Pharmacokinetics and Pharmacodynamics of Piperacillin-Tazobactam in 42 Patients Treated with Concomitant CRRT

Seth R. Bauer, Charbel Salem,† Michael J. Connor Jr.,‡ Joseph Groszek,§ Maria E. Taylor,‖ Peilin Wei,‖ Ashita J. Tolwani,‖ and William H. Fissell†§*

Departments of *Pharmacy, †Nephrology and Hypertension, Cleveland Clinic, Cleveland, Ohio; ‡Division of Pulmonary, Allergy, and Critical Care Medicine, Emory University, Atlanta, Georgia; §Department of Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; and ‖Division of Nephrology, University of Alabama, Birmingham, Alabama

Correspondence: Dr. William H. Fissell, Biomedical Engineering ND/20, 9500 Euclid Avenue, Cleveland, OH 44195. Email: fisselw@ccf.org

Summary

Background and objectives Current recommendations for piperacillin-tazobactam dosing in patients receiving continuous renal replacement therapy originate from studies with relatively few patients and lower continuous renal replacement therapy doses than commonly used today. This study measured the pharmacokinetic and pharmacodynamic characteristics of piperacillin-tazobactam in patients treated with continuous renal replacement therapy using contemporary equipment and prescriptions.

Design, setting, participants, & measurements A multicenter prospective observational study in the intensive care units of two academic medical centers was performed, enrolling patients with AKI or ESRD receiving piperacillin-tazobactam while being treated with continuous renal replacement therapy. Pregnant women, children, and patients with end stage liver disease were excluded from enrollment. Plasma and continuous renal replacement therapy effluent samples were analyzed for piperacillin and tazobactam levels using HPLC. Pharmacokinetic and pharmacodynamic parameters were calculated using standard equations. Multivariate analyses were used to examine the association of patient and continuous renal replacement therapy characteristics with piperacillin pharmacokinetic parameters.

Results Forty-two of fifty-five subjects enrolled had complete sampling. Volume of distribution (median=0.38 L/kg, intraquartile range=0.20 L/kg) and elimination rate constants (median=0.104 h^{-1}, intraquartile range=0.052 h^{-1}) were highly variable, and clinical parameters could explain only a small fraction of the large variability in pharmacokinetic parameters. Probability of target attainment for piperacillin was 83% for total drug but only 77% when the unbound fraction was considered.

Conclusions There is significant patient to patient variability in pharmacokinetic/pharmacodynamic parameters in patients receiving continuous renal replacement therapy. Many patients did not achieve pharmacodynamic targets, suggesting that therapeutic drug monitoring might optimize therapy.

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Introduction

Piperacillin-tazobactam is a frequently used antibiotic in the intensive care unit (ICU) because of its broad spectrum of activity. Optimal dosing of antibiotics improves outcomes and reduces antimicrobial resistance. Patients with severe infections often have multisystem organ failure requiring renal replacement therapy, and continuous therapies are frequently preferred over intermittent hemodialysis in intensive care patients to avoid the development or exacerbation of hypotension and maximize fluid removal. Current dosing recommendations for piperacillin-tazobactam in patients receiving continuous renal replacement therapy (CRRT) (1,2) originate from either pharmacokinetic estimates or studies with relatively few patients and lower doses of CRRT than are used today (3,4). We designed this multicenter prospective observational study to evaluate the pharmacokinetics and pharmacodynamics of piperacillin-tazobactam in patients receiving CRRT with contemporary dialysis equipment and prescriptions.

Materials and Methods

This prospective, observational study enrolled patients admitted to the ICU of two tertiary care medical centers between October of 2008 and June of 2011. The study protocol was approved by the institutional review boards at the Cleveland Clinic (CCF; Cleveland, Ohio) and the University of Alabama (UAB; Birmingham, Alabama). All patients (or their surrogate decision-makers) provided written informed consent to participate in the study.

Subjects

Patients, ≥18 years of age, who were receiving piperacillin-tazobactam with concomitant CRRT in the ICU were included. Patients were excluded if

they had end stage liver disease or were pregnant. Age, sex, weight at admission, weight at time of study entry, dialysis prescription, urine output, and location of dialysis catheter and antibiotic infusion catheter (catheter concordance) were recorded on case report forms. Illness severity scores (CCF score) were calculated from the patients' medical record (5).

