Pharmacokinetics and pharmacodynamics of continuous infusion of cefepime in cystic fibrosis patients, and stability of cefepime during simulated continuous infusion administration

By Pål Falck Sprauten

Laboratory for Clinical Pharmacy Research
University of Southern California
USA

And

Department of Pharmacology
School of Pharmacy
Faculty of Mathematics and Natural Sciences
University of Oslo
Norway

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I. PREFACE

"*Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die"*. This is a European adage folklore that makes an early reference to the common genetic disease today recognized as cystic fibrosis.

Cystic fibrosis (CF), also called mucoviscidosis, is one of the most lethal, however fortunately also the most thoroughly understood genetic diseases (1), which affects approximately 30,000 children and adults in United States (2). The disease was given its name by the first major contributor to the understanding of CF in 1938, Dorothy H. Anderson of Columbia University. She first called it "cystic fibrosis of the pancreas", based on the features she observed in pancreatic tissue from autopsies on infants and children (3).

While significant medical advances have been made over the ensuing years, there is still no cure for this disease, and treatment is only to alleviate the symptoms, and directed against the organs affected. The primary cause of death is cardiorespiratory failure caused by chronic lung infections and inflammation that finally destroys the airway. During the 1980s the fundamental physiologic defect was clearly established as the failure of cyclic-adenomonophosphate (cAMP) regulation of chloride transport caused by mutations on the Cystic Fibrosis transmembrane conductance regulator (CFTR) (4).

The survival age of patients has with new therapeutic strategies increased 3-fold from 1960 up until today, with a mean age of approximately 30 years (4). One significant pharmacological advance was the development of pancreatic supplements of enzymes, which resulted in an eradication of malnutrition as the principle cause of death (4).

Subsequent to the discovery of the CF gene in 1989, the pace of CF research has greatly accelerated. There are many new treatments under

investigation, and the most interesting one is gene therapy. If it is possible to effectively transfer a recombinant human CFTR gene into the affected cells, resulting in production of normal CFTR, there is hope that this therapy can be curative (5). The current problem is identification of a suitable vector to transfer the gene into the cells. Until such time that this hurdle can be surpassed, pharmacological management aimed at alleviating the symptoms of this disease will continue to be the mainstay of treatment.

In this thesis I describe a strategy for optimizing administration of a β-lactam antibiotic, cefepime, in treatment of CF patients. This is done by investigating both the stability and the antimicrobial effect of cefepime during continuous infusion (CI). In addition the pharmacokinetics (PK) of cefepime was assessed and pharmacodynamics (PD) of CI were compared with traditional intermittent administration. The experiments described were done during a stay at the University of Southern California School of Pharmacy, USA, in the time period October 2001- September 2002.

II. ABBREVIATIONS

ABX	Antibacterial
AIC	Akaike information criterion
ATP	Adenosidtriphosphate
ALT	Alanine aminotransferase
AST	Aspertate aminotransferase
BMI	Body Mass Index (weigh in kg/height in meters^2)
cAMP	Cyclic Adenomonophosphate
CF	Cystic fibrosis
CFP	Cefepime
CFU/g	Colonies forming units per gram
CFTR	Cystic Fibrosis Transmembrane conductance Regulator
CI	Continuous Infusion
CL_{CR}	Creatinine Clearance
CLd	Distribution clearance
CLt	Total clearance
D5W	Dextrose 5% in distilled Water
DNA	Deoxyribonucleotide acid
ELISA	Enzyme linked immunosorbent assay
FEV_1	Forced expiratory volume within 1 second
GEN-IC	Generalized information criterion
HPLC	High Pressure Liquid Chromatography
IL-8	Interleukin-8
IV	Intravenous
LPS	Lipid-polysaccharide
MAP	Maximum a posteriori
MBC	Minimal Bactericidal Concentration
MIC	Minimal Inhibitory Concentration
MS	Mass spectrometry
MW	Molecular weight, in Dalton
NCCLS	National Committee for Clinical Laboratory Standards
PAE	Post antibiotic effect

PD	Pharmacodynamic
PIP	Portable Infusion Pump
PK	Pharmacokinetic
R(t)	Rate of drug infusion
TBW	Total Body Weight
TMB	Tetramethylbenzidine
Vc	Volume of central compartment
Vp	Volume of peripheral compartment

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1. PHARMACOKINETICS AND PHARMACODYNAMICS OF CONTINUOUS INFUSION OF CEFEPIME IN CYSTIC FIBROSIS PATIENTS, AND STABILITY OF CEFEPIME DURING SIMULATED CONTINUOUS INFUSION

1.1 Abstract

Time above minimal inhibitory concentration (MIC) (T>MIC) is the PK/PD parameter that best correlates with bacterial killing for cefepime. Cefepime CI provides an efficient method of achieving T>MIC throughout the dosing interval. The purpose of this study was to determine whether cefepime exhibits sufficient stability and antibacterial activity to be given by 24-hour CI using portable infusion pumps. In addition, assess the PK and PD of CI versus intermittent infusion of cefepime in cystic fibrosis patients administered during an acute pulmonary exacerbation.

The stability of cefepime in 5% dextrose distilled water (D5W) solutions was determined for a simulated CI using a portable infusion pump (Microject 30, Sorensen Medical) worn over a period of 24-36 hours. The temperatures in the bags were measured every ½ hour. In addition the stability at different storage conditions was tested, major degradation products identified and the antibacterial activity of degraded solution was measured.

In this study, we also compared the PK and PD of traditional dosing (50mg/kg iv every 8 hour) versus CI of cefepime (100mg/kg/24hour), using standard two-stage PK modeling with ADAPT II software. The PD outcomes evaluated included sputum bacterial density, sputum interleukin-8 (IL-8) concentration and improvement in forced expiratory volume within 1 second (FEV_1).

In-vitro experiments revealed that cefepime stability at 24 hours following CI was 94.3±1.0%. The mean infusion bag temperature was 22.6±°1.5°C. Cefepime is stable for 15 days in a refrigerator and 10 hours at 37°C. The degradation includes cleavage of the R2 side chain and opening of the β-lactam ring. Antibacterial activity appeared to correlate with intact cefepime remaining in solution (r^2>0.74, p<0.001). The Arrhenius plot showed that the average temperature in the bag should not exceed 29.1°C in order to maintain 90% stability at 24 hours.

The PK analysis showed that a two-compartment model best describes cefepime observed serum concentration. The two-compartment PK parameters were calculated with MAP-Bayesian algorithm and were found to be total clearance of 2.5mL/min/kg, distribution clearance of 1.9mL/min/kg, distribution half-life of 0.53 hours, and elimination half-life of 2.8 hours. The sample size in this study was too small to be able to make any significant distinction between PD outcomes in the patients receiving CI versus those receiving intermittent dosing. It was possible to observe a trend, being that patients with T>MIC 100% of the time had a greater decline in bacterial density, and a higher decline in the inflammatory marker IL-8.

These results demonstrated that the stability of cefepime supports CI. A cold pack is necessary if the average temperature in the drug solution exceeds 29°C. Solutions of cefepime should be stored in a refrigerator if not used right away, and in a freezer if not used within 5 days. The PD results showed an indication of better clinical outcome with CI administration, however more patients are needed to show statistical significance.

1.2 Introduction

1.2.1 Pathogenesis of cystic fibrosis

Historical Perspective

The first genetic step in understanding CF was made in 1946, when the inheritance of the disease was mapped. The study showed that it was a recessive condition caused by a mutation on one single gene coding for a protein (3). Another important historic landmark was made in 1953 when it was discovered that children with CF lost an excessive amount of salt in their sweat when compared with normal children (3). This discovery resulted in a cornerstone in diagnosis of CF, to measure chloride content in perspiration. The next major advance in basic research was made in the beginning of 1980s, when biochemical investigation revealed that sweat glands failed to absorb salt from the lumen of the glands, thus making the sweat salty. At the same time a similar study of epithelial tissue in the airways showed no chloride movement and increased sodium uptake. These discoveries lead to the conclusion that some chloride channels in epithelial tissue were malfunctioning (3). The complete identification of the CF gene was achieved in 1989 (6), and with this knowledge it is possible to give a good description of the disease.

An interesting phenomenon is why such a deadly disease just did not disappear with the early death of the patients. One possible explanation that has been suggested is that there is a heterozygote advantage to the carriers of one mutant CFTR gene. In particular, carriers of the mutant CFTR gene may be more resistant to secretory diarrhea diseases (4). One experiment with a CF-mouse model showed that CF-mice did not secrete fluid in response to cholera toxin. This supports the hypothesis that CF-carriers have an advantage in surviving cholera (7).

Genetics and functions of CFTR

The CF gene is located on chromosome 7, and it codes for a protein that functions as a chloride channel called: Cystic Fibrosis Transmembrane

conductance Regulator (CFTR) (8). CFTR is an apical epithelial transmembrane protein, which is a member of the ATP Binding Cassette family. It utilizes energy from hydrolysis of ATP to undergo a conformational change that opens the channel to anions. These ion-channels have best selectivity of bromide, then chloride and least for iodide (1, 4). CFTR is regulated by phosphorylation of protein kinase A, and is mediated by cAMP (4). In the case of a malfunctioning CFTR the chloride conductance is reduced, resulting in failure of reabsorption of chloride in the sweat ducts, and failure of chloride secretion in lung tissue (4). In the sweat glands CFTR is the only available anion conductance pathway, so with lost function the lumen becomes highly electronegative and transport (absorption) ceases. The result is a high luminal salt concentration (1).

Another important electrophysiologic malfunction of the epithelial cells in CF lung tissue is the increased absorption of sodium ions. This cannot be explained by failure of the CFTR mediate chloride secretion alone, but through another function of CFTR: the regulation of other proteins. The most documented other function is its negative regulation on the amiloride-sensitive epithelial sodium channel (EnaC**)** (9)**.** CFTR lowers EnaC's probability to be in an open conformation (9). The absence of functional CFTR disrupts the negative control resulting in markedly increased sodium conductance in CF human airways (10)**.**

Recently as many as 1000 different mutations in the CF gene have been discovered (http://www.genet.sickkids.on.ca/cftr/). The most common mutation is ΔF508, and this mutation results in deletion of phenylalanine at 508^{th} amino acid at CFTR. Among white British CF patients, 70% are homozygote for this mutation (8).

The diagnosis of cystic fibrosis

The hallmark of CF is an elevated chloride content in sweat. Only 0.1% of patients with typical lung and/or involvement of other organs have normal

sweat chloride concentration (11). A sweat chloride concentration over 60 mEq/L is diagnostic of CF (4). The classical "diagnostic triad" also includes chronic sino-pulmonary disease and pancreatic insufficiency. (4). Some helpful criteria in the diagnosis are azoospermia in males and mucoid *Pseudomonas aeruginosa* in sputum culture (4).

There is also a possibility to get newborns screened for CF, so that treatment can start earlier (8). One limitation to this approach is the large number of mutations that are currently not routinely screened, due to the relatively low frequency of occurrence.

CF Lung Disease

Lung disease is of particular importance since it causes 90% of the mortality and is the primary reason for hospitalization. The mortality is caused by chronic bronchitis that develops into bronchiectasis and finally respiratory failure (5). The pathogenesis of the lung disease develops from almost normal lungs at birth into chronic infection and inflammation (4).

There is still no complete understanding of how defective CFTR gives rise to the lung disease, but some possible mechanisms do exist. The mucus that covers the airway surface is abnormally thick and impairs the ciliary clearance of the airway. This may allow bacterial infections to become established (4). In response to the infection, there is production of proinflammatory cytokines including interleukin-6, tumor necrosis factor-α, and IL-8 by alveolar macrophages and bronchial epithelial cells. IL-8 in particular is a potent neutrophil chemoattractant. (8). Due to the persistence of the lung infection there is an exaggerated inflammatory response within the airways of CF patients resulting in the release of oxidants and enzymes (i.e. neutrophil elastase), which cause structural damage to the lung tissue (3). Dying neutrophils release deoxyribonucleotide acid (DNA), which increases the viscosity of the sputum and leads to airway obstruction. The infection and inflammation

become self-sustaining, and ultimately result in bronchiectasis and respiratory failure (4).

Lung infections in children with CF typically are caused by *Staphylococcus aureus* or *H. influenza*. In contrast, in late childhood and in adults, *P aeruginosa* is the most common pathogen isolated from the airways. A problem in treatment is the growing resistance of *P. aeruginosa* to many of the existing antibiotics. In particular, *P. aeruginosa* has recently been shown to grow within biofilms that provide a barrier to antibiotics and impairs acquired host defense mechanisms (5).

Treatment of Lung Disease

The treatment of the lungs includes reducing the airway obstruction and managing the infection (5). To reduce airway obstruction, chest physiotherapy combined with postural drainage, will loosen and facilitate mobilization of mucus and improve lung clearance, especially over long periods (5, 12). Less time-consuming and newer techniques include airway oscillation (Flutter device) and high-frequency chest oscillation (ThAIRapy Vest).

A great deal of CF-patients will experience improved pulmonary function with the use of bronchodilator therapy, which should be considered when it increases the FEV_1 with 10% (13). Bronchodilators also facilitate mucus clearance (4). Additionally, reducing the viscoelasticity of sputum can assist in relieving airway obstruction. A purified recombinant human deoxyribonuclease I (rhDNase I) has demonstrated to significantly reduce sputum viscosity (14) and improve the FEV_1 (13).

Treating the infections with antibiotics has contributed to increased survival in CF-patients. Antibiotics are used both to treat exacerbations of pulmonary infections, but also as chronic maintenance therapy, either as oral or inhaled administration to increase the time between the exacerbations (5).

Appropriate treatment of exacerbations consists of parenteral administrations of 2 antibiotics for 14-21 days, along with intensified airway clearance and bronchodilator administration (13). The most common regimen is the combination of aminoglycoside and β-lactam antibiotics; however, the choice of antibiotics should always be based on identification and susceptibility of the bacteria isolated from sputum (13). To avoid resistance in bacteria and achieve synergistic effect, drug-combinations should have different mechanisms of action. The use of antibiotics in the treatment of acute pulmonary exacerbations has been shown to result in a significant decline in density of *P. aeruginosa* in sputum. In addition, the decline in bacterial density correlates with improvement in FEV_1 (15).

Since a significant contribution of the lung tissue destruction is due to inflammation, a number of anti-inflammatory compounds are currently under investigation (4). While studies have demonstrated significant benefits with long-term administration of corticosteroids, the trials were discontinued due to intolerable adverse effects including growth retardation, glucose intolerance, and cataracts. High dose ibuprofen therapy has also shown to reduce pulmonary deterioration; however, the numbers of patients studied to date are too few to provide any estimation of the relative safety of this treatment. Macrolides also exhibit potent anti-inflammatory effects and have resulted in increased survival in patients with diffuse panbronchiolitis, an illness similar to CF. There are currently several large controlled studies investigating the clinical effects of macrolides in CF-patients (8). Since the deterioration in pulmonary function begins in childhood, early therapy is desirable (5).

1.2.2 Rationale for continuous infusion of cefepime and other β-lactam antibiotics

The time that drug concentrations exceed the minimal inhibitory concentration (T>MIC) is the best PK/PD parameter to determine the efficacy of cefepime and other β-lactam antibiotics (16). This is explained by the fact that cefepime and other β-lactam antibiotics exhibit time dependent bacterial killing within the therapeutic dosing range, meaning higher serum concentration do not increase the bacterial killing. It is also important to keep the serum level well above the MIC to minimize the risk of developing resistance (17). In addition cefepime exhibits only a short post-antibacterial effect (PAE), against susceptible organisms. This is especially important in CF patients who exhibit enhanced clearance of many antibiotics (3).

CI is an efficient method of administration that will maximize T>MIC throughout the dosing interval, keeping the bacterial growth suppressed at all times. Cefepime also has a relatively short biological half-life, making it preferred to have a frequent dosing interval. Results from a few clinical trials conducted in critically ill patients and patients with CF suggest that CI may be the optimal method administration for β-lactams, since CI shows similar efficacy at a reduced dose (17-19). A lower incidence of adverse effects has been reported with CI presumably because many side effects occur at peak levels that are avoided with CI.

Other benefits of CI administration are that it minimizes the cost of treatment by using less drug while achieving the same effect compared with standard dosing (20). In addition, CI with a portable infusion pump makes it possible for patients to be more mobile and have the opportunity of being treated at home. This significantly improves quality of life for patients with CF.

