., Hannink, G., Zámecnik, P., Bomers, J., Schoots, I.G., van Basten, J. P., Debruyne, F., van Oort, I., Sedelaar, M., & Barentsz, J. (2022). Clinical implementation of pre-biopsy magnetic resonance imaging pathways for the diagnosis of prostate cancer. *BJU Int*, *129*(4), 480-490. https://doi.org/10.1111/bju.15562

James, N. (2007). Cyclooxygenase-2 inhibitors and prostate cancer. *The LANCET Oncology*.https://doi.org/10.1016/S1470-2045(07)70291-7

Janiczek, M., Szylberg, Ł., Kasperska, A., Kowalewski, A., Parol, M., Antosik, P., Radecka, B., & Marszałek, A. (2017). Immunotherapy as a Promising Treatment for Prostate Cancer: A Systematic Review. *J Immunol Res*, *2017*, 4861570. https://doi.org/10.1155/2017/4861570 Jannetto, P. (2015). *Liquid Chromatography Tandem Mass Spectrometry*. American Associationfor Clinical Chemistry. Retrieved Feb, 15 from https://www.aacc.org/cln/articles/2015/july/liquid- chromatography-tandem-mass-spectrometry

Jessome, L. L., & Volmer, D. A. (2006). Ion suppression: A major concern in mass spectrometry. *Lc Gc North America*, *24*, 498-510.

Ken Broeckhoven, K. S., Michael W. Dong. (2019). *Modern HPLC Pumps: Perspectives, Principles, and Practices* North America. https://www.chromatographyonline.com/view/modern-hplc-pumps-perspectives-

principles-and-practices

Keyes, M., Crook, J., Morton, G., Vigneault, E., Usmani, N., & Morris, W. J. (2013). Treatmentoptions for localized prostate cancer. *Can Fam Physician*, *59*(12), 1269-1274.

Khor, L. Y., Bae, K., Pollack, A., Hammond, M. E., Grignon, D. J., Venkatesan, V. M., Rosenthal, S. A., Ritter, M. A., Sandler, H. M., Hanks, G. E., Shipley, W. U., & Dicker, A. P. (2007). COX-2 expression predicts prostate-cancer outcome: analysis of data from the RTOG92-02 trial. *Lancet Oncol*, *8*(10), 912-920. https://doi.org/10.1016/s1470-2045(07)70280-2

Kim, S. Y., Lee, I. S., Park, S. L., & Lee, J. (2012). Cefepime neurotoxicity in patients with renalinsufficiency. *Ann Rehabil Med*, *36*(1), 159-162. https://doi.org/10.5535/arm.2012.36.1.159

Klimek-Turek, A., Misiołek, B., & Dzido, T. H. (2020). Comparison of the Retention and Separation Selectivity of Aromatic Hydrocarbons with Polar Groups in RP-HPLC Systems with Different Stationary Phases and Eluents. *Molecules*, *25*(21). https://doi.org/10.3390/molecules25215070

Kole, P. L., Venkatesh, G., Kotecha, J., & Sheshala, R. (2011). Recent advances in sample preparation techniques for effective bioanalytical methods. *Biomed Chromatogr*, *25*(1-2), 199-217. https://doi.org/10.1002/bmc.1560

Konermann, L., Ahadi, E., Rodriguez, A. D., & Vahidi, S. (2013). Unraveling the Mechanism of Electrospray Ionization. *Analytical Chemistry*, *85*(1), 2-9. https://doi.org/10.1021/ac302789c

Kumar, R., & Suttle, B. (2011). The Importance of PK/PD Data-Key Biological Answers Neededto Evaluate the Success of Potential Cancer Therapeutics. *Molecular Cancer Therapeutics*, *10*(11), 2028-2028. https://doi.org/10.1158/1535-7163.Mct-11-0728

Lajos Gera, D. C. C., Robert S. Hodges, Paul A. Bunn. (2013). *Flurbiprofen analogs andmethods of use in treating cancer*. Retrieved from https://patents.google.com/patent/US8575170B2/en

Lajos Gera, Z. J., Angelo D'Alessandro, and Robert S. Hodges. (2015). Development of Novel Broad Spectrum Anticancer Small Molecule Peptidomimetics with Nanomolar Activity. *AmericanPeptide Society*. https://www.researchgate.net/publication/283266332_Development_of_Novel_Broad_Spectrum_Anticancer_Small_Molecule_Peptidomimetics_with_Nanomolar_Activity

Lamoth, F., Buclin, T., Pascual, A., Vora, S., Bolay, S., Decosterd, L. A., Calandra, T., & Marchetti, O. (2010). High cefepime plasma concentrations and neurological toxicity in febrileneutropenic patients with mild impairment of renal function. *Antimicrob Agents Chemother*, *54*(10), 4360-4367. https://doi.org/10.1128/aac.01595-08

Leblanc, A. F., Huang, K. M., Uddin, M. E., Anderson, J. T., Chen, M., & Hu, S. (2018). MurinePharmacokinetic Studies. *Bio Protoc*, *8*(20). https://doi.org/10.21769/BioProtoc.3056