Antimicrobial Therapy

Patients in the study received piperacillin-tazobactam at either 3.375 g/dose (3000 mg piperacillin and 375 mg tazobactam at CCF) or 2.25 g/dose (2000 mg piperacillin and 250 mg tazobactam at UAB) as a premixed solution in 50 ml diluent (Zosyn in Galaxy containers) administered over 30 minutes scheduled every 6, 8, or 12 hours. The piperacillin-tazobactam dose and frequency were solely determined by the primary ICU service.

Dialysis Therapy

Patients received CRRT using the Gambro Prismaflex system with M-60 or M-100 dialyzer sets (acrylonitrile membranes with 0.6- and 0.9-m^2 membrane surface areas, respectively) or the NxStage System One dialysis system with the Express cartridge (polyethersulfone membrane with 1.5-m^2 membrane surface area) according to instrument availability and nephrology consulting service discretion. Patients at CCF were treated exclusively with continuous venovenous hemodialysis (CVVHD), with doses targeted to 25 ml/kg per hour. Patients at UAB exclusively received continuous venovenous hemodiafiltration (CVVHDF), with 1:1 balanced predilution replacement fluid and dialysate at a target rate of 35 ml/kg per hour to achieve a urea clearance of 25 ml/kg per hour. For this analysis, we have pooled data from CVVHD and CVVHDF, although clearance mechanisms and pharmacokinetics vary with prescribed mode of therapy. CRRT prescriptions were determined by the nephrology consulting service. Anticoagulation was achieved with an infusion of unfractionated heparin (250–500 IU/h) as tolerated or citrate regional anticoagulation (UAB only). For this manuscript, we have chosen to call waste fluids (both spent dialysate and ultrafiltrate) effluent.

Sampling

Sampling commenced after administration of at least four doses of piperacillin-tazobactam and 24 hours of concomitant uninterrupted CRRT. Patients with any interruptions in CRRT during the sampling period were excluded from analysis. Paired sets of blood and CRRT effluent samples were collected immediately before a dose (trough 1), 30 minutes after the 30-minute infusion was complete (peak), and immediately before the subsequent dose (trough 2). Blood samples were collected in 4.5-ml plasma separator vacutainer tubes. Dialysate samples were collected in 7-ml vacutainer tubes containing no additive. Because the majority of subjects were oligoanuric, no urine samples were collected or analyzed. All samples were stored on ice and processed within 60 minutes of collection. Blood samples were centrifuged at $3000 \times g$ for 10 minutes at 4°C. Dialysate samples were centrifuged identically to ensure that samples injected on the HPLC column were free of particulates. Two aliquots from each specimen were then placed in liquid nitrogen. All samples were analyzed in one laboratory (CCF). Specimens from UAB were shipped on dry ice to CCF for analysis.

HPLC Analysis

Total and free drug were analyzed by reversed-phase HPLC using an Agilent 1200 system as detailed in Supplemental Material (6). Briefly, dialysate is thawed and transferred directly into autosampler. One aliquot of plasma is ultrafiltered (300 μl, Centrifree YM-30, 2000 rotor centrifugal force [rcf] for 10 minutes at 4°C) and loaded in the autosampler, whereas another aliquot is protein-crashed in acetonitrile with internal standard (1:1 acetonitrile to aqueous phase) and centrifuged (10,000 rcf for 10 minutes). The supernatant is transferred into the autosampler. The autosampler is programmed to add internal standard to dialysate and filtered plasma (free drug) samples, whereas it dilutes acetonitrile crashed plasma (total drug) with PBS before analysis by HPLC ultraviolet absorbance (Kinetex 2.6u XB-C18 100A, PBS to acetonitrile gradient from 0:100 to 30:70 in 5 minutes). The within- and between-day coefficients of variation for piperacillin at a concentration of 100 μg/ml were 0.07% and 2.9%, respectively. The within- and between-day coefficients of variation for tazobactam at 25 μg/ml were 1.6% and 2.4%.