While the above attributes suggest many advantages of CI of β-lactams, there are a few potential drawbacks to this method of administration. Since the infusions are continuous, only compounds that have demonstrated compatibility can be infused through the same intravenous line. In addition, only β-lactams which are stable at room temperature for greater than 24 hours can be administered by CI. Finally, some data show that resistance can emerge more rapidly after CI than bolus dosing (17); however, since combination therapy is routinely prescribed for patients with CF, this is unlikely to be clinically significant.

1.2.3 Cefepime: Activity, dosing in CF/normal patients

Cefepime is a broad-spectrum semi-synthetic 4th generation cephalosporin for parenteral administration (21). It was developed by modifying the third generation cephalosporin-class (22). The classification into 4th generation is based on its broader spectrum of activity and its decreased susceptibility to certain β-lactamases (23). Cefepime is a zwitterion (see figure 1) with a net neutral charge that allows it to penetrate the outer membrane of Gram-negative bacteria faster than the third generation cephalosporins (22).

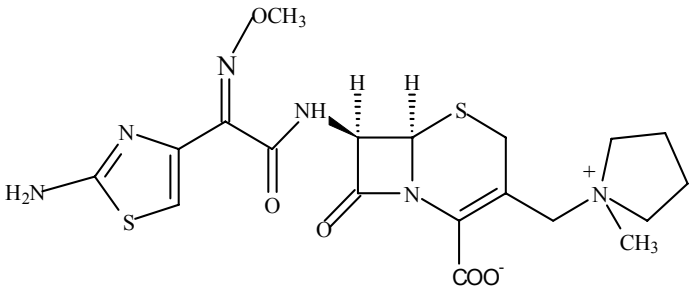


Figure 1: Cefepime with the chemical name 1- [[6R,7R)-7- [2-(2-amino-4-thiazolyl)-glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl] methyl]-1-1 methylpyrrolidinium chloride ,7^2-(Z)-(O-metyloxime), monohydrochloride, monohydrate. The molecular formula is $C_{19}H_{25}ClN_6O_5S_2 \bullet HCl \bullet H_20$

Cefepime is a bactericidal agent that acts by blocking bacterial cell-wall synthesis (21). It exhibits activity against both Gram positive and Gram-negative bacteria. Its activity against Gram-negative species separates it from the third generation cephalosporins. Cefepime has a low affinity for chromosomally encoded β-lactamases, and is highly resistance to hydrolysis by most β-lactamases (21). It has activity against the most common bacteria in CF patients, which include *Pseudomonas aeruginosa*, *Heamophilus influenzae* and *Staphylococcus aureus*, but not against the rare infection with *Stenotrophomonas maltophilia* (21).

Distribution

After parenteral administration cefepime distributes widely into extracellular fluid within tissues including bronchial mucosa and sputum(23). The average steady state volume of distribution is approximately 0.26 L/kg in healthy volunteers. Cefepime exhibits concentration independent serum protein binding of about 20% (21).

Metabolism and Excretion

Cefepime is principally eliminated unchanged via renal excretion (see figure 2) with a half-life of two hours, and total body clearance of 120 mL/min in healthy volunteers (21). Cystic fibrosis patients demonstrate increased drug clearance of a number of compounds when compared with age-matched controls. CF patients have 19% higher clearance of cefepime than normal patients, which is caused by increased renal and non-renal clearance (24). The increased clearance and the fact that *P. aeruginosa* is a less susceptible organism necessitates the use of higher doses for adequate treatment. The maximum recommended dose of cefepime, 50 mg/kg (max 2g per dose) every 8 hours, has been utilized in several studies involving cystic fibrosis patients (24, 25).

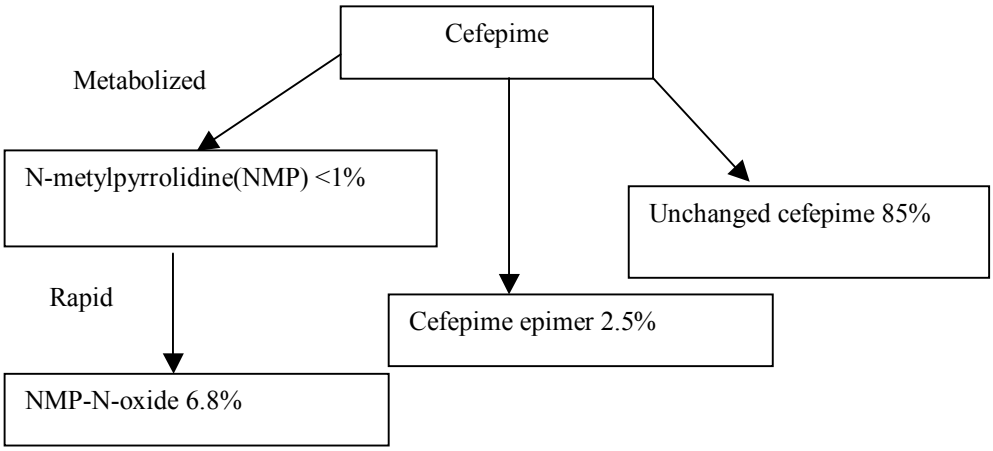


Figure 2: Disposition of cefepime in humans. 85% is excreted unchanged in urine.

Patients exhibiting renal insufficiency require dosage adjustments, and creatinine clearance can serve as a basis for dosage determination (21). The PK of cefepime does not appear to be affected by hepatic impairment (23). Cefepime exhibits linear PK over the dosing range of 250mg to 2g per day (23).

The adverse effects reported are similar to those with other parenteral cephalosporins, and cefepime is generally well tolerated (23). Headache, rash, diarrhea, nausea and vomiting have been reported for 2% of the patients, while neutropenia is present in less than 1% of patients (23). Approximately 3% of the patients discontinue drug therapy due to adverse effects (23).

1.2.4 The relevance of testing cefepime stability

In order to support CI administration of a drug it is necessary to establish that the desirable drug in solution is stable throughout the administration period. Previous studies indicate that cefepime is stable for 24 hours at room temperature (26). However there is conflicting information on

whether cefepime is stable for 24 hours at body temperature (27). The information on temperature and stability of cefepime when administered using portable infusion pumps when worn for a 24-hour period is currently unknown.

There are at least two major classes of portable pumps in clinical use today, the disposable elastomeric and the motorized peristaltic pumps. The advantage of the elastomeric pumps is that they are simple to operate and are economical; however, they appear to be more influenced by body temperature since they are worn under the clothing. In contrast, the peristaltic pumps allow more precise control of flow rate and are less influenced by body temperature since they are typically contained within an external pouch.

Therefore, we chose to study the temperature variation and stability of cefepime using a motorized portable infusion device. The purpose of this study is to determine if cefepime is stable enough to be administrated by CI using a motorized portable infusion pump, and to define optimal conditions for storage prior to administration.

2. STABILITY, DRUG-RESERVOIR TEMPERATURE AND ANTIBACTERIAL ACTIVITY OF CEFEPIME DURING CONTINUOUS INFUSION ADMINISTRATION

2.1 Scope and intent

The purpose of this study is to determine if the stability and antibacterial activity of cefepime supports CI administration.
The specific aims of this study are:

- Determine the stability of cefepime during simulated CI administration with (Microject 30, Sorensen Medical) portable infusion pump for 24-36 hours.
- Determine the average temperature with TempTrace® in the drug reservoir while using portable pump system.
- Identify potential cefepime degradation products with mass spectrometry, and determine their relative antibacterial activity.
- Determine optimal storage conditions for drug-reservoir before use.
- Determine the stability at fixed temperatures; 5°C (refrigerator), 21°C (room temperature) at 37°C (body temperature), and at 55°C to be able to make an Arrhenius plot that will provide more information on how the stability changes according to temperature.

The results of this study will provide support for clinical trials evaluating CI cefepime administration in the treatment of CF pulmonary exacerbations and nosocomial pneumonia. In addition, this data will provide information useful for the optimal storage and analysis of cefepime.

2.2 Methods

2.2.1 HPLC assay

The HPLC assay was a modification of a previous published cefepime assay (28). Chromatography was preformed isocratically on an analytical reversed-phase C-18 column with a C-18 precolumn. The mobile phase consisted of 8% v/v acetonitrile in 20mM ammonium acetate, adjusted to pH 4.9 with glacial acetic acid, all chemicals HPLC grade quality. The mobile phase was filtrated through a 47mm 0.22µm nylon membrane filter before use. Flow-rate was set at 1.0 mL/min. Cefepime was detected at its absorption UV-peak at 260nm (29). The standard curve was made with known concentrations of cefepime in HPLC-grade water, measured in triplicate.

The procedure for the samples was simple, utilizing only one dilution 1:100 with HPLC-water and filtration through a 0.2µm 4mm nylon membrane filter before injection.

2.2.2 Stability and drug-reservoir temperature during simulated continuous infusion

To best mimic the actual patient conditions during CI and estimate the stability and temperature in the infusion bag as best as possible, CI of cefepime was simulated by using a portable motorized infusion pump (Microject 30, Sorensen Medical), that provides continuous low flow rates. This pump and the drug solution reservoir are contained in two different and separated bags on the same convenient light bag system. This bag is worn like a belt, and is easy for the patients to carry around. The bag also protects the drug reservoir against light exposure. From the pump, the drug solution is pumped through a cassette and into the vein. In this study, the drug was pumped into a waste bag (same type as drug reservoir), which was also worn on the same belt. See figure 3.

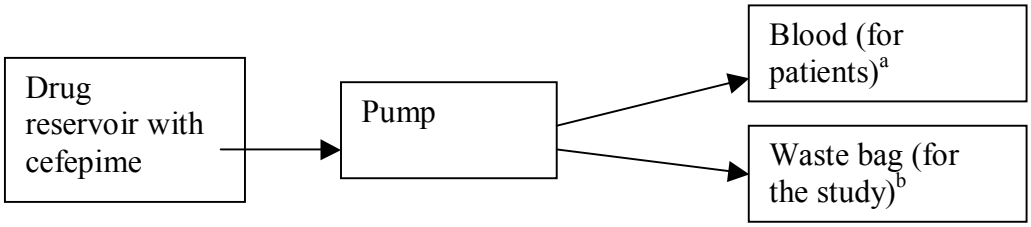


Figure 3: Schematic drawing of the infusion during CI for a real patient (a) and for the stability study(b). In this study all the components were worn different pouches on the same belt.

The dose chosen for this study was derived from prior work evaluating CI ceftazidime (30), which exhibits similar pharmacokinetics as cefepime (24, 25, 31). Cefepime is normally dosed according to the patient's weight, 100mg/kg/24hour (max 6g/24hour). One previous study demonstrated that cefepime follows first order degradation (26), we sought to confirm that different concentrations do not affect the stability. This was done by testing cefepime stability in the concentrations ranging from 2g/250mL to 6g/250mL. Cefepime solutions were made with sterile Maxipime®, containing arginine (as a buffer) in a concentration of 725-mg/g cefepime to control the pH.

Cefepime was admixed volumetrically to the accurate concentration with sterile D5W. To mimic the patient situation three individuals wore the pump and bag containing cefepime for 24-36 hours. Seven 2mL samples were extracted from the administration bag during the 24-36 hours. These samples were frozen at -70ºC until assayed. This method attempts to provide useful information on how the temperature varies in the drug reservoir and its impact on cefepime stability during a simulated CI. In addition these simulations also determines temperature variations among various individuals, and whether concentration will have impact on the cefepime stability. The measured stability after 24 hours during simulated CI will be used to determine clinical stability (> 90% cefepime remaining).

Temperature changes in the drug reservoir were measured every 30 minutes using an electronic temperature detector (TempTrace®) that was placed adjacent to the drug reservoir. The detector was then connected to a computer with software for TempTrace®, which printed out the temperatures..

2.2.3 Stability during various storage conditions

In order to simulate an actual home-case treatment situation, the drug reservoirs were stored for one or two weeks in a freezer (-15.5°C) as well as in a refrigerator (3.8°C) (for a total of four bags) before CI administration as described previously. This provides information on the stability of cefepime for home treatment, where the patients receive drug supplies for either one or two weeks at a time. It also provides data on how the patients need to store the cefepime bags prior to use.

Two additional experiments were performed to give additional information on degradation of cefepime under various conditions. The first experiment used the Arrhenius equations' to predict the degradation rate at a certain temperature, given that the degradation rate for at least one other temperature is known and that the degradation is first order. This method is commonly used to predict the shelf life of drug products. The Arrhenius equation is comprised of: $\log k = \log A - E_a/(2.303RT)$, where k is the degradation rate constant, E_a is the activation energy needed to start a reaction, A is the frequency factor assumed to be independent of temperature, R is the gas constant (8.314 J $mol^{-1}K^{-1}$) and T is the temperature in Kelvin (32).

This equation provides information about the stability of cefepime when stored under temperatures ranging from 4° to 55ºC. These different degradation rates were calculated by admixing eight infusion bags with

the same concentration (1g/50mL) of cefepime in D5W in EVA bags, and storing them at 4 different temperatures (4º, 21º, 37º and 55ºC). The respective degradation rates were calculated under these conditions. The two highest temperatures were maintained using a temperature-controlled water bath. For the remaining two samples the temperature was maintained by storing the bags in room temperature and in a refrigerator respectively. The duration for each experiment was dependent upon the temperature for which they were stored: 4ºC for 2 weeks, 21ºC for 1 week, 37ºC for 2 days and 55ºC for 8 hours. The degradation rates were calculated from the first 25% degradation, where the degradation is first order. The complete degradation does not follow first order kinetics, as discussed later. Seven samples were obtained from each bag during each experiment. The samples were frozen at –70ºC until assayed by HPLC. The information from the different degradation constants at each temperature tested was used to generate an Arrhenius plot. This plot was also used to calculate at which temperature the stability would be 90%. This temperature gives the limit of what the average temperature in the bags must be kept below to keep the drug stable enough to support CI.

The second experiment was performed to completely investigate the rate of degradation of cefepime over the entire concentration range. In addition, potential differences of cefepime stability in different bags were evaluated. Three EVA and two PVC bags with 1g/50mL cefepime were exposed to a 37°C water bath for a long enough period to degrade completely. Samples were taken twice a day for 6 days. Since the degradation rate is pH dependent, pH was measured for each sample.

2.2.4 Determination of degradation products

Putative cefepime degradation products were determined using a Micromass Quattro Ultima triple quadrupole tandem mass spectrometer (Micromass, Beverly, MA) running in positive ion mode. Briefly, drug

solutions determined to have differing degrees of parent drug degradation by HPLC were introduced into the mass spectrometer using a syringe pump (Harvard Apparatus, Holliston, MA) running at a continuous rate. Total ion signatures for each solution were obtained by averaging scans collected over a 2-minute interval. Mass ion signatures from a fresh solution, 5%, 20%, 45% and a completely degraded cefepime solution, were used to identify the degradation products. By following the appearance of new mass ions in the degraded samples and comparing the mass changes in the samples with previous studies done looking at the degradation of ceftazidime (a compound related to cefepime, see figure 4) (33, 34), the major breakdown products were identified.

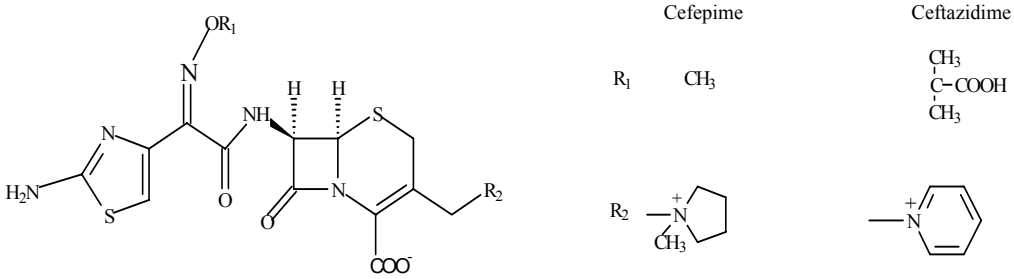


Figure 4: Chemical structures of cefepime and ceftazidime

2.2.5 Correlation between cefepime stability and in-vitro antibacterial activity

Both MICs and minimal bactericidal concentration (MBCs) were determined for a freshly prepared solution, and five solutions of cefepime containing 87% to 12% active cefepime, and varying concentrations of cefepime degradation products. All tests were performed in quadruplicate. The measured MICs were plotted against cefepime concentration remaining to determine if a relationship exists between observed MIC and percent cefepime remaining after degradation. This enables determination of whether the degradation products have any antibacterial effects.