Leslie, S. E., & John, V. H. (2008). *Chapters In The Evolution Of Chromatography* [Book]. Imperial College Press. https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,shib&db=e000xna&AN=516 744&site=eds-live&custid=uga1

Li, A. P. (2001). Screening for human ADME/Tox drug properties in drug discovery. *Drug DiscovToday*, *6*(7), 357-366. https://doi.org/10.1016/s1359-6446(01)01712-3

Li, M., Qiang, W., Wen, Z., Li, L., Wang, L., & Cheng, Z. (2019). A New Model to Describe the Single-dose Pharmacokinetics of Bevacizumab and Predict Its Multiple-Dose Pharmacokineticsin Beagle Dogs [Original Article]. *18*(3), e126161. https://doi.org/10.22037/ijpr.2019.1100716

Li, P., & Bartlett, M. G. (2014). A review of sample preparation methods for quantitation of small-molecule analytes in brain tissue by liquid chromatography tandem mass spectrometry (LC- MS/MS). *Analytical Methods*, *6*(16), 6183-6207.

Li, W., Jian, W., & Fu, Y. (2019). Basic Sample Preparation Techniques in LC-MS Bioanalysis.In *Sample Preparation in LC-MS Bioanalysis* (pp. 1-30). https://doi.org/https://doi.org/10.1002/9781119274315.ch1

Lillard Jr, J. W., Moses, K. A., Mahal, B. A., & George, D. J. (2022). Racial disparities in Blackmen with prostate cancer: A literature review. *Cancer*, *128*(21), 3787-3795. https://doi.org/https://doi.org/10.1002/cncr.34433

Lim, H.-S. (2019). Evolving role of modeling and simulation in drug development. *Transl ClinPharmacol*, *27*(1), 19-23. https://doi.org/10.12793/tcp.2019.27.1.19

Lin, K., Lipsitz, R., & Janakiraman, S. (2008). U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. In *Benefits and Harms of Prostate-SpecificAntigen Screening for Prostate Cancer: An Evidence Update for the U.S. Preventive Services Task Force*. Agency for Healthcare Research and Quality (US).

Mahmoud, S. H., & Shen, C. (2017). Augmented Renal Clearance in Critical Illness: An Important Consideration in Drug Dosing. *Pharmaceutics*, *9*(3).

Mandair, D., Rossi, R. E., Pericleous, M., Whyand, T., & Caplin, M. E. (2014). Prostate cancerand the influence of dietary factors and supplements: a systematic review. *Nutrition & Metabolism*, *11*(1), 30. https://doi.org/10.1186/1743-7075-11-30

Marsh, K., Ahmed, N., Decano, A., Dubrovskaya, Y., Jen, S.-P., Siegfried, J., Chen, X. J., &Merchan, C. (2020). Safety of intravenous push administration of beta-lactams within a healthcare system. *American Journal of Health-System Pharmacy*, *77*(9), 701-708. https://doi.org/10.1093/ajhp/zxaa044

Masich, A. M., Heavner, M. S., Gonzales, J. P., & Claeys, K. C. (2018). Pharmacokinetic/Pharmacodynamic Considerations of Beta-Lactam Antibiotics in Adult CriticallyIll Patients. *Current Infectious Disease Reports*, *20*(5), 9. https://doi.org/10.1007/s11908-018- 0613-1

McGovern, P. C., Wagenlehner, F., Gasink, L., Moeck, G., McLeroth, P. L., Beth, M., Dane, A.,& Henkel, T. (2022). 731. CERTAIN-1: A Phase 3 Study of Cefepime-Taniborbactam Efficacy and Safety in the Treatment of Complicated Urinary Tract Infections (cUTI), including Acute Pyelonephritis (AP). *Open Forum Infectious Diseases*, *9*(Supplement_2), ofac492.022. https://doi.org/10.1093/ofid/ofac492.022

Medvedovici, A., Bacalum, E., & David, V. (2018). Sample preparation for large-scale bioanalytical studies based on liquid chromatographic techniques. *Biomed Chromatogr*, *32*(1).https://doi.org/10.1002/bmc.4137

Morteza Khabiri, B. M., Jan Brezovský, Jiří Damborský, Rudiger Ettrich. (2013). Interaction oforganic solvents with protein structures at protein-solvent interface. *J Mol Model*. https://loschmidt.chemi.muni.cz/peg/wp-content/uploads/2013/11/jmm2013.pdf

Napoli, A., Alfieri, G., Scipione, R., Leonardi, A., Fierro, D., Panebianco, V., De Nunzio, C., Leonardo, C., & Catalano, C. (2020). High-intensity focused ultrasound for prostate cancer. *Expert Rev Med Devices*, *17*(5), 427-433. https://doi.org/10.1080/17434440.2020.1755258 Narain, T. A., & Sooriakumaran, P. (2022). Beyond Prostate Specific Antigen: New ProstateCancer Screening Options. *World J Mens Health*, *40*(1), 66-73. https://doi.org/10.5534/wjmh.210076

Negus, S. S., & Banks, M. L. (2018). Pharmacokinetic-Pharmacodynamic (PKPD) Analysis withDrug Discrimination. *Curr Top Behav Neurosci*, *39*, 245-259. https://doi.org/10.1007/7854_2016_36