Pharmacokinetic and Pharmacodynamic Calculations

The sampling method used allowed description of a one-compartment, first-order elimination process (4,7). Given that sampling was not performed during the distribution phase, standard first-order equations were used to calculate the pharmacokinetic (PK) parameters of interest. The percent of time that the free piperacillin plasma level was above the minimum inhibitory concentration (MIC) during the dosing interval (%T>MIC) was calculated for MIC of 16 and 64 mcg/ml using previously reported methods (8,9). A pharmacodynamic (PD) target for piperacillin of >50%T>MIC64 mcg/ml was selected as the parameter of interest based on prior publications and proportion with target achievement calculated (10). The equations used to calculate the PK and PD parameters are listed in Supplemental Material.

Statistical Analyses

Nominal data are reported as *n* (%). Continuous data are reported as mean ± SD (normally distributed data) or median and intraquartile range (IQR; non-normally distributed data). Factors independently associated with volume of distribution and the elimination rate constant were examined by multivariate linear regression. Non-normally distributed variables were log-transformed for inclusion in linear models. Parameters expected to affect clearance and volume of distribution as well as the academic medical center (CCF versus UAB) were included in *a priori*–defined linear models. Nonsignificant parameter estimates were sequentially removed from the model until the four most significant parameters remained. Univariate analyses of each parameter were then completed, and no parameters gained or lost statistical significance between univariate and multivariate models. All tests were two-tailed, and a $P<0.05$ was considered statistically significant.

Results

A total of 55 patients were enrolled in this prospective, observational study between October of 2008 and June of 2011 (Table 1). Eleven subjects had CRRT treatment interruptions between the peak and second trough, and two subjects had sample collection errors, leaving 42 patients for analysis; 6 of 42 subjects had ESRD, and the remainder had AKI. All plasma and CRRT effluent samples collected in the study were assayed for total and free piperacillin and tazobactam levels. Tazobactam data from nine subjects seemed to be affected by an interfering analyte in the HPLC chromatogram and were omitted from the tazobactam analysis.

The demographic characteristics of the study population are presented in Table 1 and are generally similar to subjects in the VA/NIH Acute Renal Failure Trial Network (ATN) and Renal Replacement Therapy Study (RENAL) trials, although 10–15 kg heavier (11,12). This study population is relatively obese compared with studies from Europe and Asia (10,13). The majority of patients were severely oligoanuric (median=38 ml/24 h, IQR=157 ml). Of the included patients, 8 patients were dialyzed with a polyethersulfone filter with a large membrane surface area (1.5 m^2), whereas 34 patients were dialyzed with an acrylonitrile filter with a relatively smaller membrane surface area (0.9 m^2 [n=32] or 0.6 m^2 [n=2]). The overall hospital mortality rate was 50.0%. PK analysis is presented in Table 2. Data from subjects treated with CVVHD and CVVHDF were pooled for analysis after a univariate analysis did not reveal an influence of mode of therapy on PK or PD parameters, although clearance mechanisms and PKs would be expected to vary with prescribed mode of CRRT. Volumes of distribution for piperacillin (median=34.5 L, IQR=30.5 L) and tazobactam (median=38.1 L, IQR=27.1 L) were large with low protein binding compared with noncritically ill patients. Elimination rate constants were similar for total and free drug, with free drug being eliminated slightly more quickly, which was expected. Tazobactam (median half-life=11.5 hours, IQR=8.4 hours) was eliminated slightly more slowly than piperacillin (median half-life=9.6 hours, IQR=4.2 hours). Total clearance was highly variable from patient to patient (piperacillin: median=64.5 ml/min, IQR=59.3 ml/min; tazobactam: median=48.3 ml/min, IQR=46.5 ml/min). Slightly under one-half of the drug clearance was through the dialysis circuit for piperacillin, and slightly more than one-half of the drug clearance was through the dialysis circuit for tazobactam. Tazobactam trough levels were all in excess of the Clinical Laboratory and Standards Institute or European Committee on Antimicrobial Susceptibility Testing thresholds for β-lactamase inhibition (4 μg/ml).