MICs were determined by using macrodilution with a fixed inoculum of 1x 10^6 colony forming units per gram (CFU/g) of a reference strain ATCC 27853 of *Pseudomonas aeruginosa* and Mueller-Hinton broth adjusted with calcium and magnesium according to the National Committee for Clinical Laboratory Standards (NCCLS). The standard inoculum was prepared by diluting the reference strain to the same turbidity as a 0.5 McFarland solution to achieve the same density, which ideally will give a solution of 1x10^6CFU/mL. The dilutions of antibiotic were prepared volumetrically in broth, which after 1:1 dilution with the inoculum gave dilutions of 5x 10^5 CFU/mL of the reference strain. Diluting the inoculum with a factor of 10^4 and counting colonies on agar after 24 hours incubation gave the exact density.

MBCs were defined as the dilution that killed over 99.9% of the bacteria. That was found by incubating the MIC-dilution and higher concentrations on 0.5% blood agar in 37°C for 24 hours determined. The colonies were counted and the lowest dilution that had killed 99.9% of the initial density was the MBC. A summary of test solutions and the MICs tested are shown in table 1.

Table 1: The second column shows the percent of intact cefepime left in the solutions tested and the third column shows the dilutions of cefepime(μg/mL) that MIC were tested..

Solution	Percent active	MIC samples
1	100%	0.5,1,2,4,6,8,10,12
2	87%	0.5,1,2,4,6,8,10,12
3	74%	0.5,1,2,4,6,8,10,12
4	45%	0.5,1,2,4,6,8,10,12
5	30%	2,4,6,8,12,16
6	12%	4,8,12,16,20,24,28,32,64

2.2.6 Statistics

Statistical analysis was performed using Graphpad Prism version 3.03 (Graphpad Software Inc. San Diego, CA). The Student's t-test was used to compare the measured stability versus the 90% stability limit, and to compare MBCs with MICs.

2.3 Results

2.3.1 HPLC assay

Assay validation

The Standard curve was linear from 25-250µg/mL, with a correlation coefficient (CV) >0.999. The intra-day and intra-day coefficients of variation were all under 3.48%. More assay information in the appendix.

2.3.2 Stability of cefepime and drug-reservoir temperatures during simulated continuous infusion

The stability of cefepime after 24 hours CI for 8 replicates of 6g/250mL was 94.2±1.1%. Four of the replicates were fresh solutions prepared for CI, and the other four replicates were taken from the experiments evaluating various storage conditions (stability from CI only was calculated by stability post infusion divided by stability after storage). Stability for the 2g/250mL and 4g/250mL were 95.0% and 92.4% respectively. When cefepime stability was evaluated after 30 hours the percent intact drug was 92.2±3.5%. Cefepime solutions administrated over 24 hours were significantly above the stability limit of 90% ($p<0.0001$).

The temperature in the drug reservoirs showed some variation during the 24 hours. Figure 5 displays the average temperature with standard

deviation of all the temperature measured during the simulated CI in all ten bags. The average temperature was 22.6±1.5°C, (mean maximum and minimum temperatures 28.0±1.9°C, and 16.6±2.5°C respectively). All of the experiments were started in the morning and ended the next morning. 87.5 % of all the average temperatures were between 20 to 25°C.

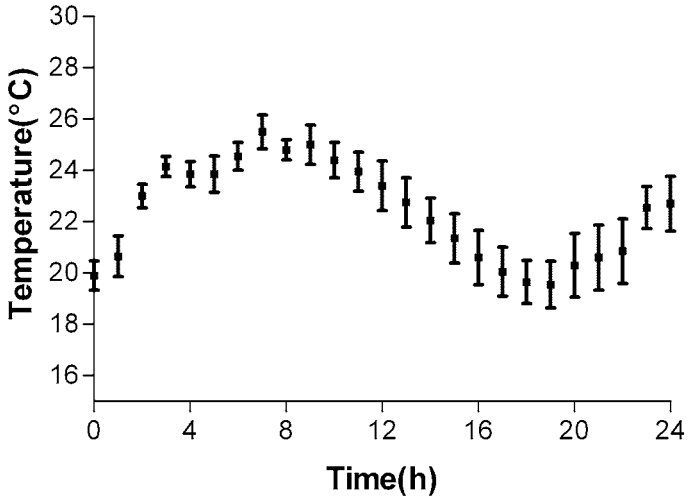


Figure 5: Variability in temperature throughout 24-hour CI period (n=10)

2.3.3 Stability of cefepime during various storage conditions

The results from all storage experiments are summarized in figure 6, which has four paired columns, representing storage condition. Shadowed columns depict the stability of cefepime after only storage conditions, while unshaded columns represent the stability after the same drug-reservoir has been worn as a simulated CI for 24 hours. The cefepime-solution was stable enough after storage and simulated CI in all experiments with the exception of the one following two weeks storage in a refrigerator. Cefepime stored at 4°C in refrigerator for two weeks was found to have 90.5% parent drug, and after the same solution was worn as simulated CI for 24 CI the parent drug concentration was declined till 85.3 %.

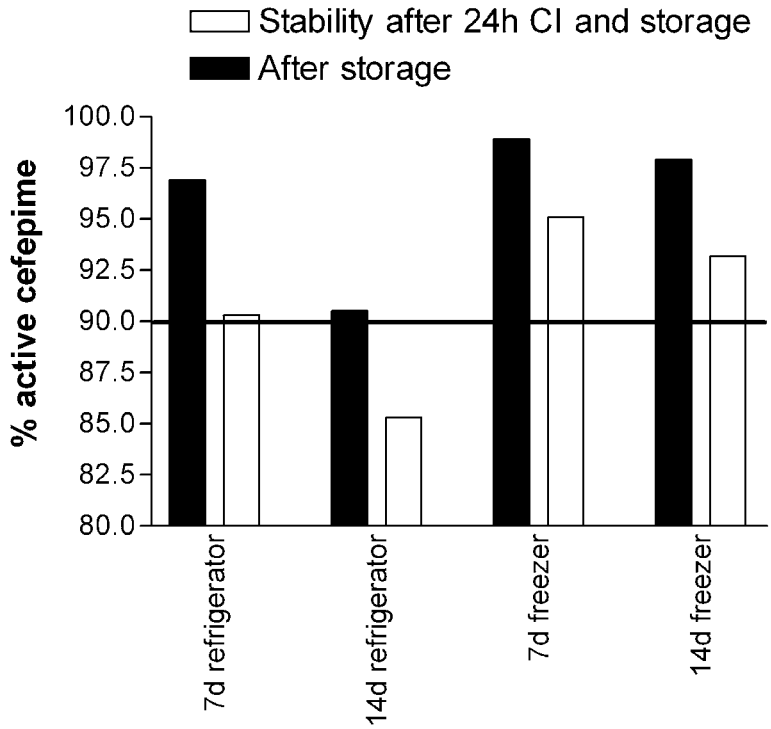


Figure 6: Stability of cefepime solution after four different storage conditions. Each paired column represents one storage condition. The left column of the two-paired columns represents the stability after the respective storage. The right of the two paired columns represents the stability of the solution after that the same drug-reservoir after storage has been used as a 24 hour simulated CI. The solid line represents the stability limit of 90%.

The complete degradation of cefepime in D5W in 37°C showed a disparity from the previously proven first order kinetics. The observed degradation and a theoretical first-order kinetic breakdown are compared in figure 7, which also show the pH in the solution while cefepime is degrading.

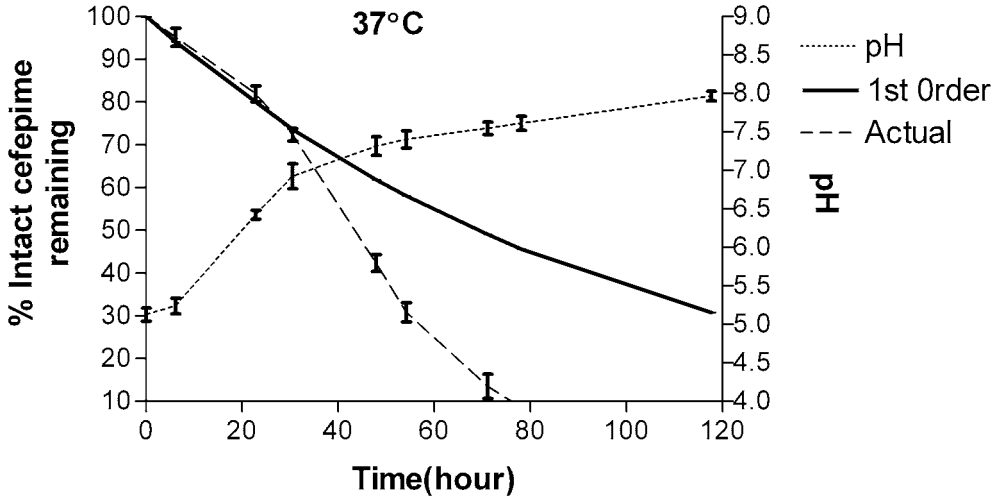


Figure 7. Degradation of cefepime at 37°C. The thick line indicates how cefepime would degrade if it follows perfect first order kinetics at 37°C. The stippled line (declining) shows the degradation observed in the experiment. Both the stability curves have values on the left Y-axis. The dotted line (increasing) shows the measured pH during the cefepime degradation, with respective values on the right Y-axis.

This is consistent with previously described information suggesting that cefepime degradation is pH-dependent (26), and is most stable in the pH range of 4 – 6. As figure 7 shows the degradation rate increases as the pH reaches a level over 6. The pH increase is due to accumulation of alkaline degradation products that increases the pH. This resulted in an increased degradation of cefepime, and a deviation from first order kinetics.

The complete degradation of cefepime in D5W from the commercial product Maxipime® follows first order kinetics in the first 25% of the degradation (r^2>0.97), while the rest of the degradation does not follow first order kinetics and is best described as an empirical breakdown. Cefepime degradation was also associated with colorimetric changes. A fresh solution of 24 mg/mL is clear, but after 10% degradation it becomes pale yellow, after 20% degradation it is yellow, after 30% and more it turns orange, and complete degradation has orange/brown color. Thus the degradation products probably have a brownish color. Cefepime solution showed less than 0.5% difference (p>0.81) in degradation between EVA

and PVC containers in the first 30.5 hours (or 70% degraded cefepime) in 37°C. After 48 hours cefepime appeared to degrade more rapidly in EVA than PVC containers, the difference in mean values was 8.0% (p= 0.038).

The stability results from all experiments at 4 different temperatures were used to calculate the Arrhenius plot seen in table 2 and figure 8.

Table 2: This table describes the 8 bags used for determining the Arrhenius plot. First column gives the temperature in Celsius, the second gives temperature in Kelvin and the third gives the reciprocal of temperature in Kelvin. The fourth column shows the measured degradation constant (k), and the fifth gives the logarithm of k. The last column gives the average of the two degradation constants.

Temp(C)	*Temp(K)*	*1/T*	*k*	*log k*	*Average*
55	328	0.003049	**0.053200**	-1.2741	-1.272
55	328	0.003049	**0.053720**	-1.2699	
37	310	0.003226	**0.009897**	-2.0045	-1.994
37	310	0.003226	**0.010380**	-1.9838	
20.8	293.8	0.003404	**0.001879**	-2.7261	-2.724
20.8	293.8	0.003404	**0.001896**	-2.7222	
1.4	274.4	0.003644	**0.000186**	-3.7317	-3.748
1.4	274.4	0.003644	**0.000172**	-3.7652	

Plotting the reprocidal of temperature against the average logarithmic of the degradation rate gives the Arrhenius plot seen in figure 8.

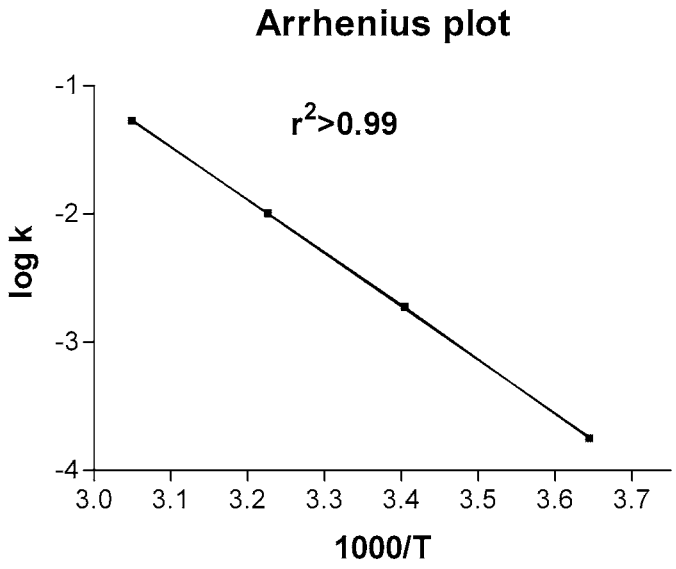


Figure 8: The Arrhenius plot of the degradation rate (k) of cefepime for four temperatures ranging from 1°C -55°C. Temperatures on the x-axes are multiplied with 1000. The four points made a straight line (r>0.99), with slope of –4.16x10^3 and intercept of 11.4. The activation energy was calculated to be 81.4KJmol^{-1}K^{-1}.

To calculate the temperature that will give 90% stability of cefepime in 24 hours the equation for a first order degradation, $C=C_0e^{-kt}$, was used. $100\%=90\% \times e^{-k \times 24h}$ was solved to give k=0.00439. The temperature that will give k=0.00439 was then solved mathematically by the equation t= 1/((11.4-log 0.00439)/4157))-273°C, since the plot is a straight line (Y=ax+b) and a is the slope and b is the intercept, and Y=log k. This equation gave the temperature that will give 90% stability in 24 hours as 29.1°C.

The Arrhenius plot was also used to predict the stability of cefepime during storage in refrigerator and freezer. This was done by first finding the log k for the wanted temperature by using the Arrhenius slope, log k = slope x 1/T + b, and then using the equation describing degradation of a first order kinetics: $C=100\% \times e^{-kt}$. The stability in refrigerator was calculated to be 95.8% after 1 week and 91.8% after 2 weeks. In the

freezer the stability after one and two weeks were 99.6% and 99.3% respectively.

2.3.4 Determination of degradation products

Mass spectrometry data indicated that degradation of cefepime includes cleavage of N-methylpyrrolidine (R2-side chain, see figure 4) and opening of the cephem (β-lactam ring) as shown in figure 9.

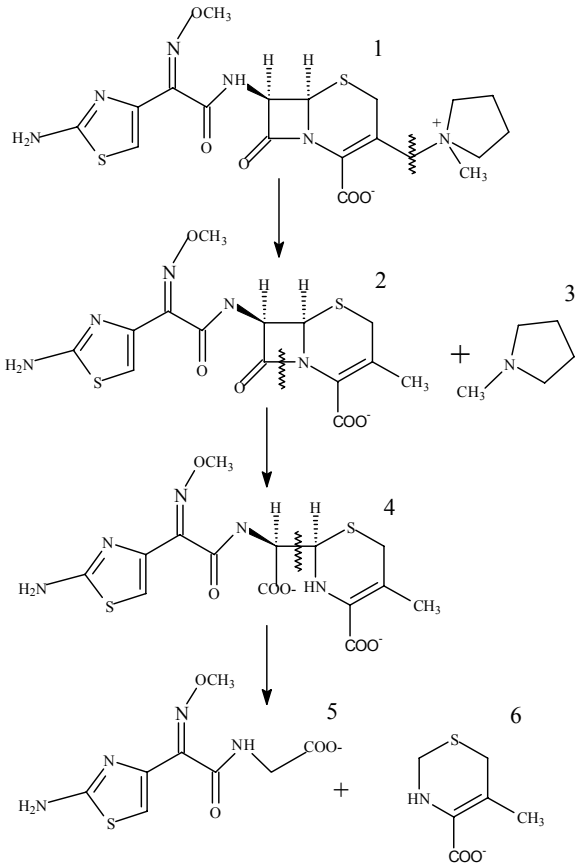


Figure 9: Shows putative main breakdown pathway of cefepime. The main degradation occurs when the β-lactam ring opens, and R2 side chain is cleaved off.

It also indicates that the ring opening occurs before the cleavage of N-methylpyrrolidine. This was observed from the relative amount of

degradation products seen in the mass scans after different amounts of cefepime have degraded. There were no observed degradation products where ring opening had occurred without N-methylpyrrolidine cleaved first. Figure 10 highlights the change in mass scans when the solution is degrading.