Nguyen, H. D., Allen, B. J., & Pow-Sang, J. M. (2013). Focal cryotherapy in the treatment oflocalized prostate cancer. *Cancer Control*, *20*(3), 177-180. https://doi.org/10.1177/107327481302000305

Nikalje Anna Pratima, R. G. (2018). Liquid Chromatography-Mass Spectrometry and Its Applications: A Brief Review. *Archives of Organic and Inorganic Chemical sciences*, *1*(1).https://lupinepublishers.com/chemistry-journal/fulltext/liquid-chromatography-mass- spectrometry-and-its-applications-a-brief-review.ID.000103.php

Nshanian, M., Lakshmanan, R., Chen, H., Ogorzalek Loo, R. R., & Loo, J. A. (2018). EnhancingSensitivity of Liquid Chromatography-Mass Spectrometry of Peptides and Proteins Using Supercharging Agents. *Int J Mass Spectrom*, *427*, 157-164. https://doi.org/10.1016/j.ijms.2017.12.006

Oczkowski, M., Dziendzikowska, K., Pasternak-Winiarska, A., Włodarek, D., & Gromadzka-Ostrowska, J. (2021). Dietary Factors and Prostate Cancer Development, Progression, andReduction. *Nutrients*, *13*(2). https://doi.org/10.3390/nu13020496

Okamoto, M. P., Nakahiro, R. K., Chin, A., & Bedikian, A. (1993). Cefepime clinical pharmacokinetics. *Clin Pharmacokinet*, *25*(2), 88-102. https://doi.org/10.2165/00003088-199325020-00002

Pais, G. M., Chang, J., Barreto, E. F., Stitt, G., Downes, K. J., Alshaer, M. H., Lesnicki, E., Panchal, V., Bruzzone, M., Bumanglag, A. V., Burke, S. N., & Scheetz, M. H. (2022). Clinical Pharmacokinetics and Pharmacodynamics of Cefepime. *Clin Pharmacokinet*, *61*(7), 929-953.https://doi.org/10.1007/s40262-022-01137-y

Pais, G. M., Chang, J., Barreto, E. F., Stitt, G., Downes, K. J., Alshaer, M. H., Lesnicki, E., Panchal, V., Bruzzone, M., Bumanglag, A. V., Burke, S. N., & Scheetz, M. H. (2022). Clinical Pharmacokinetics and Pharmacodynamics of Cefepime. *Clinical Pharmacokinetics*, *61*(7), 929-953. https://doi.org/10.1007/s40262-022-01137-y

Paner, G. P., Stadler, W. M., Hansel, D. E., Montironi, R., Lin, D. W., & Amin, M. B. (2018). Updates in the Eighth Edition of the Tumor-Node-Metastasis Staging Classification for UrologicCancers. *European Urology*, *73*(4), 560-569. https://doi.org/https://doi.org/10.1016/j.eururo.2017.12.018

Patiño, A. M., Marsh, R. H., Nilles, E. J., Baugh, C. W., Rouhani, S. A., & Kayden, S. (2018). Facing the Shortage of IV Fluids — A Hospital-Based Oral Rehydration Strategy. *New EnglandJournal of Medicine*, *378*(16), 1475-1477. https://doi.org/10.1056/NEJMp1801772

Pitt, J. J. (2009). Principles and applications of liquid chromatography-mass spectrometry inclinical biochemistry. *Clin Biochem Rev*, *30*(1), 19-34.

Polli, J. E. (2008). In vitro studies are sometimes better than conventional human pharmacokinetic in vivo studies in assessing bioequivalence of immediate-release solid oraldosage forms. *Aaps j*, *10*(2), 289-299. https://doi.org/10.1208/s12248-008-9027-6

Polson, A. G., & Fuji, R. N. (2012). The successes and limitations of preclinical studies in predicting the pharmacodynamics and safety of cell-surface-targeted biological agents in patients. *Br J Pharmacol*, *166*(5), 1600-1602. https://doi.org/10.1111/j.1476-5381.2012.01916.x

Polson, C., Sarkar, P., Incledon, B., Raguvaran, V., & Grant, R. (2003). Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B*, *785*(2), 263-275. https://doi.org/https://doi.org/10.1016/S1570-0232(02)00914-5

Price, G., & Patel, D. A. (2022). Drug Bioavailability. In *StatPearls*. StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.

Punnen, S., Pavan, N., & Parekh, D. J. (2015). Finding the Wolf in Sheep's Clothing: The 4Kscore Is a Novel Blood Test That Can Accurately Identify the Risk of Aggressive ProstateCancer. *Rev Urol*, *17*(1), 3-13.