The multivariate model explained only a small portion of the variability in volume of distribution of our single-compartment, first-order model (Table 3). Volume of distribution decreased with increasing age ($P<0.03$) and increased with weight change since admission with nearly perfect identity: for each kilogram of weight gain since admission, volume of distribution increased 1.01±0.32 L ($P<0.003$). There was no statistically significant association between volume of distribution and CRRT filter type as might arise from a first-pass effect because of drug binding to the filter material. We considered potential first-pass effects if the antibiotics are infused through a catheter near the CRRT catheter. We categorized dialysis and antibiotic catheters as on the same or opposite sides of the patient's

Table 1. Patient and treatment characteristics

Variable	Value
Subjects	
N	42
age (years)	56.8±15.5
sex (male/female)	25/17
weight (kg)	95.1±26.8
AKI	36/42
24-hour urine output (ml)	38 (157)
CCF score (*n*=36)	7.9±2.8
survival (%)	50.0
center (CCF/UAB)	19/23
Treatment	
CRRT mode (CVVHD/CVVHDF)	19/23
CRRT dose (ml/kg per hour)	25.7 (15.8)
CRRT machine (Prismaflex/NxStage)	34/8
piperacillin dose (2 g/3 g)	19/23
piperacillin dosing interval (6 h/8 h/12 h)	23/16/3
cumulative 24-hour piperacillin dose (g)	8.6±1.5

Data are presented as mean ± SD or median (intraquartile range). CCF, Cleveland Clinic, Cleveland, Ohio; UAB, University of Alabama, Birmingham, Alabama; CRRT, continuous renal replacement therapy; CVVHD, continuous venovenous hemodialysis; CVVHDF, continuous venovenous hemodiafiltration.

Table 2. Piperacillin-tazobactam pharmacokinetics in 42 intensive care unit patients receiving continuous renal replacement therapy

	Total Drug	Free Drug
Piperacillin PK parameters		
protein binding (%)	19.0±9.7	
Vd (L)	34.5 (30.5)	38.2 (26.5)
Vd (L/kg)	0.38 (0.20)	0.43 (0.26)
ke (h^{-1})	0.104 (0.052)	0.120 (0.073)
$t_{1/2}$ (hours)	9.6 (4.2)	5.8 (3.6)
clearance (ml/min)	64.5 (59.7)	78.6 (62.2)
CRRT clearance (ml/min)	27.6 (15.2)	33.2 (14.9)
Tazobactam PK parameters		
protein binding (%)	14.6 (36.5)	
Vd (L)	38.1 (27.6)	50.6 (54.1)
Vd (L/kg)	0.38 (0.33)	0.50 (0.56)
ke (h^{-1})	0.086 (0.058)	0.089 (0.063)
$t_{1/2}$ (hours)	11.5 (9.4)	7.8 (6.3)
clearance (ml/min)	48.3 (46.5)	83.6 (86.5)
CRRT clearance (ml/min)	25.7 (15.3)	35.7 (17.8)

Data are presented as mean ± SD or median (intraquartile range). PK, pharmacokinetic; CRRT, continuous renal replacement therapy.

Table 3. Clinical predictors of piperacillin total drug pharmacokinetic parameters

Multivariate Linear Regression	Parameter Estimate	Probability>|t|
Volume of distribution Vd (adjusted R^2=0.36)		
age (years)	−0.54±0.23	0.02
weight gain (kg)	1.01±0.32	0.003
current weight (kg)	0.16±0.13	0.23
CRRT filter[a]		0.38
catheter location[b]		0.80
Elimination constant Ke (adjusted R^2=0.26)		
log(CRRT dose [ml/kg per minute])	0.084±0.028	0.005
CRRT mode (CVVHD versus CVVHDF)	0.0006±0.012	0.60
sex	0.0078±0.0087	0.37
CCF score (n=36)	−0.0035±0.0031	0.27

CRRT, continuous renal replacement therapy; CVVHD, continuous venovenous hemodialysis; CVVHDF, continuous venovenous hemodiafiltration; CCF, Cleveland Clinic, Cleveland, Ohio.
[a]NxStage Express versus Gambro M60/M100.
[b]Whether dialysis access and antibiotic infusion catheter were in the same vessel.

diaphragm (catheter concordance) and tested the relation between observed volume of distribution and catheter concordance; no correlation was observed.