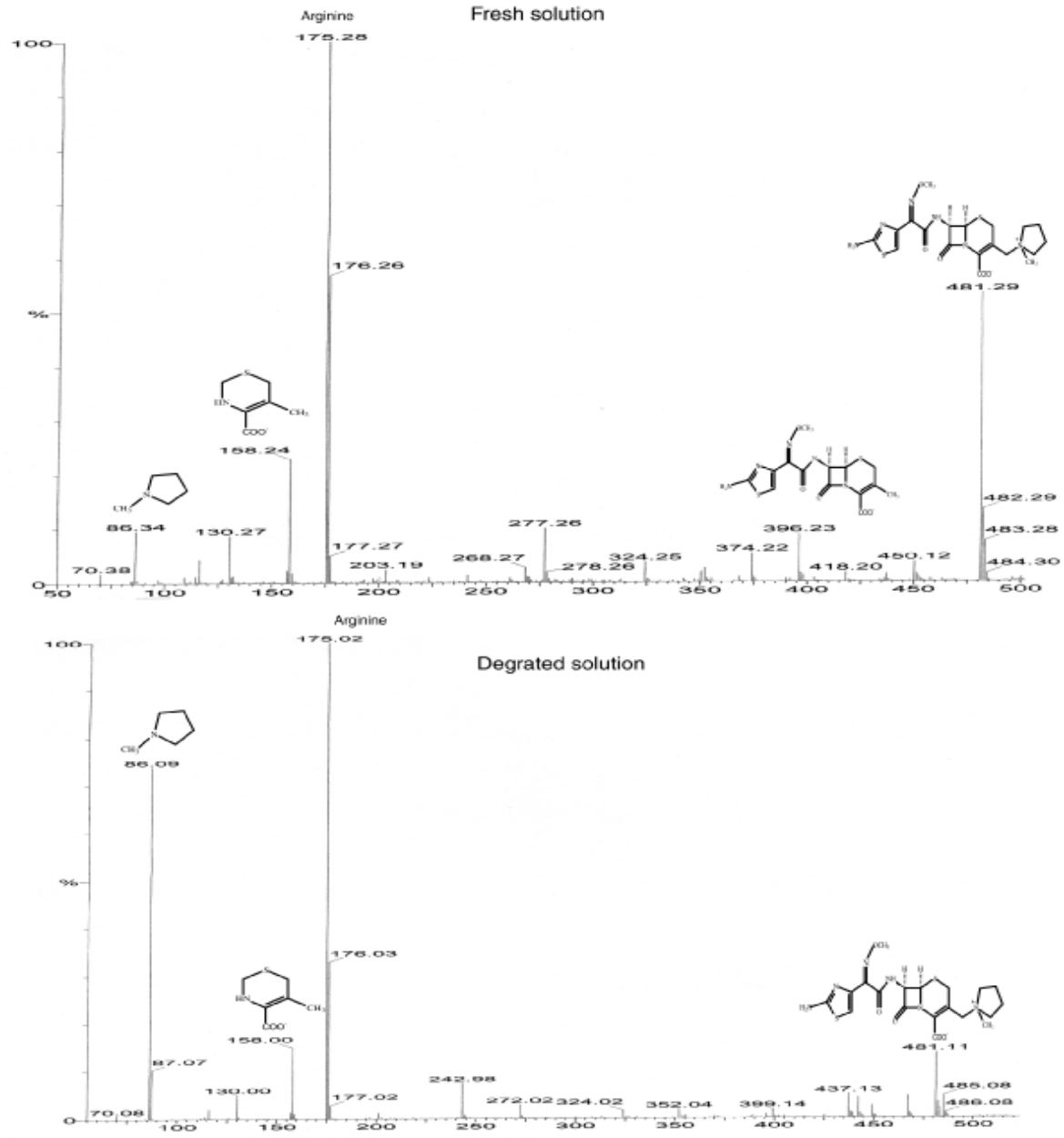


Figure 10: Changes in mass signature during degradation. On top is the mass signature of a freshly prepared cefepime solution, and on bottom is the mass signature of a degraded solution. The structures of putative degradation products are placed over their respective molecular weight (MW). MW=175 is the buffer arginine. MW+1=86 is N-methylpyrrolidine.

The mass scan of the fresh solution shows that even the fresh made solution is to some degree broken down, this can be explained since we were using commercial product that may have already been stored for some time. In addition, some degradation will occur during the freezing and thaw procedure. This experiment was done to map qualitative more than quantitative changes in cefepime and its degradation products. Cefepime (molecular weight (MW)=481) is clearly decreasing and the degradation products with MW= 158 and 86 (N-methylpyrrolidine) are increasing as a result of degradation of cefepime. Since we were primarily interested in major breakdown products, no fragmentation was performed. Structure 5, 2-[[2-amino-4-thiazolyl)((Z)-methoxyimino) acetyl] amino] acetoaldehyde, in figure 9 is a reactive amine structure and is likely broken down further in a rapid manner, since this structure was not detected at any other time of the degradation. Additional work is necessary to completely map the breakdown pathway of cefepime in water-based solutions.

2.3.5 Correlation between cefepime stability and in-vitro antibacterial activity

Cefepime exhibited excellent *in-vitro* activity against the reference strain of *P. aeruginosa* with the MICs of the freshly made solutions all measuring 4 µg/ml, which is below the NCCLS breakpoint for susceptibility. The MICs and MBCs were not significantly different from one another demonstrating the potent bactericidal activity provided by cefepime against *P. aeruginosa* ($p<0.83$). The measured MICs plotted against intact cefepime remaining in the degraded solutions are shown in figure 11.

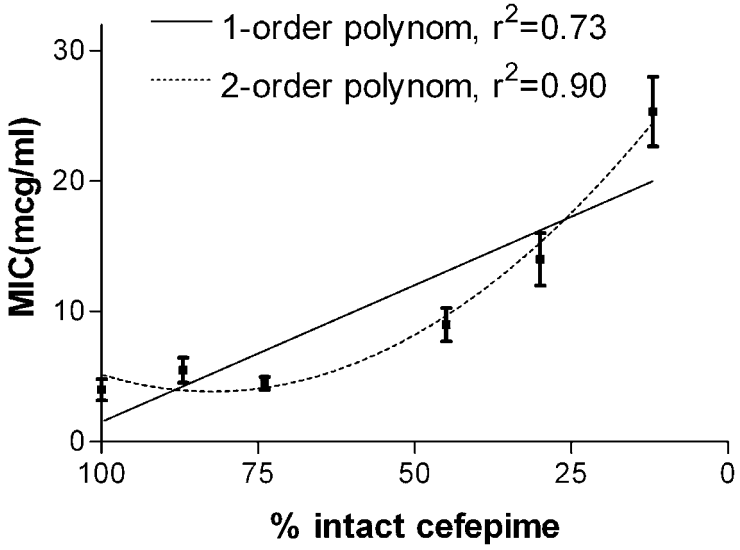


Figure 11: Antibacterial activity during cefepime degradation. Measured MICs with standard deviation on the y-axes are plotted against remaining cefepime concentration in percent on the x-axes. The solid line represents a first order polynomial fit of the observed MICs, and the stippled represents a second order polynomial fit.

The first order polynomial fitting indicates that the MICs increased linearly with decreasing amount of intact cefepime remaining (r^2>0.73, p<0.0001). This data suggests that the antibacterial activity is proportional with intact cefepime concentration, and the degradation products exhibit no antibacterial activity. The second-order polynomial (r^2=0.90) indicates that it is not a direct proportional correlation between intact cefepime and antibacterial effect.

2.4 Discussion

2.4.1 Cefepime stability and drug-reservoir temperatures

A number of prior studies have been conducted to evaluate the stability of cefepime under various conditions. The most complete study on cefepime stability evaluated the impact of pH, temperature, and buffers on cefepime degradation (26). This study demonstrated that cefepime is stable for two days at temperatures up to 30°C. Williamson et al showed that in a

peritoneal dialysis solution cefepime would be stable for two days at body temperature (37^{o}C) (35). Similarly, the manufacturer's product information states that the stability in D5W is up to 24 hours when temperatures are maintained between 20°C and 25°C (Cefepime product monograph Bristol-Myers Squibb Company, July 2000). These data indicate that cefepime should be stable when administered as a CI over 24 hours. Our data support this conclusion demonstrating that the temperature in the drug reservoir remained below 29.1°C resulting in clinically insignificant drug loss over a 24-hour interval.

In contrast, Viaene et al recently published an evaluation of cefepime stability and concluded that cefepime is not sufficiently stable to support CI administration using portable infusion pumps (27). The design of this study differs from this one in two ways: the temperature utilized in their experiment and the diluent used to admix the cefepime solution. In the study, Viaene et al. used portable elastomeric pump to evaluate the 24-hour stability of cefepime when administered as a CI. Portable elastomeric pumps are utilized commonly in the outpatient setting due to their ease of use and are relatively inexpensive. However, these pumps are typically placed under the clothing in close proximity to the body. Thus the temperature of the drug solution within the reservoir approximates normal body temperature. In the study by Viaene et al. they chose a temperature of 25°C and 37°C to simulate the conditions expected in a patient receiving cefepime via a portable elastomeric device. In this study we utilized a motorized portable infusion pump that was placed in an external pouch (separate from the drug reservoir) worn around the waist. Therefore, the higher temperature could explain the greater degradation noted in their study (10% degradation over 13 hours for 37°C and 10% degradation over 20.5 hour at 25°C) when compared with (5.7% degradation over 24 hours) in our study. Another potential contributing factor is the difference in diluents used in admixing of the cefepime solution. In the study by Viaene et al. sterile water was used as a diluent

in order to reduce the tonicity of the solution placed in the pump reservoir. Since the reservoirs for the elastomeric pumps are typically 50 or 100ml, it is necessary to prepare highly concentrated solutions of the drugs to be administered using these devices. In contrast, with the motorized portable infusion devices there are no restrictions on the volume of fluid used to deliver the medication. We are unaware of any comparative data on the stability of cefepime in sterile water versus D5W. This data demonstrates the importance of testing the stability of compounds utilizing conditions that mimic those likely encountered by a patient.

As seen in figure 5 the temperature between the experiments show little variation. The figures also show that the temperature increased to room temperature after admixing the solutions, and decreased during nighttime before they again increased in the morning. This is consistent with the fact that it is the room temperature that controls the bag temperature.

One limitation to this study is that stability was not tested at the extreme ambient temperature that might be expected during summertime. Since the drug reservoir is influenced by ambient temperature, the environment in which the patient will be receiving the drug should be taken into consideration before giving the CI. For patients who will be outside in temperatures exceeding 29°C for any length of time should place a cold pouch adjacent to the drug reservoir to ensure stability of the cefepime administered.

2.4.2 Stability of cefepime during different storage conditions

According to the results derived from the Arrhenius plot a fixed temperature of 29.1°C will give 90% stability after 24 hours. While the Arrhenius plot is more correctly used for storage at a fixed temperature than with varying temperatures, it gives an indication of stability when the

variation is relatively small as it was in this experiment. The similarity of the 24-hour stability measurements using different concentrations indicates that the concentration did not affect the stability, which is consistent with a first order process.

The in vivo stability of cefepime is to a very small degree influenced by chemical degradation. While cefepime has a biological half-life of about 2 hours (21), the chemical half-life was measured in body temperature to be about 64 hours. Practically the chemical stability does not need to be considered in vivo.

2.4.3 Degradation products

Degradation includes cleavage of the R2 side chain and opening of the β-lactam ring. This was the expected breakdown of cefepime since two related compounds ceftazidime and cefpirome have shown similar breakdown (33, 36).

One concern of CI of ceftazidime is that it breaks down to form the known toxic compound pyridine (27), this is not a concern for cefepime since pyridine is the R2 side chain only of ceftazidime (see figure 4). Cefepime has N-methylpyrrolidine as a R2 side chain, and is not broken down to pyridine. The toxicological effects N-methylpyrrolidine or the other breakdown products are currently unknown. With the recent knowledge in mind the degradation do not produce any toxic metabolite.

2.4.4 Correlation between cefepime stability and in vitro antibacterial activity

The antibacterial activity of the fresh cefepime solution was compared with degraded solutions to investigate if the degradation products exhibit antibacterial activity. If the degradation products exhibit antibacterial activity the MICs would not have increased proportionally with the intact cefepime concentration, and the MIC observed would be lower than expected. The second order polynomial provided an improved fit to the data when compared with the linear model, indicating that the relationship between intact cefepime and antibacterial activity is not proportional. However, as seen in table 1, the MICs are not continuous values and some concentration gaps are up to 50%, indicating that high variations are possible. Due to the imprecise measurements in the MICs, both fits could be possible interpretations of the data. Both models indicate that degradation products do not exhibit antibacterial activity, based on the fact that MIC is increasing during degradation.

2.5 Conclusion

CI has proven to be a more efficient method of administration than intermittent dosing for antibiotics for which the bactericidal activity best correlates with time antibiotic concentration exceed the MIC (e.g. β-lactam antibiotics). Due to fewer administrations times and reduced overall dosage, CI also has pharmacoeconomic advantages over traditional intermittent dosing of β-lactams. This study has shown that both the stability and the antibacterial activity of cefepime solutions support the use of 24 hour CI using a motorized portable infusion pump.

Our data demonstrates that cefepime in D5W is stable (>90% active) after 24 hours using a motorized portable infusion pump. Since the drug reservoir is worn in an external pouch, the temperature in the drug

reservoir more influenced by the room temperature than by the body temperature, a fact clearly indicated by the average bag temperature of 22.6±1.5°C. The use of motorized portable infusion pumps and the bag system may be more suitable for CI regimens with drugs that exhibit temperature dependent stability.

Our data demonstrates that cefepime in D5W is stable (>90% active) and maintains antibacterial activity after 24 hours using a motorized portable infusion pump ($p<0.0001$). The Arrhenius equation indicated that the drug solution must be keep in a average temperature below 29.1°C to maintain stability during a 24-hour infusion.

In the case of home treatment, the cefepime solutions must be stored in a refrigerator, since degradation of cefepime occurs rapidly at room temperature. If the supply is for more than five days, it must be kept in a freezer and allowed to thaw in a refrigerator one day before use. This is based on the average degradation of 5.8% when administered as a CI over 24 hours, and a 3% degradation over five days under refrigerator (4°C) conditions.

The buffer added in the commercial product Maxipime keeps the pH in the drug solution within the pH-range where cefepime is most stable within the clinical use.

The antibacterial activity decreases with a decreasing amount of cefepime remaining in solution, indicating that the degradation products exhibit very little or no antibacterial activity. The similarities in the MICs and MBCs in this study confirm the potent bactericidal activity of cefepime against *P. aeruginosa.*

3. PHARMACODYNAMICS AND PHARMACOKINETICS OF CONTINUOUS INFUSION VERSUS INTERMITTET INFUSION OF CEFEPIME IN PATIENTS WITH CYSTIC FIBROSIS

3.1 Scope and intent

This is a prospective, randomized comparative study with the purpose of comparing the PK and PD of continuous versus intermittent infusion of cefepime in cystic fibrosis patients, administered during an acute pulmonary exacerbation. More specific aims of this study are to compare the antibacterial and anti-inflammatory activities of continuous versus intermittent infusion cefepime in CF patients, and to determine if a relationship exists between improvement in pulmonary function and changes in airway inflammation and/or bacterial burden in CF patients treated for acute pulmonary exacerbations.

3.2 The clinical study design

The Institutional Review Board (IRB) at the University of Southern California approved this study. The inclusion criteria were:

- Age ≥ 18 years
- Confirmed diagnosis of cystic fibrosis, with either a positive sweat chloride test or an abnormal genotype.
- Being admitted for treatment of an acute pulmonary exacerbation.
- Have had a qualitative sputum culture within 6 months before the current admission for a pulmonary exacerbation. The predominant *P. aeruginosa* morphotype previously isolated was susceptible to cefepime or ceftazidime and tobramycin.
- Patients must be capable of producing sputum samples on days 1, 14 and after 1 month.

- FEV_1 20-70% of predicted for age and height.
- Have provided written informed consent.

Following criteria excluded the patient form participating in this study:

- History of hypersensitivity reaction to any cephalosporin, penicillin, carbapenem or aminoglycoside.
- Renal insufficiency (predicted creatinine clearance <60mL/min/1.73m^2), or hepatic dysfunction (ALT or AST > 3 times the upper limit of normal; bilirubin> 1,5mg/dL.
- A positive sputum culture for *Burkholderia cepacia*.
- Pregnancy or nursing.