Rajman, I. (2008). PK/PD modelling and simulations: utility in drug development. *Drug DiscovToday*, *13*(7-8), 341-346. https://doi.org/10.1016/j.drudis.2008.01.003

Rajpoot, K., Tekade, R., Sharma, M., & Tekade, M. (2021). Pharmacokinetics and biopharmaceutics: “a leader or attendant”. In (pp. 17-27). https://doi.org/10.1016/B978-0-12-814425-1.00020-6

Reddy, K., & Yachawad, A. (2016). OVERVIEW ON RECENT EXTRACTION TECHNIQUES IN

BIOANALYSIS. *International Research Journal of Pharmacy*, *7*, 15-24. https://doi.org/10.7897/2230-8407.07214

Rodrigues, G., Warde, P., Pickles, T., Crook, J., Brundage, M., Souhami, L., & Lukka, H. (2012).Pre-treatment risk stratification of prostate cancer patients: A critical review. *Can Urol Assoc J*, *6*(2), 121-127. https://doi.org/10.5489/cuaj.11085

Roehrborn, C. G. (2005). Benign prostatic hyperplasia: an overview. *Rev Urol*, *7 Suppl 9*(Suppl9), S3-s14.

Sakamoto, K. M., Kim, K. B., Verma, R., Ransick, A., Stein, B., Crews, C. M., & Deshaies, R. J.(2003). Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. *Mol Cell Proteomics*, *2*(12), 1350-1358. https://doi.org/10.1074/mcp.T300009- MCP200

Schatten, H. (2018). Brief Overview of Prostate Cancer Statistics, Grading, Diagnosis andTreatment Strategies. In H. Schatten (Ed.), *Cell & Molecular Biology of Prostate Cancer: Updates, Insights and New Frontiers* (pp. 1-14). Springer International Publishing.https://doi.org/10.1007/978-3-319-95693-0_1

Ścigalski, P., & Kosobucki, P. (2020). Recent Materials Developed for Dispersive Solid PhaseExtraction. *Molecules*, *25*(21). https://doi.org/10.3390/molecules25214869

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2022). Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians*, *72*(1), 7-33. https://doi.org/https://doi.org/10.3322/caac.21708

Singh, N., Baby, D., Rajguru, J. P., Patil, P. B., Thakkannavar, S. S., & Pujari, V. B. (2019). Inflammation and cancer. *Ann Afr Med*, *18*(3), 121-126. https://doi.org/10.4103/aam.aam_56_18

Siref, S. W. L. T. L. S.-S. A. R. I. H. S. L. E. (2022). *Prostate Cancer*. https://www.ncbi.nlm.nih.gov/books/NBK470550/#article-27833.s4

Skaar, J. R., Pagan, J. K., & Pagano, M. (2014). SCF ubiquitin ligase-targeted therapies. *NatRev Drug Discov*, *13*(12), 889-903. https://doi.org/10.1038/nrd4432

Society, A. C. (2019). *Prostate Cancer Early Detection, Diagnosis, and Staging*. https://www.cancer.org/cancer/prostate-cancer/detection-diagnosis-staging/staging.html

society, A. C. (2021). *Prostate cancer*. https://www.cancer.org/cancer/prostate-cancer/about/key-statistics.html

Song, N.-N., Zhang, S.-Y., & Liu, C.-X. (2004). Overview of factors affecting oral drug absorption. *Asian J Drug Metabolism Pharmacokinetics*, *4*, 167-176.

Starkey, E. S., & Sammons, H. M. (2015). Practical pharmacokinetics: what do you really needto know? *Arch Dis Child Educ Pract Ed*, *100*(1), 37-43. https://doi.org/10.1136/archdischild- 2013-304555

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209-249. https://doi.org/https://doi.org/10.3322/caac.21660

Surgeons, A. o. A. P. a. (2019). A History of How HPLC Became Common in Pharmaceutical Testing. https://www.aaps.ca/blog/a-history-of-how-hplc-became-common-in-pharmaceutical-testing.php#:~:text=The%20first%20commercially%20available%20HPLC,in%20the%20world% 20of%20science.

Swanson, G. P., Trevathan, S., Hammonds, K. A. P., Speights, V. O., & Hermans, M. R. (2021).Gleason Score Evolution and the Effect on Prostate Cancer Outcomes. *Am J Clin Pathol*, *155*(5), 711-717. https://doi.org/10.1093/ajcp/aqaa130

Toutain, P. L., & Bousquet-Mélou, A. (2004). Plasma terminal half-life. *J Vet Pharmacol Ther*,*27*(6), 427-439. https://doi.org/10.1111/j.1365-2885.2004.00600.x

Tsvetkov, L. M., Yeh, K. H., Lee, S. J., Sun, H., & Zhang, H. (1999). p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27.*Curr Biol*, *9*(12), 661-664. https://doi.org/10.1016/s0960-9822(99)80290-5

UK, N. H. S. (2021). *What do cancer stages and grades mean?* https://www.nhs.uk/common- health-questions/operations-tests-and-procedures/what-do-cancer-stages-and-grades-mean/ Urso, R., Blardi, P., & Giorgi, G. (2002). A short introduction to pharmacokinetics. *Eur Rev Med Pharmacol Sci*, *6*(2-3), 33-44.