Elimination rate constants were also highly variable (piperacillin: median=0.104 h^{-1}, IQR=0.052 h^{-1}; tazobactam: median=0.086 h^{-1}, IQR=0.058 h^{-1}). The only factor significantly associated with the piperacillin elimination rate constant was CRRT dose (Table 3). Of note, CRRT mode was not significantly associated with piperacillin elimination rate. When urine output was included in the regression model, urine output was not significantly associated with elimination rate constant (P>0.08), and the coefficient (2.57×10^{-5}) was very low, suggesting that the dependence, in addition to not reaching statistical significance, was not clinically significant in this population of patients with severe renal failure.

β-lactams are generally considered time-dependent antimicrobials, and the PD parameter of interest is the fraction of time that the free drug's concentration exceeds a specified breakpoint, with a typical target for piperacillin of greater than 50% (9,14). Choosing the Clinical Laboratory and Standards Institute piperacillin susceptibility breakpoint of 16 μg/ml for enterobacteraceae allowed us to calculate how many subjects achieved target attainment for specified levels of 1× and 4× MIC (Table 4) (15). All subjects achieved the more liberal goal of fT>MIC=16 μg/ml > 50%. Despite relatively low protein binding, 83% of subjects achieved the higher PD goal fT>MIC=64 μg/ml>50% if one considered the total drug concentration (free and bound). However, if one considers only the active, unbound fraction, only 77% of subjects achieved the PD target.

Discussion

To our knowledge, this manuscript presents PK/PD data on piperacillin-tazobactam in CRRT from the largest number of patients reported to date. The data support the findings of prior investigators. Our primary conclusion is that patient to patient variability in drug clearance makes prospective dose calculation challenging for patients receiving concomitant piperacillin-tazobactam and CRRT.

PK parameters for piperacillin in our study resembled those parameters reported in the work by Seyler *et al.* (10), and they are notably differ from those parameters published in a commonly used prescribing guide ("The Green Book") (16). Protein binding was lower, volume of distribution was higher, and half-life was longer than described in this prescribing guide (16). Half-lives measured in our study resembled those values measured by Valtonen *et al.* (17) for 2 L/h CVVHDF effluent, but they were shorter than those values reported in the work by Arzuaga *et al.* (3,18). Total and extracorporeal clearance was higher in our study (75 versus 50 ml/min; 29.7 versus 11.45 ml/min, respectively) than reported in the work by Arzuaga *et al.* (3,18) for patients with severe renal failure on predilution CVVH with much lower effluent rates than used here (3). Compared with the measurements in the work by Mueller et al. (4), our patients had lower elimination rate constants for both piperacillin and tazobactam.

Our PD data resembled the data of others, suggesting that the probability of target attainment (probability of reaching fT>MIC=64 μg/ml>50%) was not 100% (10,19,20).

Measurements of tissue levels for β-lactams are generally, at best, one-half to one-quarter of plasma levels and in septic patients, possibly much lower (18,21–23). The relatively low probability of target attainment raises significant concerns regarding response to infections and development of antimicrobial resistance.

The prospective determination of drug dosing in patients with fluctuating intrinsic and extracorporeal clearances is highly challenging (16,24,25). The work by Taccone et al. (26) correctly points out that β-lactam antibiotics may be underdosed in early sepsis, although a minority of the subjects in that study had acute renal failure. Ideally, the practitioner would be able to use clinical observations, such as age, weight, creatinine clearance, and others, to prospectively calculate a dose that would achieve desired PD targets. Unfortunately, the data presented here suggest that this approach may not be feasible. For example, our

Table 4. Piperacillin/tazobactam pharmacodynamics (median [interquartile range])

	Piperacillin		Tazobactam	
	Total	Free	Total	Free
Peak	135 (78.4)	115 (62.1)	20.9 (16.2)	16.3 (16.4)
Trough	66.2 (39.3)	54.8 (35.2)	11.7 (9.7)	9.0 (12.6)
fT>MIC=64 μg/ml>50%	83%	77%		

MIC, minimum inhibitory concentration.

observations of the weight-normalized volume of distribution for piperacillin showed an IQR (0.20 L/kg) that was nearly one-half of the median value of 0.38 L/kg. This finding creates a challenge for the practitioner in selecting the appropriate value to calculate a weight-based loading dose. More confusingly, weight gain since admission was much more strongly associated with volume of distribution than was the actual weight at time of study entry (Table 3). With only 42 patients in our study, it would be difficult to construct a model based on more predictor variables than the five that we selected (age, weight gain, current weight, filter type, and catheter concordance). Even with this limited sample size, our best fit linear regression explained less than one-half of the variance in the observed volume of distribution.