The patients enrolled in the study was randomized to receive cefepime as:
- A continuous infusion with the rate 100mg/kg/24hours (maximum 6g/day) in dextrose 5% in water, with a loading dose of 15mg/kg (maximum 1g). **Or**
- Intermittent infusion of 50mg/kg IV every 8 hours (maximum 6g/day). Each dose was infused over 30 min.

The combination of an antipseudomonal β-lactam and an aminoglycoside are routinely prescribed for treatment of acute pulmonary exacerbations in patients with CF. In this trial intermittent or CI cefepime was administrated in combination with intravenous (IV) tobramycin 5mg/kg every 12 hour. Tobramycin dosage was adjusted to achieve peak concentration between 10-15 μg/mL and trough concentration less than 1μg/mL. Since tobramycin and cefepime are incompatible (37) a double lumen central venous access device (PICC line) was utilized in patients receiving CI.

The predicted serum concentration time profiles of CI and intermittent cefepime administration are illustrated in figure 12. With an intermittent regimen, cefepime serum concentrations drop below an MIC of 8 μg/mL, which represents the NCCLS breakpoint for susceptibility.

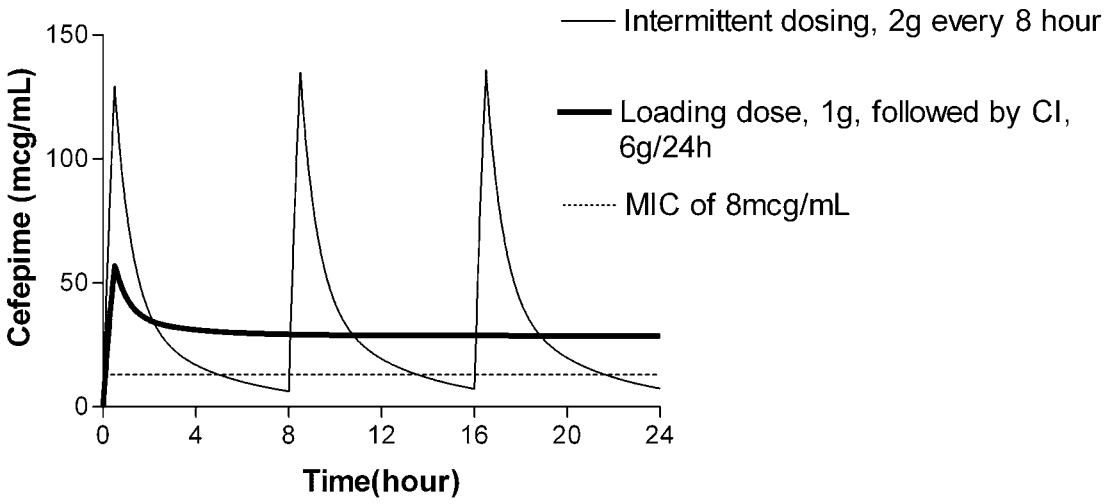


Figure 12: This figure illustrates a comparison of the concentration-time curve of CI 6g/24 hours with intermittent 2g every 8 hours. The MIC of 8μg/mL represents the NCCLS breakpoint for susceptibility to cefepime. The values are calculated using the simulation module in ADAPTII PK program, with mean parameter values derived from prior studies in CF patients receiving intermittent administration of cefepime (24).

The outcomes assessed in this study were:

- Antibacterial effect, which was assessed by quantifying sputum bacterial densities.
- Anti-inflammatory effect, which was assessed by comparing IL-8 concentrations in urine and sputum. IL-8 is a proinflammatory cytokine that is secreted by different cells such as monocytes, neutrophils, endothelial cells, fibroblast after activation, and by mitogen-stimulated T lymphocytes. IL-8 is a potent neutrophil chemoattractant.
- Pulmonary function was assessed using spirometry obtained prior to initiation, at the completion of therapy (14 days), and at 1-month follow up.

To ensure safety during this study, the patients received routine monitoring of renal function by measurement of serum creatinine and hepatic function by measurement of AST, ALT, bilirubin and alkaline

phosphatase every fourth day during therapy. Patients were questioned about adverse events on a daily basis and any patient who developed a rash or other serious adverse effects attributed to cefepime were discontinued from the study.

3.3 Materials and methods

3.3.1 HPLC assay

The HPLC assay was a modification of a previous published cefepime assay (28). Chromatography was preformed isocratically on an analytical reversed-phase Adsorbsphere C18 at 250nm, with 100µg ampicillin as internal standard. The mobile phase consisted of 8% v/v acetonitrile in 20mM ammonium with pH 4.0 The standard curve was calculated in quadruples, and inter-day covariation was measured for the three analysis days. All the patients' samples were injected in duplicates. More HPLC assay details are listed in appendix B.

3.3.2 Sampling

Blood

Serial blood samples were taken from the forearm vein contralateral to the site of drug administration. Samples were taken right before the dose was given and at the following times after completion of the infusion (loading dose in those receiving CI): 0, 0.25, 0.5, 2, 4, and 6 hours. In addition, blood samples were collected mornings on days 5, 9 and 14 for the CI patients. Fresh blood was allowed to clot on ice after sampling before it was centrifuged, and then the serum was separated and stored at -70° C until assayed. The catheter that was used to obtain the blood sample was flushed with 3mL of 0.9% saline before and after each sample was obtained. In addition, blood samples were obtained as a part of routine

care to quantify the erythrocyte sedimentation rate prior to treatment, at completion of therapy, and at 1-month follow up.

Urine

Three 3-mL portions of urine were obtained prior to initiation, at the completion of therapy, and at 1-month follow up. The urine samples were stored at –70°C until assayed for IL-8 by ELISA.

Sputum

Sputum samples were obtained after the patient first removed the saliva, then coughing up the sputum. If needed, sputum induction using inhaled hypertonic saline was used. Samples were obtained prior to initiation, at the completion of therapy, and at 1-month follow up. The sputum was weighted and admixed with four times the weight of 10% Sputasol® and three times the weight with a phosphate buffer solution. Sputasol® is a supplement for the liquefaction of sputum samples before microbiological testing. This mixture was made homogenous by mixing and then shaking at 37°C in a water bath for 5 minutes. This cycle was repeated two additional times. The solution was centrifuged at 1000rpm until the pellet was solid (about 10minutes). Then the supernatant was separated from the pellet and transferred into small vials. The pellet was discarded. The supernatant was again centrifuged at 13000 for 15min. This supernatant was frozen at -70°C until assayed by ELISA for IL-8 concentration.

Bacterial organism identification was performed by plating on selective media according to NCCLS guidelines. Bacterial susceptibility was determined using a quantitative disk diffusion assay (E-test, AB Biodisk). Sputum bacterial density was determined by plating serially diluted sputum samples and counting the colonies using a microscope. The sputum bacterial densities were performed by the Microbial Research Laboratory at the University of Southern California. Organism identification and in-vitro susceptibility testing were performed at the Clinical Microbiology at USC University Hospital.

3.3.3 Pharmacodynamic/pharmacokinetic modeling

Pharmacokinetics

The pharmacokinetic analysis was performed using ADAPT II software (Biomedical Simulations Resource, University of Southern California, Los Angeles) (38). ADAPT II provides a modeling package for fitting concentration data from individuals using a standard two-stage parametric approach. In this study serum concentrations were fitted to both one- and two compartment models (see figure 13) using both maximum likelihood analysis and the maximum *a-posteori* (MAP) Bayesian methods.

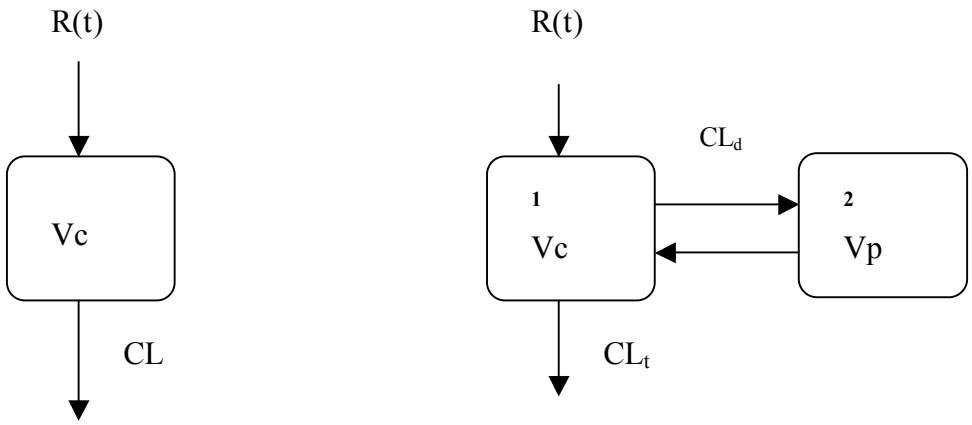


Figure 13: One- and two-compartment models. Left side is the one-compartment model with the central compartment with volume V_c, and clearance CL. The model on the right is the two-compartment model, V_c is the volume of the central compartment (1), Vp is the volume of the peripheral compartment (2). CL_d is the distribution clearance, and CL_t is the total clearance. R(t) refers to the rate of drug input for both models.

The maximum likelihood method fits the curve by minimizing the difference between the model estimated concentrations and the observed concentration, and calculates the parameters from that model. The MAP Bayesian method minimizes the difference between the observed and model estimated concentrations, as well as the difference between the model-estimated values and the population parameter estimates. This is

achieved since MAP-Bayesian starts out with selected mean parameter value estimates and their standard deviations as *a-priori* values. These initial values are used as priors to update the parameters using the MAP-Bayesian algorithm.

The *a-priori* values for MAP-Bayesian analysis of the 1-compartment model were taken from a previous pharmacokinetic study on cefepime (24). No previous pharmacokinetic study using a 2-compartment analysis has been reported with cefepime, so the *a-priori* estimates were taken from a 2-compartment study on ceftazidime (also adult CF patients) (39), a drug that is very similar to cefepime (also se figure 4). The *a-priori* values for both the 1- and 2-compartment models are summarized in appendix C.

A fixed linear variance model (variance= 0.1+ 0.1*concentration) was used. Model discrimination was based on the generalized information criterion (GEN-IC) and the r-squared values for the MAP-Bayesian method, and on the Akaike information criterion (AIC) for the maximum likelihood method. The pharmacokinetic parameters obtained from this analysis were volume of central compartment (V_c) and peripheral compartment (V_p), clearance (CL) and distribution clearance ($CL_{d)}$). Using standard equations, distribution half time ($t½^{\alpha}$) and elimination half-life ($t½^{\beta}$) were derived.

Pharmacodynamics

The PK/PD parameter time over MIC (T>MIC) has previously been shown to be the best parameter to correlate with the antibacterial effect of β-lactams (16). To investigate if there is a relationship between the PK/PD parameter T>MIC and clinical effect of the treatment, T>MIC was compared with the density changes in *P. aeruginosa* and total bacteria, the change in IL-8 concentration, and change in FEV_1 from start until end of treatment. The pulmonary function marker FEV_1 was compared with the

decrease in IL-8 concentration. FEV_1 is a marker for pulmonary function, and IL-8 is a marker for pulmonary inflammation.

The MIC in the PK/PD parameter T>MIC refers to the highest MIC for cefepime of all the *P. aeruginosa* pathogens in the sputum. The time above MIC was calculated form the ADAPT data-files using the model that best described the serum concentration time course for each patient.

3.3.4 ELISA assay

Enzyme linked immunosorbent assay (ELISA) is a very important serological test for antigens and antibody (40). The kit used here determines IL-8 concentration and is an enzyme amplified sensitivity immunoassay (IL-8 EASIA produced by Biosource, Belgium).

The manufacturer already absorbed a specific monoclonal antibody directed to bind distinct epitopes of IL-8 onto the 96 wells on the microtiter plate. This sensitized the wells to IL-8. Standard or sputum sample was added to the wells, allowing the IL-8 and antibody specific for IL-8 to react and bind. Next, a monoclonal antibody specific to IL-8 labeled with horseradish peroxidase was added. A two-hour incubation in room temperature and using a horizontal shaker, allowed a sandwich-formation, consisting of antibody-enzyme, IL-8 and the anchored antibody. The microtiter tray was then washed with buffer containing preservatives to remove all unbound enzyme linked antibody. To be able to measure the IL-8 concentrations a chromogen solution (tetrametylbenzidine (TMB) in acetate/citrate buffer with H_2O_2) was added and then the solution was incubated at room temperature for 30min. The horseradish peroxidase converts TMB into a colored product. Since the horseradish peroxidase was present in the same concentration as IL-8, the light density from this solution is proportional with IL-8 concentration. The absorptions of visible light at 450nm (reference filter

at 620nm) were measured with a Tean Spectra. A standard curve was made from standard solution provided by the kit from 0-726pg/mL.

The sputum samples were diluted 1:100 (to get the sample concentration within the standard curve), and together with the dilution during processing, the total dilution of sputum samples was 1:700. Therefore the measured concentrations were multiplied by 700 to give correct concentration.

3.3.5 Statistical methods

The descriptive statistics of the patient population were calculated from each subject's age, height, total body weight, BMI, and predicted creatinine clearance. Statistical analysis was performed using Graphpad Prism version 3.03 (Graphpad Software Inc. San Diego, CA). Differences between the clinical measurements from prior to treatment to the end of treatment were calculated with paired student-t test. The Mann-Whitney nonparametric U-test was used to discriminate between 1- and 2-compartment models, and a nonparametric t-test was also used to assess differences between clinical outcomes and measured parameters. A nonparametric student t-test was chosen since the sample size was small and will not possess a Gaussian Bell-shaped distribution.

3.4 Results

3.4.1 HPLC assay

The standard curve was linear in the range from 2.5-125µg/mL with a correlation factor above 0.999. The recovery from serum was on average 77.0%. The intraday and interday coefficients of variation ranged from 2.99-4.90%, and 7.5%. More details from the HPLC-assay are described in the appendix B.

3.4.2 Pharmacokinetics

A total of eight patients have been enrolled in this study to date. Two patients were dropped form the study (one CI and one intermittent), one because of nausea and the other due to a pump failure. These two patients had serum samples taken, and their PK was calculated, but no clinical outcomes were assessed. Of the six patients that completed the study, four of them were given intermittent administration and two were given CI. The two groups of patients and their demographics and clinical parameters are given in table 3, and each patient's demographics and calculated parameters are shown in a table in appendix C.

Table 3: Patient demographics of the two groups of patients. The groups are divided into CI and intermittent.

Parameter	CI	Intermittent	Total	Range
Males/Females	1/2	2/3	3/5	
Age (years)	38.0±11	33.0±5	34.9±7.7	26-50
Height (cm)	166±8.5	171±8.0	169±8.3	158-185
TBW (kg)	43.4±4.8	64.2±17	56±18	38.4-83.5
BMI (kg/m^2)	16.1±0.8	22.2±4.4	19.9±4.7	15.4-25.8
CL_{CR}(mL/min)	98.8±44	124.0±21	115±30	58-146

TBW: total body weight, BMI: body mass index, CL_{CR}: creatinine clearance (calculated with Cockcroft & Gault)

The PK of cefepime was better described with a two-compartment model than with a one-compartment model for both the maximum likelihood and the MAP-Bayesian methods, as shown by the higher r^2 values and lower AIC and GEN-IC values seen in table 4 and 5, and all the patient fittings in figure 14 and 15. There was less bias in the MAP-Bayesian 2-compartment fitting than in the 1-compartment fitting, as seen in figure 16. This figure shows the median percent error between the estimated and the observed values for each patient. The r-squared was significantly better for the two-compartment model ($p<0.01$, pared two-way t-test) and the GEN-IC was significantly higher for the two-compartment model

($p<0.03$, paired one-way t-test). Patient 3 showed an unreasonable serum over time curve, and investigation revealed that there was trouble adjusting the pump rate just after initiation of dosing. This patient's PK was not calculated because of this, but steady state was later achieved and PD was calculated.

Table 4: The r-squared and AIC values used in model discrimination using a one- or two-compartment mode with maximum likelihood fitting model.

	One-compartment		Two compartment	
Patient	**r^2**	**AIC**	**r^2**	**AIC**
#1	0.819	42.414	0.986	30.885
#2	0.947	41.608	1.000	32.860
#4	0.846	48.312	0.977	38.519
#5	0.950	97.336	0.992	38.589
#6	0.819	42.414	0.986	30.885
#7	0.888	100.620	0.976	54.957
#8	0.872	66.131	0.984	41.747

Table 5: The r-squared and GEN-IC values used in model discrimination using a one- or two-compartment MAP-Bayesian model.