USFDA. (2018). *Bioanalytical Method Validation Guidance for Industry*. Retrieved from https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for- Industry.pdf

Vaddady, P. K., Lee, R. E., & Meibohm, B. (2010). In vitro pharmacokinetic/pharmacodynamic models in anti-infective drug development: focus on TB. *Future Med Chem*, *2*(8), 1355-1369. https://doi.org/10.4155/fmc.10.224

Vikse, K. L., & Scott McIndoe, J. (2018). Ionization methods for the mass spectrometry of organometallic compounds. *J Mass Spectrom*, *53*(10), 1026-1034.https://doi.org/10.1002/jms.4286

Visser, W. C. H., de Jong, H., Steyaert, S., Melchers, W. J. G., Mulders, P. F. A., & Schalken, J. A. (2022). Clinical use of the mRNA urinary biomarker SelectMDx test for prostate cancer. *Prostate Cancer and Prostatic Diseases*, *25*(3), 583-589. https://doi.org/10.1038/s41391-022-00562-1

Wang, Y., Sun, J., Qiao, J., Ouyang, J., & Na, N. (2018). A “Soft” and “Hard” Ionization Method for Comprehensive Studies of Molecules. *Analytical Chemistry*, *90*(24), 14095-14099. https://doi.org/10.1021/acs.analchem.8b04437

Wang, Z., Butner, J. D., Cristini, V., & Deisboeck, T. S. (2015). Integrated PK-PD and agent-based modeling in oncology. *J Pharmacokinet Pharmacodyn*, *42*(2), 179-189. https://doi.org/10.1007/s10928-015-9403-7

Wawrzyniak, R., Kosnowska, A., Macioszek, S., Bartoszewski, R., & Jan Markuszewski, M.(2018). New plasma preparation approach to enrich metabolome coverage in untargeted metabolomics: plasma protein bound hydrophobic metabolite release with proteinase K. *Scientific Reports*, *8*(1), 9541. https://doi.org/10.1038/s41598-018-27983-0

Wei, Y., Kobayashi, T., Lu, Y., Vogel, M., Zhang, R., Wu, J., Gao, Y., Lin, L., Zhu, Q., Ye, L.,Horie, S., Wang, X., & Li, T. (2022). Comparison of Survival Outcomes and Risk Factors Between Ductal Carcinoma of the Prostate and Acinar Adenocarcinoma of the Prostate: A Population-based Propensity Score-matching Study. *Eur Urol Open Sci*, *46*, 88-95. https://doi.org/10.1016/j.euros.2022.10.013

White, J., Shenoy, B. V., Tutrone, R. F., Karsh, L. I., Saltzstein, D. R., Harmon, W. J., Broyles, D. L., Roddy, T. E., Lofaro, L. R., Paoli, C. J., Denham, D., & Reynolds, M. A. (2018). Clinicalutility of the Prostate Health Index (phi) for biopsy decision management in a large group urology practice setting. *Prostate Cancer and Prostatic Diseases*, *21*(1), 78-84. https://doi.org/10.1038/s41391-017-0008-7

Winger, B. E., Light-Wahl, K. J., Ogorzalek Loo, R. R., Udseth, H. R., & Smith, R. D. (1993).Observation and implications of high mass-to-charge ratio ions from electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom*, *4*(7), 536-545. https://doi.org/10.1016/1044-0305(93)85015-p

Wirth, M. P., Hakenberg, O. W., & Froehner, M. (2007). Antiandrogens in the Treatment ofProstate Cancer. *European Urology*, *51*(2), 306-314. https://doi.org/https://doi.org/10.1016/j.eururo.2006.08.043

Yahav, D., Paul, M., Fraser, A., Sarid, N., & Leibovici, L. (2007). Efficacy and safety of cefepime: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *7*(5), 338-348. https://doi.org/https://doi.org/10.1016/S1473-3099(07)70109-3

Yılmaz, Ö., Bayer, B., Bekçi, H., Uba, A. I., Cumaoğlu, A., Yelekçi, K., & Küçükgüzel Ş, G. (2020). Synthesis, Anticancer Activity on Prostate Cancer Cell Lines and Molecular ModelingStudies of Flurbiprofen-Thioether Derivatives as Potential Target of MetAP (Type II). *Med Chem*, *16*(6), 735-749. https://doi.org/10.2174/1573406415666190613162322

Zhang, L., Xie, H., Wang, Y., Wang, H., Hu, J., & Zhang, G. (2022). Pharmacodynamic Parameters of Pharmacokinetic/Pharmacodynamic (PK/PD) Integration Models [Review].*Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.860472

Žuvela, P., Skoczylas, M., Jay Liu, J., Bączek, T., Kaliszan, R., Wong, M. W., & Buszewski, B. (2019). Column Characterization and Selection Systems in Reversed-Phase High-PerformanceLiquid Chromatography. *Chemical Reviews*, *119*(6), 3674-3729. https://doi.org/10.1021/acs.chemrev.8b00246

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TABLES

Table 3.1 Calibration curves of cefepime in human plasma (n = 4).

Analyte	Slope ± SD	Intercept ± SD	R^2 ± SD
Cefepime HCl	0.00488 ± 0.0002	0.00118 ± 0.00090	0.9991 ± 0.00047

Table 3.2 Accuracy and precision of cefepime in human plasma.