A similar challenge lies in prospectively calculating a piperacillin dosing interval from clearance estimates. Extracorporeal clearance accounted for slightly less than one-half of the total clearance, a larger fraction than reported by other investigators. The SD was approximately 50% of the mean total clearance for total and free drug (76.6±37.9 and 90.0±45.0 ml/min, respectively). We examined factors that might contribute to extrinsic drug clearance, such as CRRT dose and CRRT mode. We used illness severity as estimated by the CCF (based on the work by Paganini *et al.* [5]) scoring system as an estimate of organ dysfunction and possibly, impaired intrinsic clearance. In our limited analysis, only prescribed CRRT dose was significantly associated with elimination rate constant and not CCF score or mode of CRRT.

The datasets for tazobactam closely resembled the datasets for piperacillin with larger confidence intervals. Tazobactam concentrations in blood were lower than for piperacillin, although the lower limits of detection and quantitation were the same; therefore, we attribute some of the broader confidence intervals to a lower signal/noise ratio for tazobactam.

Our study has several significant limitations that deserve attention in considering the interpretation and generalizability of our results. We limited our consideration solely to situations where CRRT had not been interrupted during the observation period in an effort to attenuate potential sources of variability. This limitation is closely related to a second limitation of our study, which is that we initiated sampling after four doses had been administered in an effort to measure PD parameters in a situation close to steady state. In this sampling, we were somewhat successful, because the mean difference between the two troughs was only 1.8 μg/ml. However, the time period of most importance may be the first few doses. Neither center routinely calculated or administered loading doses of piperacillin, although importantly, the PKs that we observed would support such a practice. The most significant limitation of the analysis is the oversimplified PK model dictated by our three time point sampling strategy. We, as well as institutional review boards, had significant concerns regarding repeated blood sampling in this critically ill population. As a result of our trough–peak–trough sampling strategy, we had only two data points (peak and trough) with which to estimate clearance. Although previous data have supported a first-order elimination process of piperacillin, we cannot confirm this assumption with our data. As such, the variability noted in our data could reflect a poor fit of the model that we used.

Therapeutic drug monitoring is commonplace for certain antimicrobial antibiotics, such as vancomycin or gentamicin, but could be overwhelming to attempt for every antimicrobial in every patient. Several groups have shown the feasibility of β-lactam therapeutic drug monitoring in the general ICU population (27–29), and our data suggest that this monitoring may be particularly important in patients on CRRT. Our data suggest that a piperacillin dose of 9 g/d (piperacillin/tazobactam=3.375 g every 8 hours) may result in failure to reach the PD goal, and practitioners should consider using doses above 9 g/d in practice. In the absence of therapeutic drug monitoring for piperacillin, doses should be individualized based on severity of illness, patient characteristics (weight gain), and CRRT dose.

The ATN and RENAL studies have motivated investigators to look beyond small solute clearance (11,12,30,31). There may be room for improvement in care of critically ill patients with renal failure by optimizing antibiotic dose and timing of therapy. Studies to determine which patients are at highest risk of treatment failure may be helpful in selecting patients who will benefit from patient-specific dosing strategies.

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Disclosures

None.

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Piperacillin-tazobactam Chromatographic Analysis Detailed Methods

Collection

Blood is collected in PST Lithium Heparin Blood Collection tubes (#367963 - BD, Franklin Lakes, NJ) and dialysate is collected in blood collection tubes with no additives (#366431 - BD, Franklin Lakes, NJ). Samples are centrifuged, (10 min, 4ºC, 3,000 rcf), resultant plasma and dialysate are aliquoted into cryovials (1400 uL - 430488 – Corning, Corning, NY), and stored in liquid nitrogen within 1 hr of collection. Samples that are from centers outside the Cleveland clinic are shipped overnight on dry ice.