	One-compartment		Two-Compartment	
Patient	**r^2**	**GEN-IC**	**r^2**	**GEN-IC**
#1	0.981	51.342	0.988	42.147
#2	0.948	38.375	0.986	32.677
#4	0.841	96.893	0.968	84.009
#5	0.848	38.847	0.957	42.700
#6	0.955	93.249	0.990	50.171
#7	0.819	37.407	0.964	33.586
#8	0.890	101.00	0.978	73.356

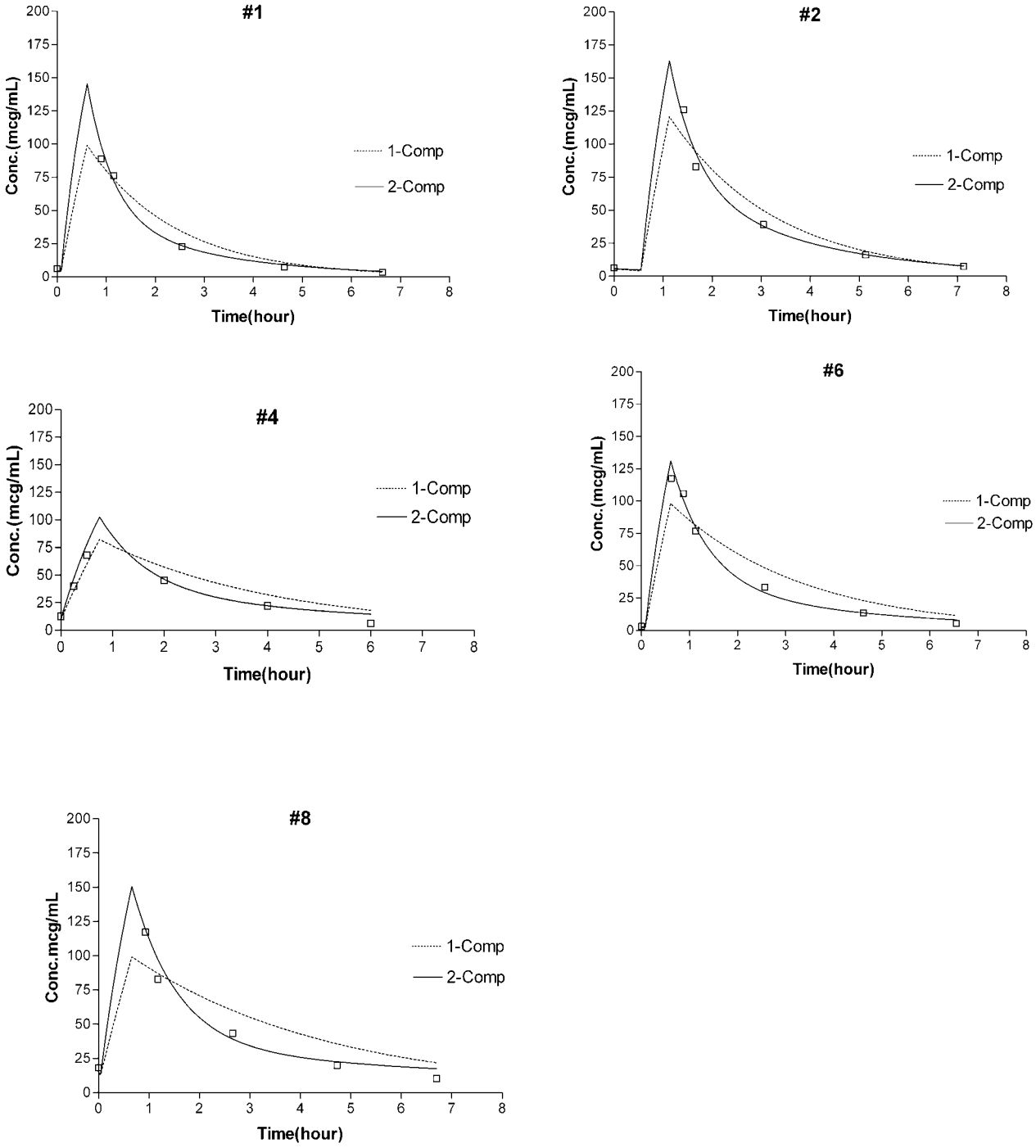


Figure 14: Individual serum concentration versus time for the patients receiving intermitted dosing. The dashed line represents the 1-compartment fitting of the measured concentrations, while the solid line represents the 2-compartment fitting. The PK of patient #3 were not calculated because of a pump rate problem.

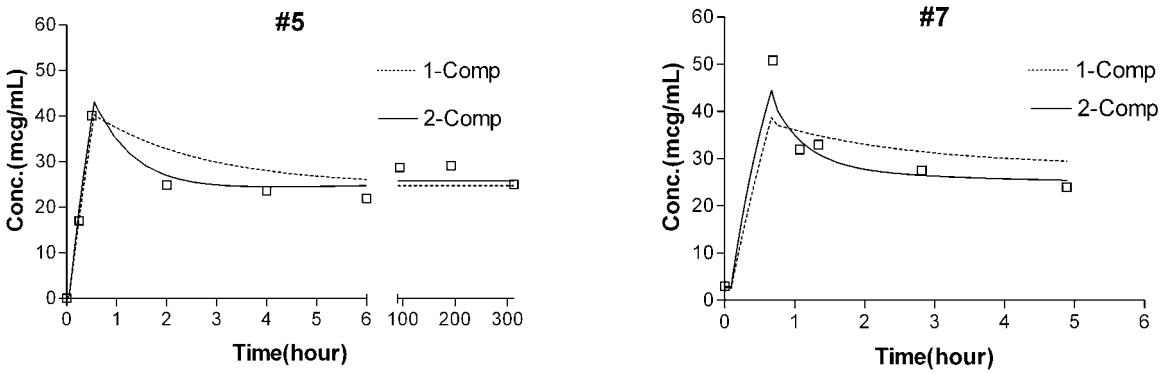


Figure 15: Individual serum concentration versus time for the patients receiving CI. The dashed line represents the 1-compartment fitting of the measured concentration, while the solid line represents the 2-compartment fitting. #7 has only serum concentration until 6 hours because that patient was discontinued from the study.

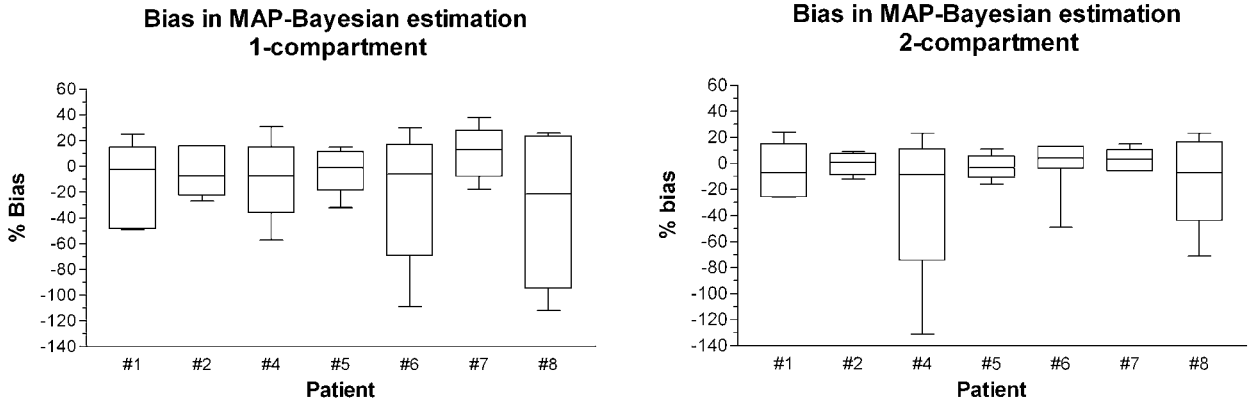


Figure 16: Boxes and Whiskers of bias in the MAP-Bayesian estimation. The figure on the left is for a 1-compartment model and the figure on the right is for 2-compartment model. The line is the median percent error in the estimation, the boxes are the interquartile intervals, and the whiskers are the maximum and minimum values.

Using the 2-compartment model, all the patients' parameters were calculated using the MAP Bayesian method. A summary of all the PK parameters is shown in table 6.

Table 6: PK parameters of the seven patients determined after both intermittent and CI cefepime administration using two-compartment MAP-Bayesian method.

Parameter	Median	Interquartile range
CLt mL/min/kg)	2.48	(1.87-2.92)
CLd(mL/min/kg)	1.91	(1.28-1.98)
$t_{1/2}^{\alpha}$ (h)	0.534	(0.37-0.62)
$t_{1/2}^{\beta}$ (h)	2.82	(1.74-4.22)
Vc (L/kg)	0.314	(0.27-0.37)
Vp (L/kg)	0.198	(0.10-0.25)
T>MIC (%)**	92.55	(30.2-100)*
Css,avg(µg/mL)	25.30	(24.8-25.8)*
Cmax (µg/mL)	137.4	(111.8-46.2)*
Cmin (µg/mL)	5.7	(2.07-13.2)*

** Mean T>MIC is only calculated from the intermittent dosing, since all the CI patients had T>MIC 100% because of the nature of this administration form.
* Since the range contains too few patients to give the interquartile range, the range is shown as maximum and minimum value.
CLt : total clearance, CLd: distribution clearance, $t_{1/2}^{\alpha}$:distribution half-life, $t_{1/2}^{\beta}$: elimination half-life, Vc: volume of central compartment, Vp: volume of peripheral compartment, T>MIC: time above MIC, Css,avg: concentration of steady-state for the patients on CI, Cmax: maximum serum concentration, Cmin: minimum serum concentration, AUC: area under the serum concentration curve.

3.4.3 IL-8 concentrations

The standard curve was linear from 0-726pg/mL (r^2>0.98), and according to the manufacture the lowest detectible concentration was 0.7pg/mL. The CV% from the duplicate standard curve concentrations ranged from 2.5-13.7%. The control solutions that were provided by the kit were measured

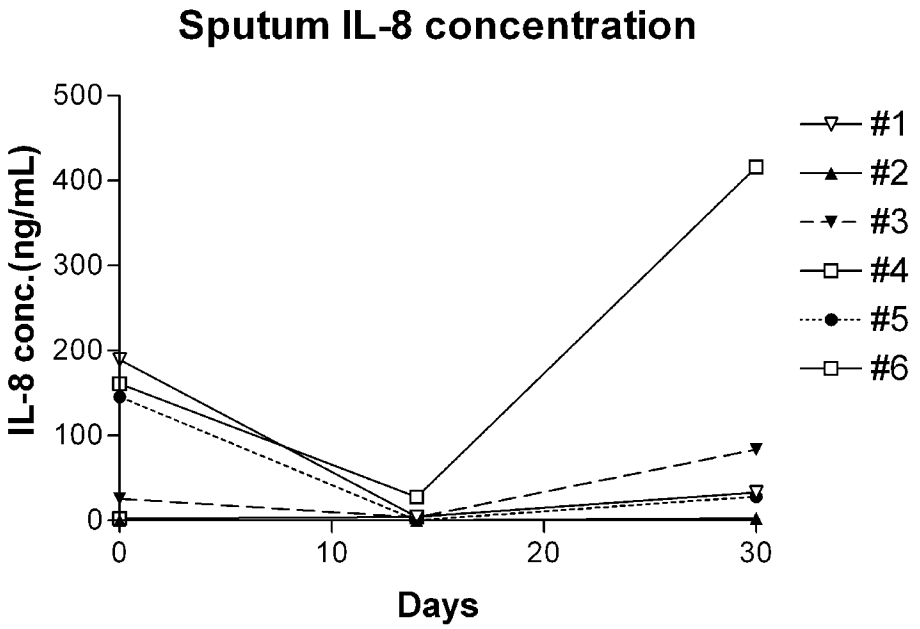
to be: the 97pg/mL control was measured to be 109.8±1.2pg/mL (+13% compared to control), and 425pg/mL was measured to be 505.9±27pg/mL (+19% compared to control). The average CV% from the duplicate sample measurements was 6.2%, ranging from 0.9-19.4%.

All the urine samples had too low IL-8 concentration to be measured.

The results from all the sputum samples obtained from the patients who completed the study are shown in table 7 and figure 17.

Table 7: IL-8 concentration in ng/mL in patient sputum taken before treatment (0 days), after the end of treatment (14 days), and follow up (1 month). Nd= not detectable. The thesis was completed before patient 6 had its 1-month follow-up.

Patient	0 days	14 days	1 month
#1	189.3	4.3	33
#2	nd	Nd	2.7
#3	25.7	3.7	83.5
#4	161.1	27.7	416.1
#5	145.8	Nd	28.2
#6	2.3	4.5	



Graph 17: IL-8 concentration in ng/mL showed as a function of time after started treatment. IL-8 concentrations were measured on three days; before treatment (0 days), after treatment (14 days), and follow up (1month).

All the patients except one showed a decrease in the IL-8 level during the treatment, and an increase after treatment was complete. The IL-8 concentration showed an average decrease of 45% during the 14 days treatment, but this difference was not found to be statistically significant ($p<0.075$, pared t-test, $p<0.04$ for one-way t-test).

3.4.4 Pulmonary function (FEV_1)

The average increase in FEV_1 during the 14 days treatment was 10% for all patients. The improvement in FEV_1 during treatment was not statistically significant ($p<0.15$ paired two-way t-test, $p<0.05$, paired one-way t-test). All the FEV_1 changes are shown in figure 18.

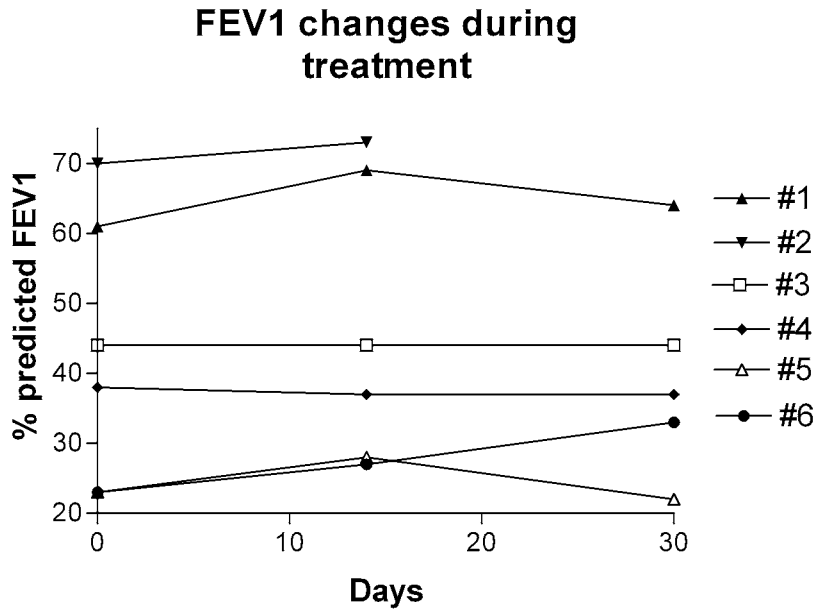


Figure 18: Pulmonary function shown as percent predicted FEV_1. This figure shows the changes in FEV_1 in all the 6 patients during the treatment (first 14days) and up until the follow up 1month later. All the patients showed a increase or the same FEV_1 except one patient (#4) during treatment (day 0-14).

The patients with the best FEV_1 improvement seemed to have a higher decrease in IL-8 concentration as shown in figure 19.

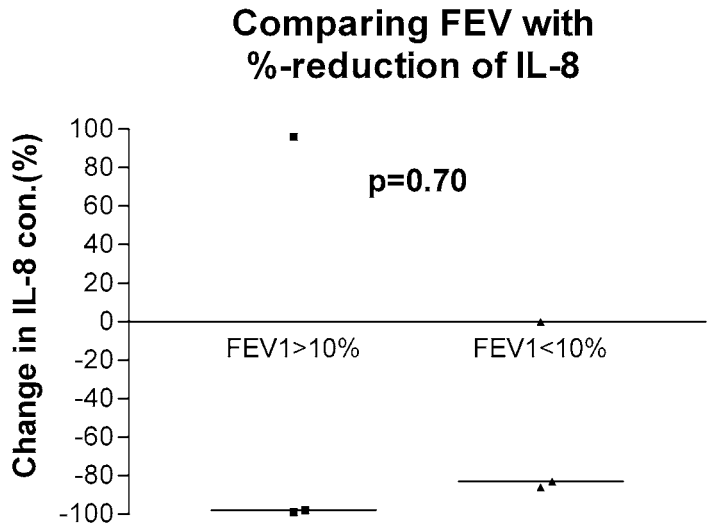


Figure 19: Comparing changes in FEV_1 with percent changes in IL-8 concentration. Patients are grouped into two groups; one with an increase of >10% in FEV, and those patients that did not achieve 10% improvement in FEV_1. There are three patients in each group. The line represents the median.