Matrix	Nominal concentration	Intra-day (n=5)			Inter-day (n=15)		
	(µg/ml)	Measured concentration ± SD(µg/ml)	RSD (%)	Accuracy (RE %)	Measured concentration ± SD(µg/ml)	RSD (%)	Accuracy (RE %)
Human plasma	10 (LLOQ)	9.77±0.10	1.01	2.28	10.21±0.58	5.69	2.13
	25 (LQC)	25.12±0.13	0.51	0.47	25.31±0.61	2.41	1.26
	75 (MQC)	73.76±1.41	1.92	1.66	73.80±1.00	1.35	1.61
	190 (HQC)	187.31±1.87	1.00	1.42	187.74±3.23	1.72	1.19

Table 3.3 Recovery (n = 3) of cefepime.

Matrix	Concentration (µg/ml)	Absolute recovery (%)	Relative recovery (%)
Human plasma	25.0 (LQC)	77.36±1.25	84.77±1.37
	75.0 (MQC)	77.55±0.94	8375±1.02
	190.0 (HQC)	84.04±1.35	88.92±1.43

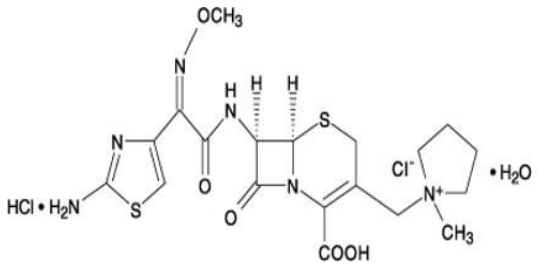
Table 3.4 Stability studies of cefepime: autosampler stability (n = 3), bench top stability (n = 3), and freeze-thaw cycle 3stability (n = 3).

Matrix	Stability	Nominal conc. (µg/ml)	Measured conc. ± SD (ug/ml)	RSD (%)	RE (%)
Human Plasma	Autosampler	25	25.82±0.03	0.11	3.27
		190	187.26±1.99	1.06	1.44
	Bench-top	25	25.22±0.12	0.47	0.88
		190	184.85±1.58	0.86	2.71
	Freeze-thaw	25	23.59±0.29	1.25	5.66
		190	184.98±0.67	0.36	2.64

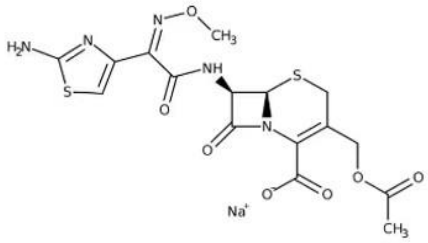
Table 3.5 Summary of PK parameters in ICU patients.

Parameters	Pk values
Cmax (µg/mL)	136.8
Tmax (minute)	5
AUC (µg.min/ml)	459.8
Elimination rate constant (per hour)	0.3 ± 0.1
Volume of distribution (Liter)	18.8 ± 2.9
Elimination half-life (hour)	2.9 ± 0.9
Clearance (Liter/hour)	4.8 ± 1.6

FIGURES



Cefepime HCl



Cefotaxime Sodium

Figure 3.1 Chemical Structures of Cefepime HCl (MW = 517.50 g/mol), and Cefotaxime Sodium (MW = 477.50 g/mol).