Calibration Standards

Five level standards are prepared (by mass) each day HPLC analysis is run. Each standard contains Piperacillin sodium salt (Sigma, St. Louis – P8396) and Tazobactam (LKT Laboratories, Inc., Montreal, Quebec – T0298). Calibration levels are prepared by diluting a fresh 1 mg/mL stock of each drug with PBS elution buffer at 0.5, 2, 25, 100, and 200 ug/mL for Piperacillin and 0.5, 2, 6, 25, 50 ug/mL for Tazobactam. Stocks were diluted on a balance to have precision to 0.01ug/mL when constructing a calibration curve. Penicillin-G (BP914 – Fisher, Fair Lawn, NJ) is prepared at 1 mg/mL and used as an internal standard (IS). One vial containing IS is placed in the autosampler. The autosampler is programmed to wash the needle, draw 1uL of IS from reservoir vial, then draw 9 uL from the sample or level standard.

Dialysate Preparation

Dialysate samples are thawed and pipetted directly into 96-well autosampler plate (#3363 - Costar, Corning, NY) and individually covered with a piercable lid (MP53001 - MicronicUSA, Aston, PA).

Free Drug Preparation

Plasma samples are thawed and aliquoted (300 uL) into centrifugal filter units (Centrifree YM-30 - Millipore, Ireland) and centrifuged with a fixed angle rotor (10 min., 4ºC, 2,000 rcf). All the filtrate is transferred to the 96-well autosampler plate.

Total Drug Preparation

HPLC grade acetonitrile (A998SK-4 – Fisher, Fair Lawn, NJ) is mixed 10:1 (v/v) with internal standard. This mixture is pipetted into micro-centrifuge tubes (200 uL). Thawed plasma samples are added drop-wise (163.6 uL) yielding a 1:1 mixture of acetonitrile to aqueous phase (IS+Sample). Each tube is vortex mixed (~5 seconds), allowed to stand (~1 min.), then centrifuged (10 min., 10,000 rcf). The resulting supernatant is pipetted directly into a 96-well autosampler plate and immediately capped with a piercable lid. Once in the temperature controlled autosampler, each sample is diluted prior to injection to mitigate poor peak shape from sample versus mobile phase differences. The autosampler needle is used to draw and eject sample (20 uL) into a remote 'mixing well' (14230238 – Fisherbrand, Pittsburgh, PA). Then an aliquot of mobile phase 'A' (40 uL - from a vial inside the autosampler) is added to the sample in the remote mixing well. In between each step the autosampler needle is washed to eliminate carry-over or contamination. The autosampler does a series of fast draws and ejects to mix sample and diluent, then injects 10 uL onto the column for analysis.

These added dilution steps increase the time to make an injection, but allow analysis without the need for solid phase extraction or liquid-liquid extraction. Level standards are processed identically to total drug patient samples yielding a unique calibration curve for total drug and eliminating the need for multipliers and/or dilution factors.

Chromatographic Conditions

The chromatographic system consists of a degasser (G13122A), binary pump (G1312B), a chilled autosampler (G1367D and G1330B), column heater (G1316A), and diode array detector (G1315C) all from Agilent (Santa Clara, CA). Separation is achieved on a Kinetex 2.6µ XB-C18 100Å, 100 x 3.00 mm column with a KrudKatcher inline filter (00D-4496-Y0 and AF0-8497 – Phenomenex, Torrance, CA) both kept in the column oven at 30ºC. The autosampler is kept at 4ºC. Mobile phase A: phosphate buffered saline (PBS - P4417 – Sigma, St. Louis, MO) with 5% HPLC grade methanol (A452-4 – Fisher, Fair Lawn, NJ). Mobile phase B: HPLC grade acetonitrile with 0.1% trifluoroacetic acid (HB9813-4 – Fisher, Fair Lawn, NJ). Gradient elution is performed at a flowrate of 0.8 mL/min run from 0% B to 30% B over 5 minutes. The mobile phase is returned to 0% B over 0.1 minutes and allowed to equilibrate for 1.9 minutes for a total run time of 7 min. Antibiotics and internal standards (IS) are quantified by UV absorbance at 214 nm.

Assay Characteristics

The chromatographic assay has a lower limit of quantitation for piperacillin or tazobactam of 1 µg/ml for the total drug assay and 0.5 µg/ml for free drug and dialysate. The within-day and between-day coefficients of variation for piperacillin at a concentration of 100 µg/ml were 0.07% and 2.9%, respectively. The within-day and between-day coefficients of variation for tazobactam at 25 µg/ml were 1.6% and 2.4%.