3.4.5 Bacterial density

To be able to investigate pulmonary bacteria flora, all typical mouth and skin bacteria were excluded from the total bacterial count. The six patients showed a decrease in bacterial density during the 14 days of treatment ($p<0.69$, two tailed, $p<0.35$, one tailed, student t-test). The decrease in pseudomonas for all patients was minimal. The total bacterial density and Pseudomonas density are shown in graph 20:

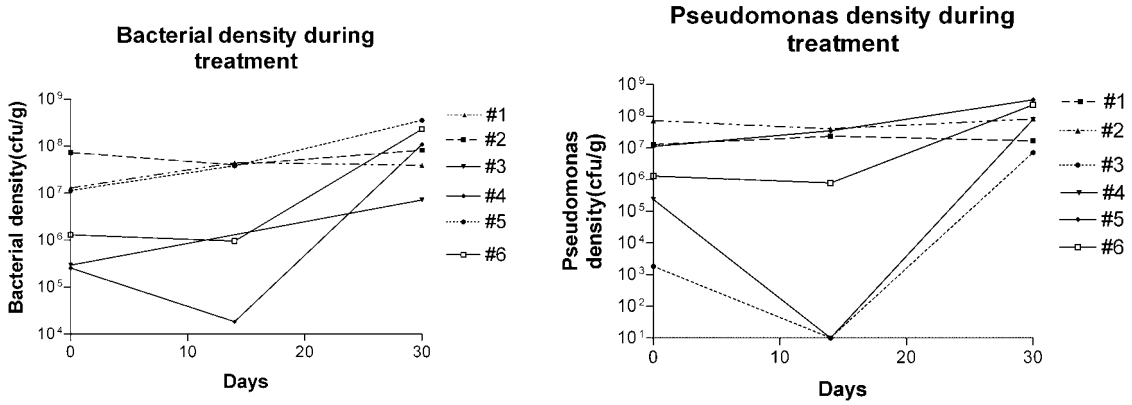


Figure 20: Both the total bacterial (left) and P. aeruginosa *(right) density during the treatment is shown with cfu/g sputum on the Y-axis. The bacterial count was done on 3 days; before treatment (0d), after treatment (14d) and follow-up (30d).*

There was an indication that a decrease in *P. aeruginosa* correlated with the decrease in IL-8 concentration ($p=0.25$) as seen in figure 21.

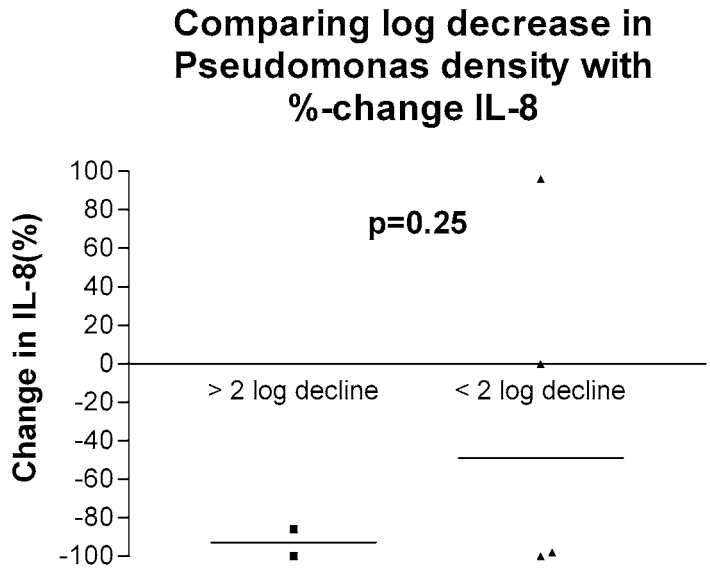


Figure 21: Comparing the logarithmic decline in pseudomonas with the change in IL-8 concentration. The group of two patients with more than 2 logarithmic decline of pseudomonas density was compared with a group of 4 patients with less than a logarithmic decrease of 2 in pseudomonas density with their respective change in IL-8 concentration. The line represents the median.

Figure 22 shows that there was no correlation between the logarithmic decrease of *P. aeruginosa* and improvement of pulmonary function. The result shows that the group with the least change in pseudomonas density had a better increase in FEV_1, but the difference was not significant.

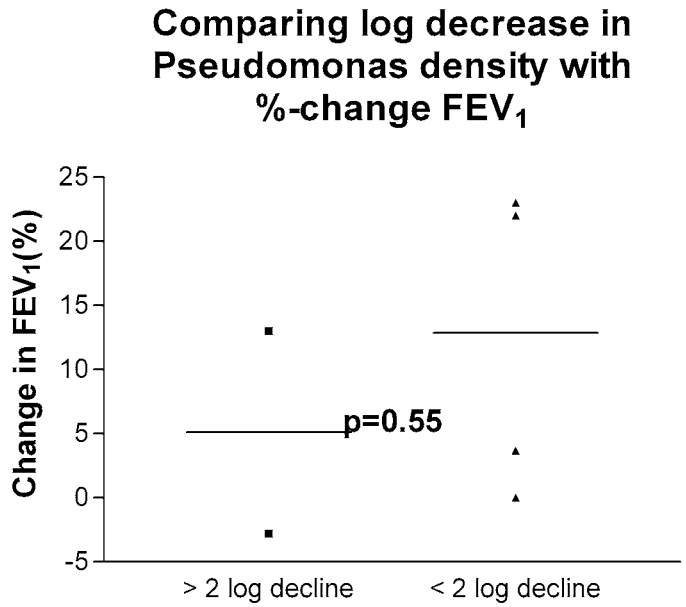


Figure 22: The six patients were divided into two groups, the left group had a logarithmic decline of pseudomonas density greater then 2, and the group on the right had a logarithmic of pseudomonas density decline of less than 2. The two groups were compared with the change in FEV_1 on the Y-axis. The line is the median.

3.4.6 Time over MIC

The time the serum concentrations were over MIC was 100% for the patients receiving CI, for the intermittent patients the average T>MIC was 92.6%. To investigate the possible correlation between T>MIC and clinical effect, the patients were divided into two groups: one with the 3 patients achieving T>MIC 100% of the time, and the other group that did not achieve T>MIC 100% of the time. There were three patients in each group. These two groups were then compared with logarithmic decrease in both total bacterial and *P. aeruginosa* density. The group with most time over MIC showed a greater decrease in both total bacterial and *P. aeruginosa* density. The comparison is shown in figure 23:

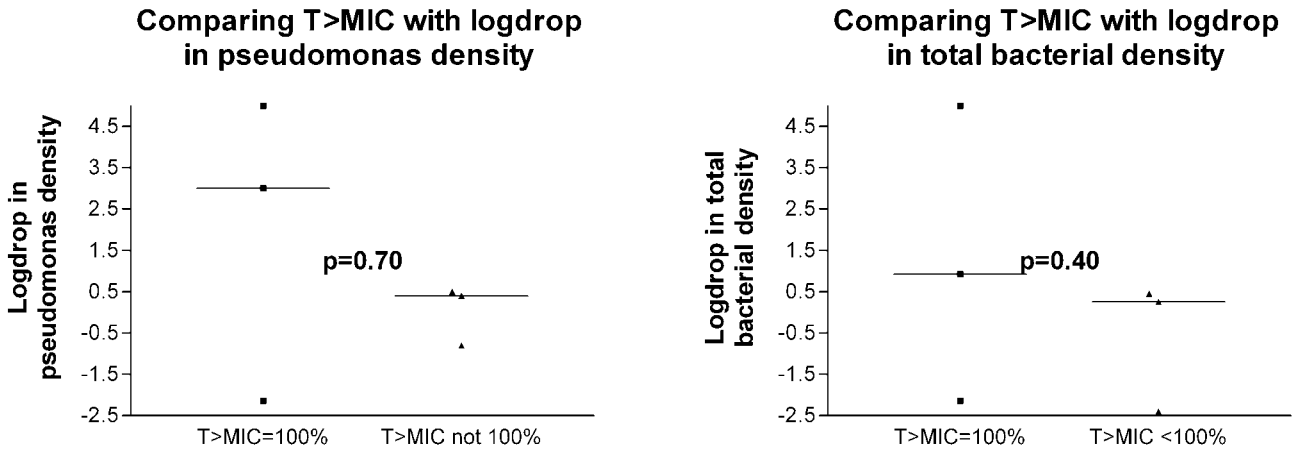


Figure 23: The comparison of two groups divided into those patients who achieved T>MIC 100% and those that did not and their effect on lowering the total bacterial or P. aeruginosa density during the treatment. The group that achieved T>MIC 100% of the time showed a higher decline in both total bacterial density and P. aeruginosa density. The line represents the median.

The same groups were also compared with the ability to lower the IL-8 concentration in sputum. Figure 24 shows that the group with T>MIC 100% had a greater decline in IL-8 concentration than the group that did not achieve T>MIC 100% (p=0.4).

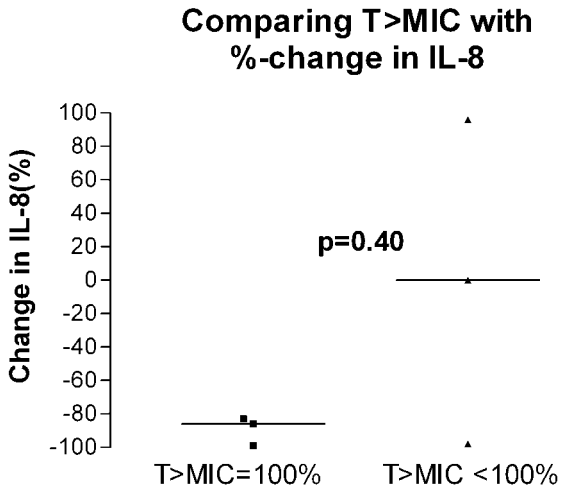


Figure 24: This figure compares the correlation between the parameter T>MIC with the decline in IL-8 concentration. To the left side are the 3 patients who achieved T>MIC 100%. The points are how much the IL-8 concentration changed for those patients. To the right are those 3 patients who did not achieve T>MIC 100% of the time with their respective change in sputum IL-8 concentration on the Y-axis. The line is the median of groups.

The group with T>MIC of 100% had less improvement in FEV_1 than the group with T>MIC <100% (p=0.7) as seen in figure 25.

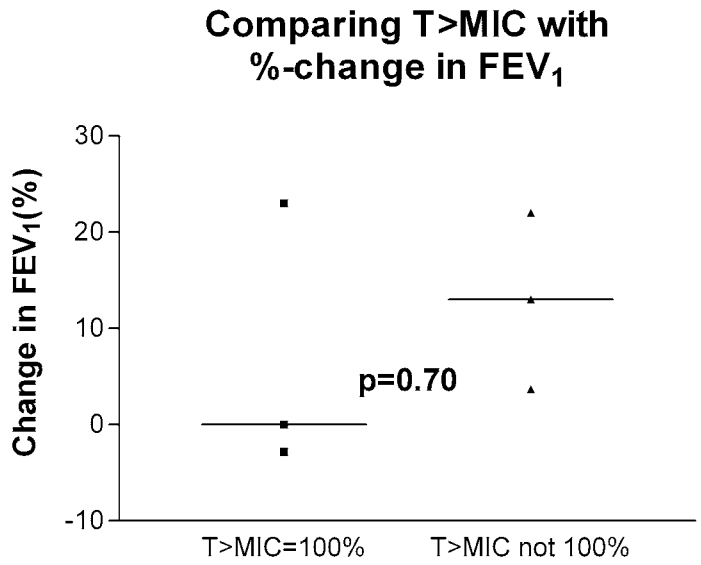


Figure 25: This figure compares the T>MIC with change in FEV_1 in percent. The three patients with T>MIC 100% of the time are shown at the left side and the other three patients are on the right side. The patient's respective percent changes in FEV_1 are shown on the Y-axis. The line represents the median.

3.5 Discussion

3.5.1 Pharmacokinetics

The maximum likelihood gave a very good fit with respect to the measured serum concentrations. But many of the calculated parameters had an unlikely value, for example an elimination half-life of 30 hours, and peripheral volume of 130L. This is much higher than previous kinetic analysis has found with half-life of 2 hours and distribution volume of 25L (25). This model was rejected and MAP-Bayesian modeling was used instead. Since MAP-Bayesian minimizes the sum of errors from the measured serum concentrations with the previous populating data, the

parameters get a more probable value given that the population parameters values are true. All the PK parameters for the individual patients are listed in table 9 in appendix C.

The MAP-Bayesian method gave significantly better fitting when assuming a 2-compartment model. This was expected since cefepime has shown tissue distribution (21). Since this is the first investigation of the actual values of the parameters from a 2-compartment model of cefepime, the a-posteriors values were taken from a study on ceftazidime (30). Figure 16 shows that there was no systematic error in the MAP-Bayesian fitting model. The parameters found in this study we similar to two other studies on non-compartment cefepime pharmacokinetic (24, 25), and to the product information (21), and are summarized in table 8:

Table 8: Shows similarities between this study outcome and previous PK results.

Parameter	**This study**	**Study1 (Arguedas)**	**Study 2 (Huls)**	**Product Info**
Tot Clearance	2.5(ml/min/kg)	3.0±1.5(ml/min/kg)	3.4±1.4(ml/min/kg)	120±8(ml/min/kg)
Cmax (µg/mL)	137(µg/mL)	141±35(µg/mL)		164±25(µg/mL)

To achieve best possible parameter estimates of a 2-compartment model it is important to get two or more samples early after the serum concentration peak. As seen in figure 14 especially patient #4 and #1 had their samples taken late after the peak. This might result in a fitting that underestimates the peak concentration and thereby underestimates the clearance, as observed in low clearance for both patients #4 and #1.

The ADAPT II software used to calculate the PK values assumes a parametric distribution. Since the number of patients was few, parametric distribution is not likely. Therefore, median parameter values were reported.

3.5.2 Pulmonary inflammation

Two other articles have evaluated IL-8 concentrations in CF patients. One found that the mean value was 102.4ng/mL, with a 90% range of 63.2-219ng/mL (41). The other found a mean of 18.0±12.2 ng/mL (42). Both these articles show that our measurements were similar to the previous found concentrations. The high follow up IL-8 concentration in sputum of patient #5 can be explained since the patient also had a high density of mucoid *Pseudomonas* at that time. This is reasonable since inflammatory cells produce IL-8 as a result from interaction with the lipid-polysaccharide (LPS) in *P. aeruginosa* cell wall.

The IL-8 concentration in urine samples was too low to be detected with the IL-8 assay kit that was used.

As shown in figure 17 all the patients except one showed a significant decrease in IL-8. The one patient that did not have a decrease had a very low IL-8 baseline, making improvement difficult. This same patient (#3) also had the highest percent-predicted FEV_1 at admission, and it is possible that this patient did not have a real exacerbation, making it difficult to achieve clinical improvement. The rest of the patients showed that the inflammation decreases during the treatment; as discussed before this can be an indirect effect of a decreasing *P. aeruginosa* density.

Two patients (#5 and #1) showed an increase in *P. aeruginosa* and a decline in IL-8 concentration. This indicates that the treatment lowers the pulmonary inflammation with a different mechanism than just the decline in P-aeruginosa density as discussed previously.

3.5.3 Pulmonary function

The patients showed an average increase in FEV_1 of 10% from admission. A previous study with cefepime and clinical improvement in CF patients showed an absolute increase of 10% in FEV_1 (24, 25). An absolute increase of 10% in FEV_1 (percent predicted for age and weight) is thought to give a clinical difference. None of the patients accomplished a 10% absolute increase FEV_1. The lower increase in pulmonary function may be a result the lower decrease in bacterial density than expected as discussed later.

3.5.4 Bacterial density

Cefepime is an anti-pseudomonal drug and all the patients except two showed a decrease in *P. aeruginosa* density. The average decrease in Pseudomonas density was 44% for 5 of the six patients. However the sixth patient (#5) had a large increase in mucoid pseudomonas that draws the total average down. One of the patients (#1) that had an increase in Pseudomonas density had a high baseline of FEV_1 compared with the others, indicating the possibility that the patient did not have an exacerbation open. The other patient (#5) that did not experience a decline in pseudomonas density had a high increase in mucoid Pseudomonas during the treatment that could be attributed to potential resistant to cefepime. Since the other bacteria were eradicated, it is easier for the mucoid strain to grow. In addition the pseudomonas isolate from patient #5 was only intermediately susceptible to cefepime. This occurred since at admission the most recent susceptibility data was used, this was done since susceptibility tests cannot give a rapid result. Since that last susceptibility testing the bacteria have become partly resistance to cefepime and poor outcome came as expected.