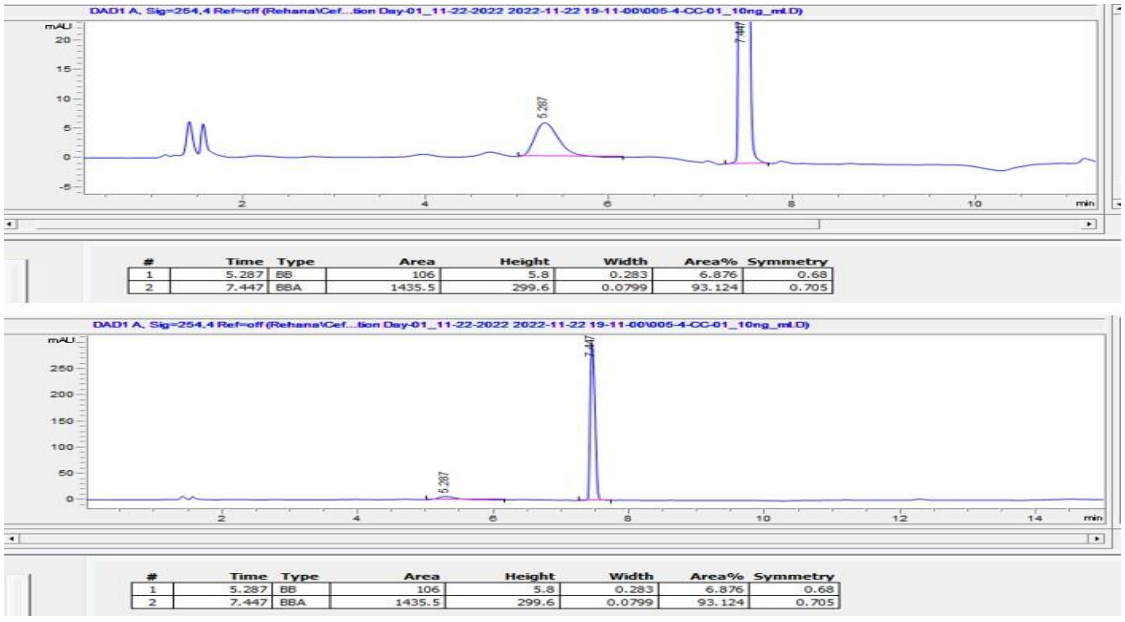
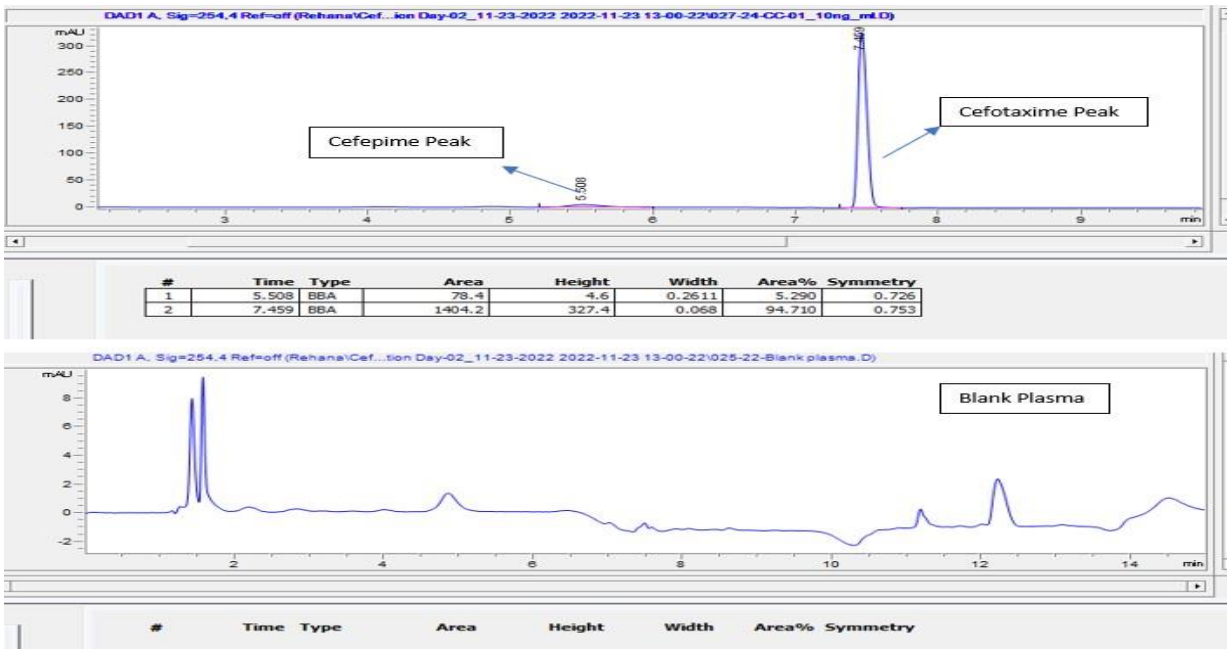


Figure 3.2 Representative chromatograms of cefepime HCl and Cefotaxime sodium (IS) in human plasma at LLOQ level (10 µg/mL), Retention times for analytes are shown in minutes.



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	5.508	BBA	78.4	4.6	0.2611	5.290	0.726
2	7.459	BBA	1404.2	327.4	0.068	94.710	0.753

Figure 3.3 Representative chromatograms of cefepime HCl and internal standard selectivity in blank plasma, and plasma at LLOQ of method (10 µg/mL), Retention times for analytes are shown in minutes.

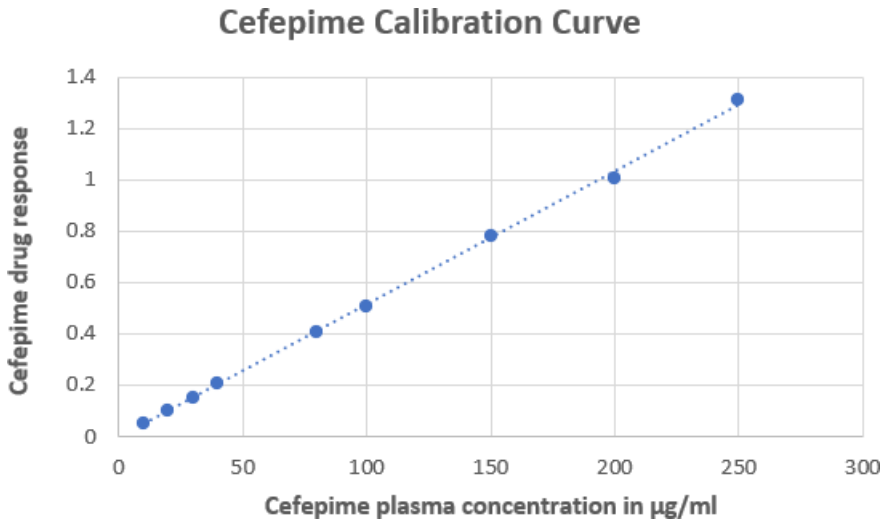


Figure 3.4 Calibration curve graph of Cefepime HCl using 1/x weighted linear regression model.

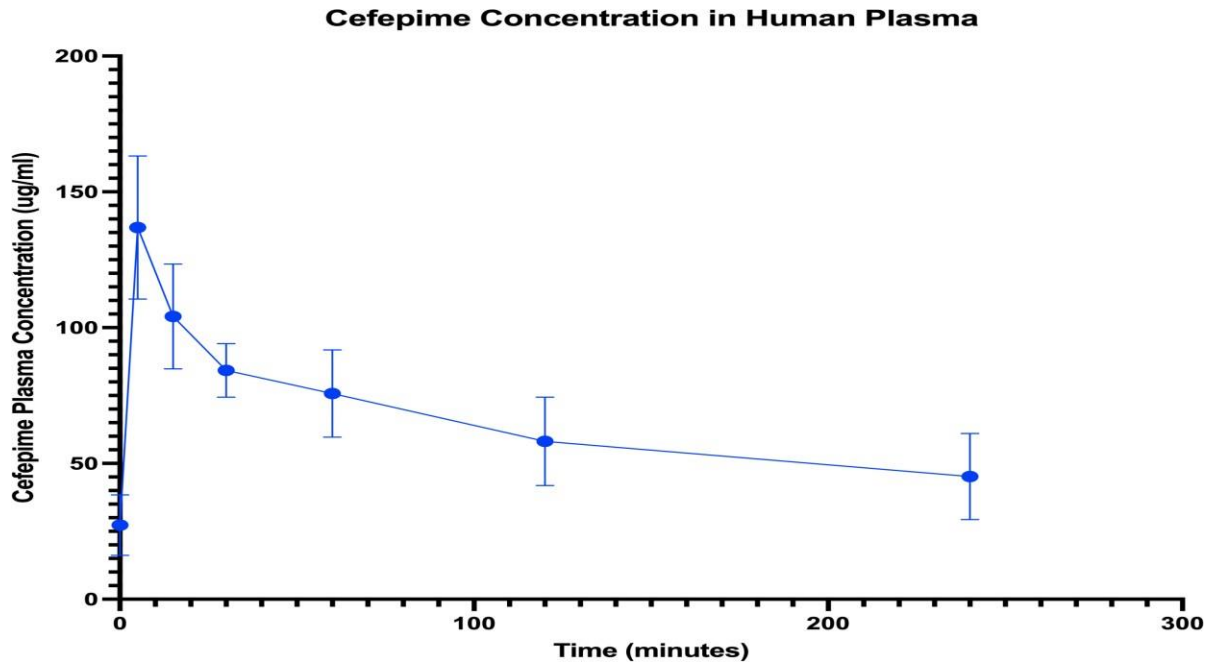
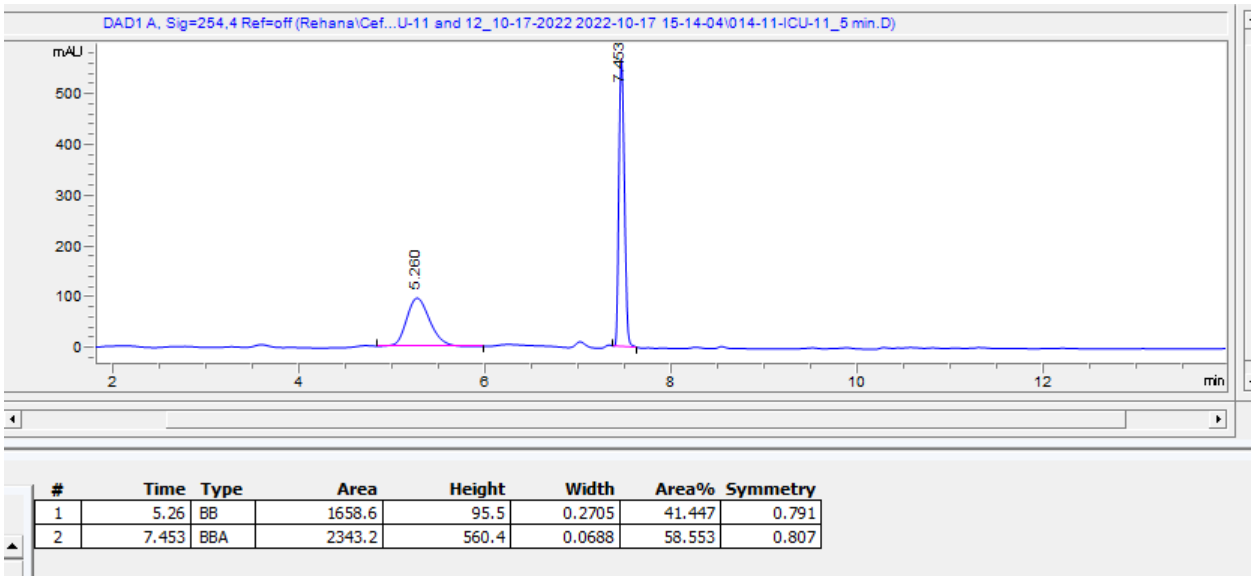


Figure 3.5 Mean plasma concentration-time curves of cefepime in ICU patients after administration of 1-2g of IV push injection over 5-minute period.



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	5.26	BB	1658.6	95.5	0.2705	41.447	0.791
2	7.453	BBA	2343.2	560.4	0.0688	58.553	0.807

Figure 3.6 Representative chromatograms of Cefepime HCl extracted from ICU patients' plasma samples.