A similar relationship demonstrating a decline in total bacterial density with time was noted. This was excepted since cefepime exhibits activity against gram-negative and gram-positive bacteria. Strains of *Xanthomonas maltophilia* the cefepime exhibited no antibacterial effect; however, strains of *Staphylococcus aureus*, other Pseudomonas strains and *Nocardia farcinica*, were eradicated.

A greater decline in pseudomonas density was expected based on a previous study of cefepime that showed a logarithmic decline after 14 days treatment (24). In addition, cefepime has good bronchial penetration (21). One possible explanation is the fact that mucoid *P. aeruginosa* exists within biofilms in patients with CF. Considering the age and severity of pulmonary disease in our adult CF population it is possible that a greater number of patients in this study were infected with *P. aeruginosa* within biofilms. Traditional in-vitro susceptibility testing is performed on planktonic forms of the bacteria that do not correlate well with activity against bacterial biofilms. Additionally, these findings could be a result of acquired resistance during treatment, and thereby reduced the effect of the antibacterial treatment. Increased resistance during treatment has been reported previously (24), and in this study no investigation of the susceptibility was done after the end of treatment.

The degree of decrease in *P. aeruginosa* has previously been proven to correlate significantly with the degree of improvement in FEV_1 (15). A similar correlation was not seen in this study, as seen in figure 25. The reason could be that this study only had six patients. Additionally, this finding could be explained by the fact that the observed decline in Pseudomonas density was lower than expected.

This study demonstrated ($p<0.25$) that the greater decline in pseudomonas density resulted in a greater decline in IL-8 concentration after treatment was completed, as seen in figure 21. As discussed previously this could

have a direct explanation, since IL-8 is produced in response to LPS from *P. aeruginosa*.

3.5.5 Time above MIC

The main outcome of this study is whether CI has better clinical effect than traditional intermittent dosing. Since only two patients have received CI administration to date, patients were dividend into two groups regarding the time the serum concentrations were above MIC. One group included the patients with time >MIC 100% of the dosing interval, and the other group included those that did not. This gave 2 groups with three patients in each, since one of the patients (#4) had estimated serum concentration above the MIC of 4 mg/mL throughout the dosing interval despite receiving intermittent dosing.

The relationship between T>MIC and change in bacterial density is depicted in figure 23, which shows that the group with T>MIC 100% had a higher decrease in both *P. aeruginosa*- and total bacterial- densities, although the difference was not statistically significant. The same relationship between T>MIC has previously been shown by other investigators, as mentioned in the introduction in 1.2.2. The effect pseudomonas has on IL-8 concentration was seen in the result in figure 24, which shows that patients with T>MIC 100% showed a greater decline in sputum IL-8 concentration than those with T>MIC <100% of the time. The parameter T>MIC did not show any correlation ($p<0.7$) with the pulmonary function seen in FEV_1. It has previous been shown that the bacterial density corresponds with pulmonary function (15). The fact that a greater T>MIC results in more significant decreases in the pseudomonas density can been seen as an indication that patients receiving CI may have a better clinical outcome.

Previously it has been shown that time over 4 times the MIC correlates best with β-lactams effect (43). The reason that T>4*MIC was not used in this interim analysis due to the few numbers of patients included in the present analysis.

3.5.6 Limitations of this study

Since this thesis had a one-year time limit, the data were analyzed before all the patients of the study were enrolled. The results showed several meaningful trends, however because of small sample size there was no statistical significance noted at this time.

The enrollment of patients was slower than expected due to resistant bacterial isolates to the cefepime treatment. This led to only eight candidates, of which six completed the study. The nature of the randomization of the drug administration gave only two patients with CI and four receiving intermittent administration.

3.6 Conclusions and future directions

Cefepime was best described using a 2-compartment model. MAP-Bayesian estimation provided parameter values consistent with previous reports.

The marker for inflammation, IL-8, had too low of a concentration in urine to be detected. In sputum the IL-8 concentration was much higher, and IL-8 concentration decreased during treatment. This decrease in inflammation can be explained the reduction in *P. aeruginosa* density.

CI has the advantage over intermittent dosing by achieving time over MIC 100% of the dosing interval. This study had too few patients to be able to make definitive conclusions; however, it appears that maximizing T>MIC resulted in greater antibacterial effects. This was seen by the greater reduction in bacterial load, and inflammatory mediators in patients who sustained serum concentrations above the MIC throughout the dosing interval. Obviously since CI will always have higher T>MIC than intermittent, this could be seen as an indication that CI is more effective administration way for cefepime and possible other β-lactams.

Later when more patients are enrolled, the same tests can be used and provide much higher significance, and perhaps more powerful conclusions can be drawn.

V. APPENDIX A: STABILITY TESTING; HPLC ASSAY AND EQUIPMENT

HPLC assay

The intra-day coefficient of variation, measured with triplicates, ranged from 0.05%-1.15%, and inter-day during the 23 analytical days ranged from 1.33%-3.48%.

Cefepime was eluted after 7.8-8.6 minutes. The assay lower part of the standard curve was 25µg/mL. At this concentration the coefficient of variation was 0.54%.

None of the degradation products interfered with the cefepime peak, as shown in figure 26:

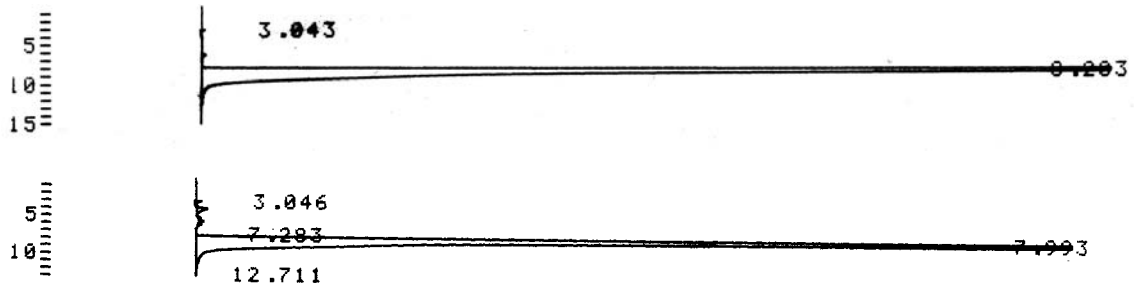


Figure 26: On the top is the chromatogram of freshly made cefepime solution, on the bottom is 9.7% of the cefepime broken down, and degradation products (retention time 3-6min) has no interference with the cefepime area.

HPLC equipment:

Pump: Hitachi, L-6200 Intelligent pump.

Detector: Hitachi, L-4200, UV-VIS Detector.

Autosampler: Hitachi AS-2000.

Integrator: Hitachi D-2500 Chromato-integrator.

Recycler: Alltech.

Column: C-18 column (250x4.6mm) with 4.6µm diameter particles from J&W Scientific.

Mass spectrometry:

Micromass Quattro Ultima triple quadrupole tandem mass spectrometer (Micromass, Beverly, MA).

Chemicals:

Cefepime: Maxipime® from Elan. Lot 1A42352, 0M28658 and 1F37831.

Sterile dextrose 5% from Abbott laboratories.

Ammonium Acetate, HPLC grade from Fisher Chemical.

Acetonitrile, HPLC grade from EM science.

Acetic acid, HPLC grade from J.T Baker.

0.2 µm 47mm nylon membrane filters from Gelman sciences.

0.2 µm 4mm nylon membrane filters from Nalgene.

Continuous infusions equipment:

Portable infusion pump: Microject 30, made of Sorensen Medical, West Jordan UT.

Drug reservoir and waste bag: MediBag™, made of EVA, 250mL from Sorenson medical.

Microject® cassette: filter, from Sorenson medical.

McGaw HyperFormer® PCA mixing container 250mL.

Black belt bag for the pump, drug reservoir and waste bag.

Temperature measurement:

TempTrace®, electronic temperature reader from Dickson.

Software for TempTrace; DicksonWare for windows V2.2LC.

Other:

Waterbath from Precision Scientific Co.

pH-meter type 320 from Corning

VI. APPENDIX B: HLPC ASSAY FOR SERUM SAMPLES AND EQUIPMENT

HPLC-assay

The serum processing consisted of adding 200µL of acetronitrile to the serum, then vortexing the mixture and centrifugation at 14000rmp for 15min. This step was done to precipitate and separate proteins in serum. The supernatant was then evaporated to dryness and reconstituted into 500µL of water. 50µL of this solution was injected into the HPLC. The mobile phase consisted of 8% v/v acetonitrile in 20mM ammonium acetate, adjusted to pH 4.0 with glacial acetic acid, all HPLC grade quality. The mobile phase was filtrated through a 47mm 0.22µm nylon membrane filter before use. To accomplish less interference from serum components, cefepime was detected at 250nm instead of its peak absorption at 260nm (29). The internal standard, ampicillin, was chosen because of its similar structure with cefepime and because of its availability. Ampicillin had a much lower absorption at 250nm and 100µg ampicillin was added to each solution to get absorption similar to the middle range of the standard curve. The co administration of Tobramycin has low or no UV absorption (44), and did not interfere with the determination of cefepime and ampicillin.

After each sample containing serum, the HPLC was washed for 10 minutes with acetonitrile to remove any possible compounds form serum that might be plugged in the column. Then it was flushed 10 minutes with the mobile phase to get the system homogenous again.
The standard curve is shown in figure 27.

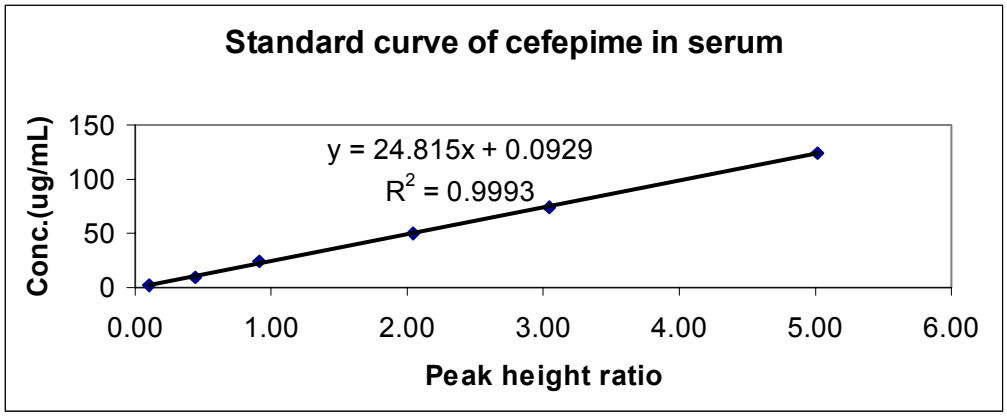


Figure 27: The standard curve of cefepime in serum: The peak height between cefepime and the internal standard ampicillin is plotted on the x-axis and the belonging cefepime concentrations are on the y-axis.

A typical patients chromatogram is shown in figure 28.

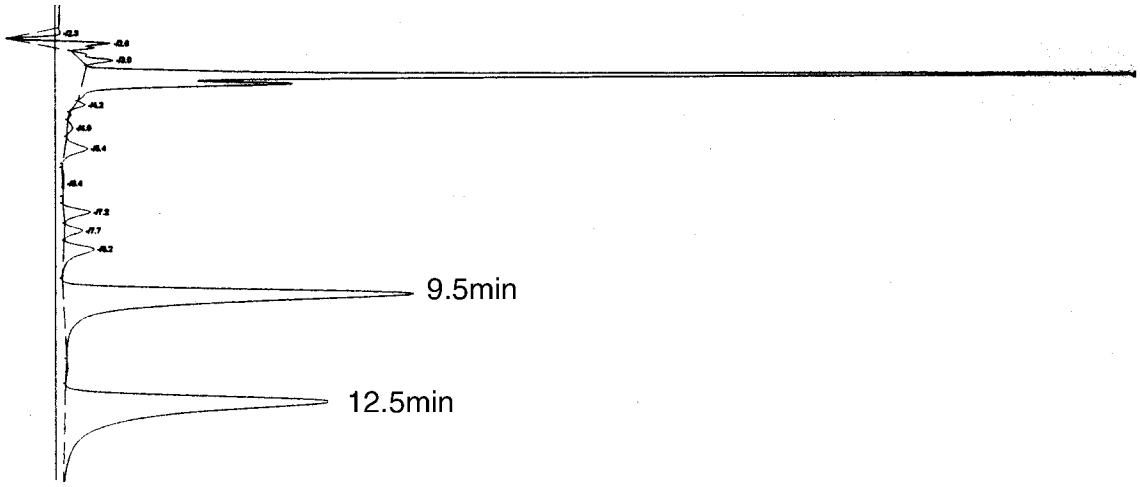


Figure 28: A typical chromatogram. Cefepime is eluted in 9.5 minutes with baseline separation from serum components with retention time 1-8minuts and also baseline separation with the internal standard (ampicillin) with retention time 12.5 minutes.

HPLC equipment:

Pump: Perkin-Elmer series 410 LC pump.

Detector: Perkin-Elmer, LC 90 UV, spectrophotometric detector.

Autosampler: Waters 715 Ultra WISP sample processor.

Column: Alltech Adsorbsphere C18 250x 4.6mm.

Chemicals:

Cefepime: Analytical grade lot. no 1C42905L from Bristol-Myers Squibb.

Ampicillin: Analytical grade lot.no.60120 from Bristol-Myers Squibb.

Ammonium Acetate, HPLC grade from Fisher Chemical.

Acetonitrile, HPLC grade from EM science.

Acetic acid, HPLC grade from J.T Baker.

VII. APPENDIX C: PHARMACOKINETIC PARAMETERS

***A-priori* values:**

For the 1-compartment MAP-Bayesian modeling (24):

Total body clearance: 3.01±1.46 mL/min/kg

Volume of distribution: 0.32±0.10L/kg

For the 2-compartment MAP-Bayesian modeling (39):

Distribution half life: 0.38±0.18 h

Elimination half life: 1.84±0.76 h

Volume of central compartment: 0.19±0.03 L/kg

Total body clearance: 8.74±1.86 L/h

Volume of peripheral compartment and distribution clearance was calculated using these parameters.

Patients' demographics and parameters calculated with a 2-compartment MAP-Bayesian model:

Table 9: *1*= DF508, Clearance was calculated with Cockroft & Gault equation. IM: Intermittent, PK: Pharmacokinetics, Patient #3 had flow rate problems and PK parameters were not calculated.*

Patient #	1	2	3	4	5	6	7	8
Genotype	1*/1*	1*/R117H	1*	1*/D11528	N1303K	1*/W1282X	1*/1*	
Dose	IM(PK only)	IM	CI(PK only)	IM	CI	IM	CI	IM
Age	26	36	50	38	36	37	28	28
Height (cm)	165	170	158	170	165	185	175	163
Weight (kg)	48.8	71.8	38.4	72.4	44.9	83.5	47	44.6
BMI(kg/m^2)	17.9	25.59	15.4	25.81	17	25.07	15.93	16.8
CrCl(mL/min)	131	110	58	147	92	133	146	99
Adm FEV_1	53%, 1.67	26%, 0.65	61%, 1.92	70%, 2.18	38%, 1.77	23%, 0.82	44%, 1.28	23%, 0.98
CLt (L/h)	2.480	1.870		1.540	2.579	3.368	2.920	2.217
CLd (L/h)	1.278	1.293		1.158	1.976	1.925	2.333	1.908
T1/2 α (hour)	0.367	0.386		0.668	0.581	0.534	0.320	0.620
T1/2 β (hour)	1.738	1.897		4.216	3.276	2.819	1.644	6.608
Vc (L)	0.271	0.271		0.414	0.370	0.329	0.214	0.314
Vp (L)	0.104	0.092		0.198	0.245	0.249	0.125	0.528
T>MIC(%)	88.000	30.200	100	100.000	100.000	97.1%	-	-
AUC								
Cavg,ss(µg/mL)	-	-		-	25.800	-	24.800	-
Cmax(µg/mL)	137.420	146.240		111.820	-	127.990	-	141.930
Cmin(ug/mL)	2.060	3.982		10.140	-	5.679	-	13.195

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